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Application and Value of Contrast-enhanced Ultrasound (CEUS) in Focal Liver Lesions

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1. List of abbreviations

AFP	Alpha– fetoprote
CECT	Contrast– enhanced computed tomography
CEMRI	Contrast– enhanced magnetic resonance imaging
CEUS	Contrast– enhanced ultrasound
CT	Computed Tomography
FLL	Focal liver lesion
FNH	Focal nodular hyperplasia
HCC	Hepatocellular carcinoma
MI	Mechanical index
MRI	Magnetic Resonance Imaging
NAFLD	Non-alcoholic fatty liver disease
NPV	Negative predictive value
PPV	Positive predictive value
SF6	Sulfur hexafluoride
UCA	Ultrasound contrast agent

2. List of publications

2.1 The diagnostic value of contrast-enhanced ultrasound (CEUS) for assessing hepatocellular carcinoma compared to histopathology; a retrospective single-center analysis of 119 patients

Constantin Marschner[1], **Lan Zhang[1]**, Vincent Schwarze, Wiebke Völckers,

Matthias Frank Froelich, Niklas Freiherr von Münchhausen, Moritz Ludwig Schnitzer, Thomas Geyer, Matthias Philipp Fabritius, Johannes Rübenthaler, Dirk-André Clevert

Clinical Hemorheology and Microcirculation. DOI：10.3233/CH-209221

2.2 Long-term study analysis of contrast-enhanced ultrasound in the diagnosis of focal nodular hyperplasia

Giovanna Negrão de Figueiredo-Miller, Katharina Müller-Peltzer, Vincent Schwarze, Constantin Marschner, **Lan Zhang**, Johannes Rübenthaler, Timo Siepmann, Ben Min-Woo Illigens, Dirk-André Clevert

Clinical Hemorheology and Microcirculation. DOI 10.3233/CH-190710

3. The contribution to the publications

3.1 Contribution to publication I

(1): Recorded and collected the data in Picture Archiving and Communication System (PASC) of our institution.

(2): Analyze the collected and recorded data, and analyze the main conclusions in the publication.

(3) : Participate in the writing and editing of manuscript.

3.2 Contribution to publication II

(1): Recorded and collected the data in PASC of our institution.

(2): Analyze the collected and recorded data, and analyze the main conclusions in the publication.

(3): Participate in the writing and editing of manuscript.

4. Introduction

4.1 Contrast agents for ultrasound

Currently, ultrasound is the best choice to examine focal liver lesions (FLL), because the technique has a variety of advantages, such as real–time imaging, lack of radiation, and low cost [1,2]. Two-dimensional ultrasound and color Doppler ultrasound can make the definite diagnosis of typical hyperechoic hemangiomas and focal nodular hyperplasia（FNH）with spoken-wheel enhancement [3–9]. However, for atypical or complex liver lesions, the accuracy of the final definitive diagnosis may be limited. Contrast-enhanced ultrasound (CEUS) is an imaging method that has been applied in Europe and Asia for more than 20 years, and injection of contrast microbubbles can improve the diagnostic accuracy of FLL [10].

More than two decades later, the era of microbubbles as an ultrasound contrast agent (UCA) has arrived, and more than 50 countries support this application [11–13]. The main requirement of UCAs for clinical work is that they can be administered intravenously and completely pass through the heart and lung channels [14–17]. Its widespread application stems from the fact that gas-filled microbubbles are about the same size as erythrocytes in diameter and can circulate freely in the vascular system [18].

The exploration of contrast agent microbubbles has led to the establishment of many new areas of ultrasound imaging technology, such as liver, kidney and breast [3,19–21]. The microbubbles contain small spherical gases with low solubility in the blood, such as the perfluorocarbon (surrounded by a thin and biocompatible shell, which is typically lipid, protein and polymer can also be used). Microbubbles suspended in saline are injected into peripheral veins, such as the anticubital veins [13]. In general, the contrast agent dose of focal liver nodules is between 1.2 ml and 2.4 ml and contains millions of microbubbles [22,23]. UCAs can increase the effect of echo in the blood by 500 to 1,000 times. Therefore, contrast agents allow the users to easily observe the microvascular distribution in tissues or focal lesions [24]. After

diffusion of the gas into the blood for about 5 minutes, the small shell materials can be metabolized. Simultaneously, the gas in the microbubbles is expelled from the body through respiration.

4.2 Development and introduction of UCA

4.2.1 Free gas bubble

Gramiak and Shah [25] firstly used stirred saline and glucose solutions to enhance blood echoes in 1968. The larger microbubbles in these solutions can be effectively filtered through the lungs and are unstable. Therefore, there are so many limitations to the application of free gas. At present, free gas bubbles are rarely used as a contrast agent, except for accidental use to distinguish cardiac shunts [26].

4.2.2 First-generation agents

The first-generation of UCA contained microbubbles, called air-filled microbubble contrast agents, which dissolved in the blood when exposed to sound pressure in the ultrasound field. As a result, the first-generation of contrast agents can exist in the blood for a very short time. An example of a first-generation agent is Levovist, which was developed by the Schering company in Germany [27,28] and was the first intravascular contrast agent (consisting of microcrystalline galactose particles and 0.1% palmitic acid). After dissolving in blood, galactose degrades in the microparticles, thus creating an irregular adherent surface for the microbubbles (the diameter is 3–4 um). A number of early studies on Levovist have demonstrated that it can increase grayscale and color Doppler signals at sufficient concentrations when use in non–linear imaging examination modes.

4.2.3 Second-generation agents

In the 1990s, researchers replaced the air in microbubbles with low-diffusion and low-saturation fluorinated gas, which can significantly prolong the survival time of the microbubbles in the body. Furthermore, the fluorinated gas was difficult to dissolve in the blood. Because the advantage of its low solubility, the second

generation of UCA were generated and widely applied. Bubbles oscillate when exposed to ultrasonic beams (bubbles are compressed during the positive pressure generated by the ultrasound and expand during the negative pressure phase). The compression of gas is smaller than the expansion, causing a non-linear echo. The enhanced mode is quite similar to the intravenous contrast agents applied in CT and MRI, which greatly affect the backscattering of ultrasound and increase the contrast of blood vessels. The second-generation UCA is represented by SonoVue (including microbubbles and a phospholipid shell). Simultaneously, phospholipid shell is filled with sulfur hexafluoride (SF6) gas [29–31]. Currently, SonoVue is the most commonly used UCA.

4.3 Advantages and contraindications of UCA

4.3.1 Safety

UCAs can be safely used in a variety of applications with minimal risk to patients. They are not expelled from the body through the urinary or digestive system and safe for patients with hepatic and renal failure. Because of no toxic effect can be found on the heart, liver and kidneys, there is no need to conduct a blood test before injections of UCA. The incidence of allergic reactions to UCA is very low [32,33]. Several millions of ultrasound-contrast injections for clinical diagnosis have been administered around the world. They are well-tolerated and have excellent safety records. In the wide use of SonoVue, the incidence of allergic reaction is 1: 7,000 [34], which is much lower than other imaging contrast agents [35]. No relevant death have been reported in the literature.

4.3.2 Compared with CT and MRI contrast agents

CT and MRI contrast agents cannot be used for patients with renal failure or contraindications to iodine. CEUS allows for real-time imaging, dynamic, and repeat examinations. In addition, compared with other contrast agents, UCAs are more cost-effective. Their incidence of allergic reactions is lower than CT and MRI contrast agents because UCA does not contain the iodine. The injection dose of UCA is very

low (usually1.2–2.4ml), while contrast-enhanced CT (CECT) requires a high-pressure pump to quickly inject the contrast agent into the vein [12,36]. Furthermore, the injection dose is large at 80–100ml.

4.3.3 Contraindications of UCA

Just like other imaging contrast agents, there are some contraindications to UCAs. Contraindications include: allergies to contents of UCA; heart disease with right-to-left shunts; severe pulmonary hypertension, uncontrolled hypertension or adult with respiratory distress; severe cardiac dysfunction, arrhythmia or chronic obstructive pulmonary disease; and acute myocardial infarction [37–40]. The safety for pregnant and lactating women is not clear, so it is prohibited to use these agents in these populations.

With the ongoing development of CEUS examination, due to advantages such as safety, real-time imaging, cost-effectiveness and good tolerance, the technology is accepted by an increasing number of patients with a high diagnostic accuracy [4，41,42].

4.4 Clinical application of CEUS in the diagnosis of FLLs

In most clinics, ultrasound is the primary choice for patients with FLLs to determine the nature of the focal liver diseases [43]. In addition, liver lesions is usually detected during unintentional ultrasound screening [44].

Once a liver lesion is detected, the most important issue is often to distinguish between benign and malignant. However, discovering and diagnosing FLLs by non-enhanced ultrasound are limited by grayscale mode and microvascular blood flow, and therefore the sensitivity and specificity of non-enhanced ultrasound are generally not as good as in CT and MRI [3,45–50], two contrast-enhanced methods that can be used to better diagnose and differentiate focal liver tumors. A large number of experimental researches illustrated that the accuracy of CEUS in the diagnosis of hepatic tumors can match to that of CECT and CEMRI. Therefore, CEUS imaging is increasingly trusted by patients and doctors. Next, I will introduce in detail the

application of CEUS in the diagnosis of hepatic tumors as well as the imaging basis of CEUS of the liver.

4.4.1 Configuration of contrast agent

Levovist (Schering AG, Berlin, Germany), Sonazoid (Daiichi, Sankyo, Tokyo, Japan), Definity (Lantheus Medical Imaging, North Billerica, MA), SonoVue（Bracco, Milan, Italy）and Optison (GE Healthcare, Buckinghamshire, UK) currently used in clinics as UCAs, and there are specific requirements of them for clinical application. At present, SonoVue is most often used in the clinics and clinical experiments [51–53]. The standard product contains 25 g white lyophilized powder, a glass vial filled with 59 mg SF6 gas and a disposable sterile syringe.

The configuration process of SonoVue is shown as figure 1. During use, the 5 ml physiological saline (0.9% NaCl) is injected into a vial that contains SF6 lyophilized powder and shaken for 20 s until the contents of the bottle are mixed uniformly to form a milky white microbubble suspension liquid. Its concentration is 8 ul of SF6 per ml, and its suspension is ph4.5–7.5. In addition, it is isotonic with human plasma. The configured suspension can be placed for 6 hours at room temperature, and the use effect is stable.

Figure1. The configuration process of SonoVue

4.4.2 Route and method of contrast agent injection

At present, UCA is routinely injected into peripheral superficial veins, and most of them are anticubital veins. In special cases, intravenous catheter needles can be used. The diameter of the injection needle should be greater than 18G to avoid damage to the microbubbles caused by mechanical shock when injecting. The injection method for contrast agent is mainly bolus injection, which involves injecting the contrast agent into the blood vessel at the fastest speed (<5 s) and immediately injecting 5 ml of 0.9% saline [54]. This method allows the contrast agent to enter the lesion faster and at a higher concentration, enabling effective observation of the dynamic changes of the contrast enhancement [55,56].

4.4.3 Operation and method of CEUS

Routine two-dimensional grayscale ultrasound examination of the liver can confirm the main target of CEUS. Meanwhile, Doppler technology can be used for a preliminarily observation of the blood supply in the lesion area.

After the target lesion is identified, the probe position is essentially fixed, and the imaging mode is switched to the CEUS specific imaging mode.

Firstly, it is necessary to use a specific imaging mode with a low mechanical index (MI) [57]. Then, according to the location and scope of the observation area, one must choose the appropriate MI (usually<0.3) [58,59], then quickly inject the specified amount of UCA followed by 5–10 ml 0.9% saline into the veins. Timing begins at the same time as the contrast agent injection [14–17]. Due to the continuous and dynamic characteristics of CEUS, dynamic images of each vascular phase are recorded.

The operator selects a dual-mode that displays both the fundamental image and the contrast harmonic image simultaneously to guarantee that the lesion of interest is always in the scanning section during the examination [60].

Usually, a single injection of contrast agent is sufficient to make an accurate diagnosis of a hepatic lesion. If necessary, the agent can be reinjected after the complete

disappearance of microbubbles. The required interval between two injections usually more than 15 min.

After the completion of CEUS examination, the imaging mode is switched to the fundamental ultrasonic state. Color Doppler can be used to detect the lesion again to make up for the deficiency of blood flow in the lesion by grayscale ultrasound with harmonic frequency wave.

4.4.4 Phases of CEUS in liver

After the injection of UCA through peripheral veins, the sequence of contrast agents in the normal liver is usually: hepatic arteries and its branches, main portal veins and branches, superficial and deep parts of the liver parenchyma and finally hepatic veins. As the hepatic tissue is supplied by dual blood supply, from the portal vein (70% –75%) and hepatic artery (25%–30%), CEUS can allow the user to define and observe three blood vessel phases, including the arterial phase, portal phase and delayed phase [61,62]. Tissue enhancement of the hepatic arterial blood supply often begins 10–20 s after peripheral intravenous injection and sustains for 10–25 s. 2 minutes after the injection of UCA, the portal phase is followed. The last phase continues till the cancellation of UCA from the liver parenchyma, which occurs about 5 mins after injection with SonoVue [63]. This last phase is different from the equilibrium period of extracellular CT and MRI enhanced agents [64,65].

Based on the characteristic enhancement types, CEUS in liver can clearly enhance the differentiation and diagnosis of hepatic tumors with good diagnostic consistency compared with CECT and CEMRI [66-68]. CEUS shows the microvascular distribution of liver tissue and liver tumors, and different enhancement modes in different phases can provide important information for clinical diagnosis.

4.4.5 Analysis method of CEUS imaging

Followed by the injection of contrast agent, the interpretation and analysis of CEUS imaging should be performed based on the following aspects: time of enhancement, location of enhancement, enhancement patterns, intensity of enhancement,

performance at peak enhancement, enhancement duration and dynamic change of enhancement. Each of which is discussed below.

Time of enhancement. This usually refers to the period during which the contrast agent enters the region of interest after the injection of the contrast agent, that is to say, the time when the contrast agent arrives [69]. For FLL, the initial enhancement time is often different from the surrounding liver parenchyma [70,71].

Location of enhancement. This is the place and range where the contrast agent appears, such as the surrounding or the center [72]. Determining the extent of lesion enhancement is of great help in understanding the size and extent of the tumor.

Enhancement patterns. This refers to the dynamic mode of the contrast agent when it begins to enter the organ or lesion, such as overall enhancement, centripetal enhancement, radioactive enhancement or branch-like enhancement.

Intensity of enhancement. This is the echo intensity of the contrast agent entering the region of interest, and it often needs to be compared with the surrounding liver parenchyma.

Peak performance of enhancement. This refers to the sonographic manifestations when the contrast enhancement reaches the strongest, such as uniform enhancement, ring enhancement, peripheral nodular enhancement, patchy enhancement, honeycomb enhancement or no enhancement. It has important value for differential diagnosis of FLL.

Duration of enhancement refers to the time from contrast enhancement to absolute disappearance of contrast agents. It varies for different hepatic lesions.

Dynamic change of enhancement. During the contrast enhancement process, the enhancement intensity of the lesion, the enhancement method and other dynamic processes may change with time. For example, hepatic hemangiomas enhance with peripheral nodules during the portal phase. Simultaneously, hepatic cell carcinomas (HCC) rapidly wash in during the arterial phase and out during the portal phase.

4.4.6 Indications for CEUS in liver

According to the user guidelines for SonoVue recommended by the European Federation of Societies for Ultrasound in Medicine and Biology [73–80], the indications for CEUS of liver are as follows:

1. Qualitative diagnosis of FLL inconclusive or detected coincidentally on routine ultrasound.

2. Lesions or suspicious lesions based on chronic hepatitis or cirrhosis.

3. When there are no abnormal findings or there are unclear conclusions in conventional ultrasound, CT or MRI, but there is a small lesion in the liver in clinical suspicion (such as elevated tumor markers).

4. When examining the intrahepatic lesions, the results of conventional ultrasound, CT and MRI are inconsistent or unclear.

5. To examine and diagnose the nature of portal embolism.

6. Patients with suspected liver trauma.

7. Lesions that are not clearly displayed on conventional ultrasound can be guided for localization and puncture.

8. Immediate and postoperative efficacy evaluation after local treatment of liver tumor (such as radiofrequency and microwave ablation).

9. Patients with contraindications to CECT and CEMRI, it can be used as a long-term follow-up monitoring method for tumors.

4.5 The expression of FLLs in CEUS and related medical and imaging foundations

FLLs are often detected unintentionally [81]. It is reported that 5% of the world's population suffers from this type of diseases. The most common benign liver tumor is hemangioma [82–87], followed by FNH [22]. Among these malignant liver tumors, HCC is the most common one [88,89]. Further examination of liver diseases varies

from hospital to hospital, and the results of the examination will depend on the imaging technology available and the needs and limitations of the patient. As the treatment and management of FLLs are different [90,91], their identification and diagnosis are very important. For example, liver hemangiomas and simple cysts require conservative observation, while liver malignancies require surgical resection or chemotherapy. The following is an introduction to CEUS and medical imaging for common benign and malignant liver tumors, including the diagnostic value and application of CEUS in liver nodules, taking FNH and HCC as examples.

4.5.1 FNH

4.5.1.1 Epidemiology and histopathologic features

FNH is the second most common benign hepatic tumor with an 1-3% incidence [92,93]. FNH is usually detected by chance in asymptomatic young women. The ratio of female patients to male patients is 8:1, and the typical age is 30–50 years old [94]. They might undergo imaging examinations for related reasons, and the nodules are usually single. FNH is considered to be caused by a congenital vascular abnormality and does not carry the risk of transforming into a malignant tumor [95,96]. Therefore, conservative treatment rather than surgical resection is recommended [97] (follow-up with ultrasound examination is the most appropriate management method). However, imaging characteristics is similar to those of some liver malignancies, so accurate diagnosis is essential.

Pathologically, FNH is composed of liver cells, bile ducts, Kuffer cells and other normal liver tissues. The typical FNH is mainly considered to be a benign liver mass formed by the proliferation and regeneration of the congenital artery located in the center of the lesion [22]. The typical pathological manifestation of FNH is a central satellite scar containing a large artery with radial branches of blood vessels that extend to the periphery of the mass through fibrous separation [98]. This type of FNH has no envelope but has boundaries. The liver lobular structure is normal. There are thick-walled blood vessels and proliferating small bile ducts in the fibrous compartment.

4.5.1.2 Multimodality imaging – ultrasound and colour Doppler ultrasound

In two-dimensional grayscale ultrasound, compared with the surrounding liver tissue, FNH usually exhibits uniform isoecho or only slight hyperecho or hypoecho. High-frequency probes increase resolution so that radial fibrous separations in FNH can be observed. A typical color Doppler image of FNH is characterized by arterialized high blood flow signals with a central nourishing artery [99]. The blood flow velocity of most of FNH is higher than that of surrounding normal tissues, and it shows a spoke-wheel blood flow is accompanied by central blood vessel radiation distribution to the periphery of the tumor. A combination of two-dimensional and color Doppler ultrasound can offer an accurate diagnosis in some cases of typical FNH [100].

In general, it is difficult to distinguish the atypical or small FNH (<3 cm) from other liver lesions in two-dimensional ultrasound, especially liver malignant nodules and liver adenomas. Because of the completely different treatments for them–FNH can be conservatively observed, while malignant liver nodules and large liver adenomas require surgery or radiofrequency ablation–it is important and critical to make a definite diagnosis.

4.5.1.3 Multimodality imaging – CEUS

FNH can be categorized into three types: Wermke Type Ia FNH, the Wermke Type Ib FNH and Wermke Type II. Wermke Type Ia is a typical FHN with a spoke-wheel sign, and there is a central scar in this type. On the other hand, Wermke Type Ib FNH may show disordered blood vessels. In this type, the central artery can be eccentrically moved to the edge of the FNH. Wermke Type II is telangiectatic or atypical FNH with nodular diffusion enhancement. The figure 2 shows typical enhancement of FNHs at CEUS.

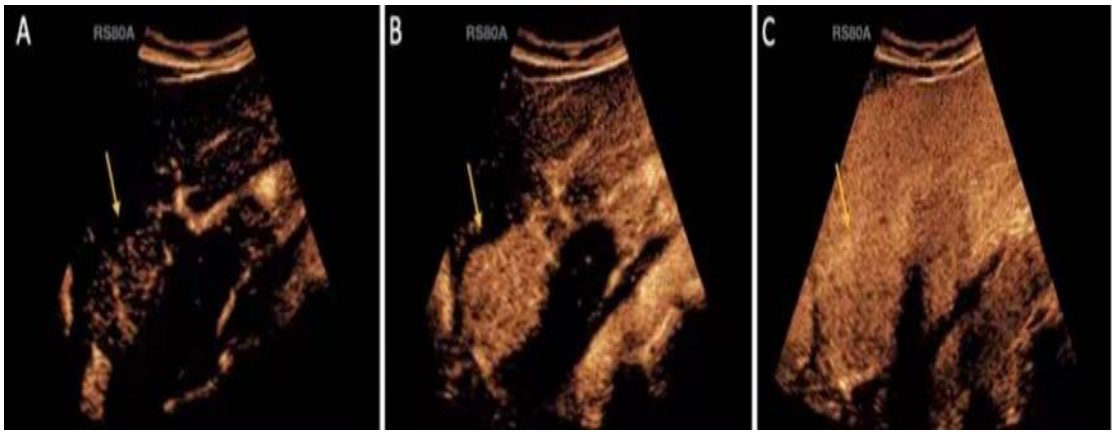

Figure 2. Typical enhancement of FNH at CEUS. A and B: arterial phase indicates the centrifugal enhancement of FNH. C: iso-enhancement of FNH in the portal and late phase.

Most types of FNH can detect early arterial hyperperfusion. Typically, the arterial phase shows a central or eccentric stellate or spoke-wheel enhancement: that is, the entire nodule shows radial enhancement from the center [5,66,70,101]. The portal phase and the delayed phase can show equal enhancement or even hyper enhancement, and occasionally, hypoenhancement patterns can be seen. The wash-out phenomenon may be the result of microbubble destruction which is caused by long-term inspection.

In FNH with a diameter of $\leqslant$3 cm, the spoke-wheel enhancement mode in the center is observed less [102], but the diffuse contrast enhancement in the arterial phase can be quickly observed. During the delayed phase, the central scar may be clearly visible, showing a central hypoechoic zone [103]. The reason is that the microbubble contrast agent is a pure intravascular contrast agent and does not leak any components into the interstitial space. Leaking into the interstitial space is a process of CECT and CEMRI leading to enhancement with a scar [104]. In addition, the central scar is a feature that is always detected in relatively large FNH (> 3 cm) and usually shows an typical centrifugal enhancement pattern [3]. At the same time, feeding artery is another noticeable sign of FNH; however, it is often not considered as a characteristic of FNH because it is also observed in other types of FLLs.

The contrast enhancement performance of typical FNH can be summarized as including:

1. Spoke-wheel enhancement pattern

2. Feeding artery

3. Central scar

Atypical FNH may also be found [8,105]. For example, it has been reported in a small number of cases that the contrast agent is washed out in the portal and delayed phase, and due to excessive enhancement in the arterial and portal phases, it may even show malignant features. This may be an uncommon manifestation, but it is very complicated to the distinguish between FNH and malignant lesions. In these cases, supplementary methods and even histological examinations are usually required.

4.5.1.4 Multimodality imaging – CECT and CEMRI

FNH may show ill-defined borders and isodensity or low density in non-enhanced CT, sometimes with evidence of low-density central scars [106]. Exogenous growth or distorted tumor contour are seen in about 30% of the nodules. Following injection of contrast-enhanced agent, the feeding arteries are seen within the tumor, and the peripheral drainage veins and peripheral pseudocapsule could be noticed. In the arterial phase, FNH typically exhibits hyper-attenuation that is relatively close to the liver parenchyma and hypo-attenuation scars in the center. The manifestations are usually equal or hyper-attenuation in the portal phase or delayed phase. More specifically, during the interstitial phase, the contrast agent is in fibronectin-like tissue, and the central scar more often exhibits hyper- or iso-attenuation compared with the surrounding liver parenchyma.

FNH shows iso- or slightly hyper- or even hypo-signal in non-enhanced T2-weighted sequences. However, the central scar probably show low or high signal depending on the the components of contrast agent. After injection of gadolinium-based contrast agent, nodules appear high signal in the arterial phase [1,107,108], which is the characteristic manifestation of FNH in MRI examination. A gradual decrease in contrast enhancement, leading to iso-signal, can be figured out in the portal phase and the interstitial phase.

In short, CEUS, which is widely used in clinics, can identify FNH in artery phase, portal phase, and late phase in real time, thereby helping to quickly diagnose newly discovered FNH mainly detected by ultrasound. Many previous studies have used CEMRI and CECT as diagnostic criteria to evaluate the accuracy of CEUS in the diagnosis of FNH. In publication II, MRI is used as the standard for FNH diagnosis. The long-term study confirmed that CEUS has 97% sensitivity, 76% specificity, 93% PPV and 89% NPV compared with MRI. These findings indicate that CEUS is practicable and a fast examination tool compared with MRI in the purpose for evaluating the diagnosis of FNH in daily clinical practice.

4.5.2 HCC

4.5.2.1 Epidemiology and histopathologic features

At present, primary liver cancer is the sixth commonest cancer in the world and third in cancer mortality [109,110]. There are about 500,000 new cases around the world every year. Primary liver cancer is mainly divided into HCC, intrahepatic cholangiocarcinoma, and mixed liver cancer, with HCC accounting for about 90% [111–113]. More than 80% of HCC are secondary to conditions such as chronic alcohol consumption, hepatitis B, hepatitis C, NAFLD and cirrhosis [69,114]. NAFLD is related to lifestyle problems in Western countries, including obesity, diabetes and etc. According to related reports, the incidence in Western countries has increased in this century and is predicted to continuously rise [115]. The incidence in patients with liver cirrhosis is 16 times higher than that in patients with non-cirrhosis (2%–6.6% versus 0.4% respectively). The occurrence of HCC may also be related to genetic diseases including Wilson's disease and α-1-antitrypsin deficiency [116]. In the cirrhotic liver, the formation of hepatic cancer follows a multi-step process, progressing from hypertrophic regenerative nodules and low-grade dysplastic nodules to high-grade dysplastic nodules and finally to HCC [91]. Liver cancer can be well-differentiated, moderately differentiated or poorly differentiated [117]. Some previous studies indicated that CEUS probably help distinguish between poorly, moderately and well-differentiated HCC subtypes [118–120].

In the early stage of HCC, the tumor is about 1–2 cm, and some patients still have no typical symptoms. It is very difficult to make an accurate diagnosis, so most tumors are found by serum alpha-fetoprote (AFP) screening. However, the sensitivity of AFP to diagnose HCC is 39–64%, and the specificity is 76–91% [121,122]. Globally, using CT or MRI for liver cancer screening is uncommon, while ultrasound can be used for screening. The unique performance of HCC in CEUS plays a very important role in its diagnosis.

4.5.2.2 Multimodality imaging – the ultrasound and colour Doppler ultrasound

The typical HCC is easily detected by conventional ultrasound mainly based on the characteristic manifestations, such as hypoechoic and dark rings around it. There are various manifestations of atypical HCC. For example, when HCC is hyperechoic and there is no dark ring around it, it is difficult to identify and detect with conventional ultrasound. Diffuse liver cancer sometimes only manifests the various intensities of intrahepatic echo, and it is difficult to distinguish specific cancer nodules.

In the color Doppler ultrasound image, most HCC are manifested as rich blood flow signals. Color Doppler ultrasound can significantly improve the accuracy of ultrasound diagnosis of liver cancer by detecting the blood flow signals in HCC and the measurement of blood flow parameters (such as resistance index and pulsatility index). Color Doppler also has value in distinguishing common portal vein thrombosis and tumor thrombus. Portal vein tumor thrombus can be detected in arteries to nourish blood vessels, but portal vein thrombosis displays no such phenomenon.

4.5.2.3 Multimodality imaging – CEUS

The main purpose of using CEUS is for preventing the advancement of HCC by monitoring high-risk patients (such as those with cirrhosis) or to figure out lesions with high specificity and sensitivity [69]. In CEUS, the diagnostic imaging feature is early arterial hyperperfusion, which is subsequently washed out in the portal and delayed phases [123–125]. More specifically, the ultrasound contrast enhancement

method in typical HCC shows that lesions in the early arterial phase are in a state of hyper-perfusion–that is, hyperenhancement-while the portal and delayed phases show hypo- enhancement (fast wash-in and wash-out mode). UCA shows that the microbubbles quickly enters the tumor micro-vessels during the arterial phase, which makes the tumor rapidly enhance, showing fast and high enhancement performance. However, portal phase and delayed phase showed rapid deduction and hypo-enhancement because of a significant decrease in portal blood supply. Figure 3 shows sonomorphological appearance of a histopathologically verified HCC.

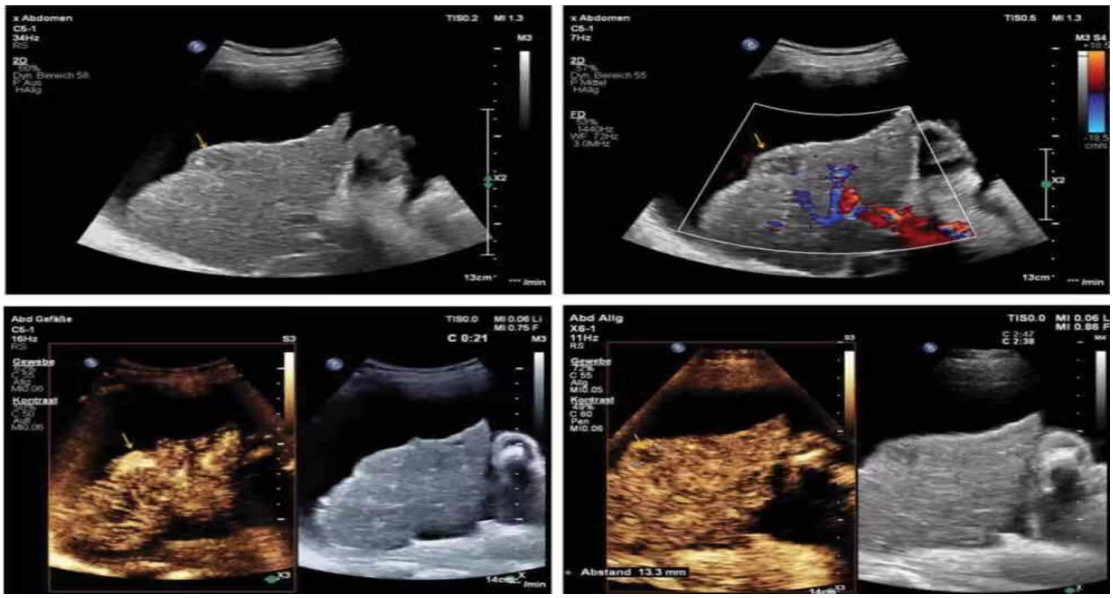

Figure 3. Sonomorphological appearance of a histopathologically verified HCC

If the contrast enhancement method in HCC is observed in disorder, it means that the formation of new blood vessels in the tumor is obvious. Regenerative nodules can also show hyper-enhancement in the arterial phase but often show iso-enhancement in the portal and late phases, in contrast to HCC.

When liver cirrhosis is extremely heterogeneous, it is difficult to find HCC. The application of low MI and real-time CEUS in the early stage of the artery phase is characterized by obvious hyper-enhancement and wash-out during late phase, and it thus may improve the diagnosis rate of HCC with liver cirrhosis [1,126-128].

4.5.2.4 HCC diagnosis based on histopathological results

Our work aims to analyzes the diagnostic value of CEUS and corresponding histopathological results. In addition to CECT and CEMRI, a few studies have used CEUS as an effective non-invasive tool for detecting and evaluating intratumoral microperfusion [129–131]. Although ultrasonography can make a statistically significant difference in detecting between HCC, FNH and metastatic liver cancer [132–134], there are some limitations in distinguishing the differences between HCC and intrahepatic cholangiocarcinoma [125-127]. In the context of hemodynamic changes in patients with liver cirrhosis, NAFLD or non–alcoholic steatohepatitis, insufficient view or unable to evaluate deep liver lesions can increase the difficulty of HCC diagnosis. In some significant conferences, including the American Association for the Study of Liver Diseases, the European Association for the Study of Liver Diseases, and the Asia Pacific Association for the Study of Liver Diseases, researchers have reached a broad consensus on the diagnosis of HCC. Imaging methods is for clinical monitoring of high-risk patients for six months. Ultrasound and CEUS constitute the first recommended method with a sensitivity of up to 80% and a specificity of more than 90%. In publication I, taking pathological results as the gold standard, CEUS showed 96.6% sensitivity, 63.9% specificity, 86.7% PPV and 88.5% NPV in detecting liver lesions suspected of HCC. Based on Cohen's Kappa coefficient (k=0,659), CEUS showed huge cross-modal consistency compared with histopathological findings. If there is suspicious or unclear liver disease, it is recommended to perform CECT, CEMRI and biopsy. And the following histopathological analysis is practical for diagnose HCC, especially for unclear cases, and can verify suspected liver masses. Immunohistochemical and molecular characteristics can then be further tested [135–137].

5. Publication I

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The diagnostic value of contrast-enhanced ultrasound (CEUS) for assessing hepatocellular carcinoma compared to histopathology; a retrospective single-center analysis of 119 patients[#]

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Abstract.
BACKGROUND: HCC as the 6th most common tumor entity with the fourth highest mortality and an increasing prevalence especially due to today's lifestyle acquires a high attention in the clinical setting. Beside CECT and CEMRI, CEUS depicts a dynamic, low-risk and radiation free imaging method that finds its use mainly in screening and active surveillance programs.
PURPOSE: The aim of the retrospective study was to evaluate the diagnostic value of CEUS in correlation to pathologic findings.
MATERIALS AND METHODS: Between 2004 and 2018 a total number of 119 patients were included in this retrospective single-center study. Every patient underwent CEUS in addition to a native B-mode and Color-Doppler scan. After given informed consent *SonoVue®* (Bracco, Milan, Italy), a second-generation blood-pool agent, was used as contrast medium. Every examination was performed and interpreted by a single experienced radiologist (EFSUMB level 3). A low mechanical index (MI) of <0,2 was chosen to obtain a good imaging quality.
RESULTS: All 119 included patients received CEUS followed by a renal biopsy for inter-modality comparison. In correlation to the pathology results, CEUS showed a diagnostic sensitivity of 96,6%, a specificity of 63,9%, a PPV of 86,7% and a NPV of 88,5% by detecting liver lesions suspicious for HCC. According to the Cohen's Kappa coefficient (k = 0,659) CEUS shows a strong inter-modality agreement in comparison to the histopathological finding.
CONCLUSION: With a high sensitivity and a strong cross-modality comparability to histopathology, the CEUS is highly effective in the detection of suspicious HCC lesions.

Keywords: HCC, liver, CEUS, histopathology

[1]Co-first authorship: Both authors contributed equally to the manuscript.

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1. Introduction

According to the International Agency for Research on Cancer (IARC), liver cancer represented the sixth most common cancer with the fourth highest mortality in 2018 worldwide. Subdivided between men and women, the mortality was 2nd for men and 6th for women. The hepatocellular carcinoma (HCC) reflects the most common de novo liver malignancy in patients arising in a cirrhotic liver [1, 2]. Approximately 85% of primary liver tumors are HCC [3–5] with chronic alcohol consumption, hepatitis B, hepatitis C and non-alcoholic fatty liver disease (NAFLD) as their major risk factors [2, 3, 6]. NAFLD describes an entity that, due to its association with today's western lifestyle problems such as obesity, diabetes and metabolic syndrome, has increased in incidence in Western nations in recent decades and is expected to continue to increase in the future [2, 7]. In addition, the development of HCC can also be associated with hereditary diseases such as hemochromatosis, Wilson's disease and α-1-antitrypsin deficiency [8]. Hepatocarcinogenesis is based on a multi-step process which develops in the cirrhotic liver from hypertrophic regenerative nodules and low grade dysplastic nodules to high grade dysplastic nodules and finally to HCC [1]. The HCC itself is further subdivided into well-, moderately- and poor-differentiated HCC [1, 9].

The primary goal is to prevent the development of an HCC lesion or to detect even small lesions with a high specificity and sensitivity by monitoring risk patients, e.g. patients with liver cirrhosis or patients with hepatitis B but without detectable cirrhosis [5]. Within CEUS, the pathognomonic imaging features are an early arterial hyper-perfusion, followed by wash-out during the delayed venous phase [10–17].

Within the various leading societies including the American Association for the Study of Liver Diseases (AASLD), the European Association for the Study of the Liver (EASL) and the Asian Pacific Association for the Study of the Liver (APASL), there is broad agreement that clinical monitoring of high-risk patients at six month intervals should be carried out using imaging procedures whereas ultrasound (US) being the recommended modality with an sensitivity up to 80% and a specificity of more than 90%. The additive determination of special biomarkers, e.g. AFP, is still a matter of recent debate by having possibly a lower benefit than initially expected due to suboptimal cost-effectiveness for routine surveillance of early HCC [3, 5, 18–22]. In case of suspicious or unclear liver lesions, diagnostic imaging should be expanded. In this case, multiphase contrast-enhanced computed tomography (CE-CT) and contrast-enhanced magnetic resonance imaging (CE-MRI) are recommended as well as a performing a biopsy followed by histopathological analysis. The histopathological analysis has its high value especially in unclear cases either to confirm or to reject the diagnosis of suspicion and nowadays for further detection of immunohistochemical and molecular characteristics [3, 23–25].

2. Materials and methods

The performed single-center study was approved by our institutional ethical committee and all data were gained according to the principles expressed in the Declaration of Helsinki/Edinburgh 2002.

All CEUS examinations were performed and subsequently analyzed by a single well experienced radiologist (EFSUMB level 3). The included patients were examined with high-end up-to-date ultrasound systems by using adequate CEUS protocols (GE Healthcare LOGIQ L9, Milwaukee, Wisconsin, USA; Siemens Ultrasound Sequoia, ACUSON Sequoia, Mountain View, California, USA; Philips Ultrasound iU22, EPIQ 7, Seattle, Washington, USA). As contrast medium we used *SonoVue*® (Bracco, Milan, Italy), a second generation blood-pool contrast agent with an only intravascular distribution pattern. To avoid an early destruction of the injected microbubbles the examinations were performed

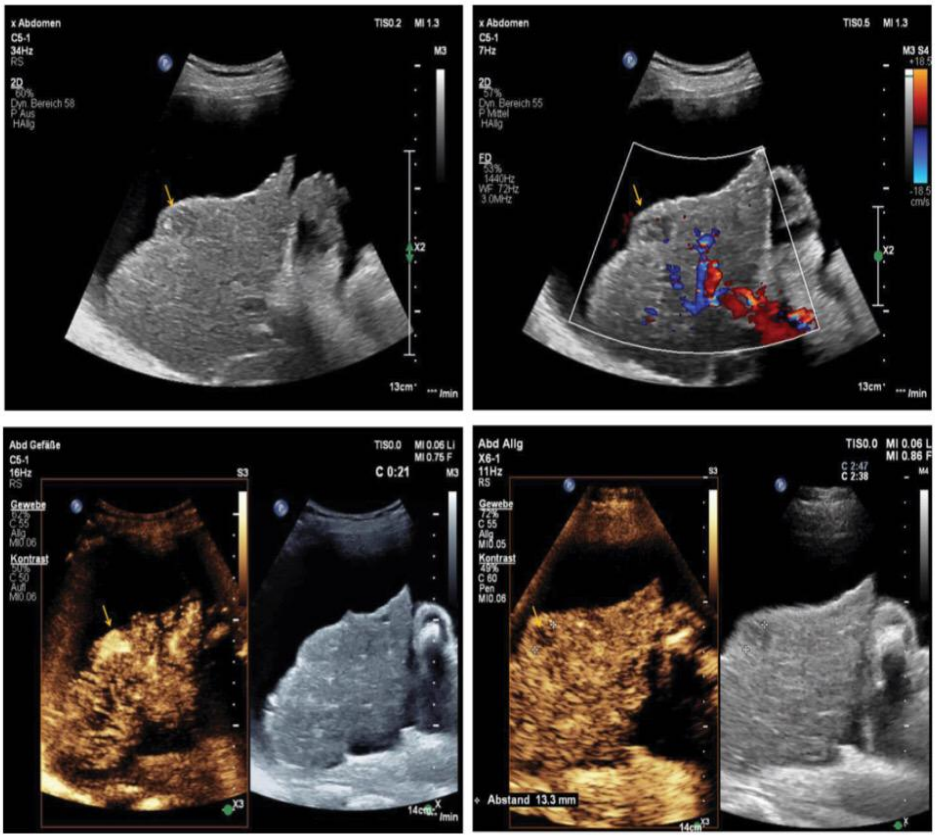

Fig. 1. Sonomorphological appearance of a histopathologically verified hepatocellular carcinoma.

with a low mechanical index (<0,2). Over a peripheral venous access, a total volume of 1,2–1,5 ml *SonoVue*® followed by 5 to 10 ml of sterile 0,9 % sodium chloride solution were applied.

In this present study 119 patients were included with a mean age of 62 and a range between 20 and 88 years. The period of investigation was between 2004 and 2018. Before performing B-mode, Color Doppler and CEUS scan, oral and written informed consent were obtained by every patient. No adverse side effects were registered due to the applied contrast medium and sufficient imaging quality was acquired in every single examination. The patient files were stored in the local archiving system of our institution to allow further analyses and a precise interpretation of the gained data. After performing CEUS the patients underwent a renal biopsy followed by an extensive histological interpretation of the collected material. CEUS and pathological data were retrieved from the in-house Picture Archiving and Communication System (PACS) and were correlated to the histopathological report written by pathologists of our institution.

To evaluate the inter-modality agreement the Cohen's kappa statistic was calculated. In this scale values less than 0.2 indicate a positive but only poor agreement, values of 0.2–0.4 indicate a weak agreement whereas values between 0.4–0.6 indicate a clear, 0.6–0.8 a strong and values greater than 0.8 an excellent agreement (Fig. 1).

A 49-year old patient with hepatic cirrhosis shows a suspicious subcapuslar hypoechoic lesion in native B-mode (a). The lesion does not feature hypervascularization in Color Doppler sonography (b). Early arterial contrast enhancement (c) and venous wash-out (d) registered during CEUS.

3. Results

Between 2004 and 2018 a total number of 119 patients with a suspected HCC lesion underwent 124 CEUS examinations followed by a renal biopsy. The patient population is subdivided into 84 male (70,6 %) and 35 female patients (19,4 %) with a mean age of 62 and a range between 20 – 88 years.

In detecting HCC-suspicious lesions CEUS showed a sensitivity of 96,6 % and a specificity of 63,9 %. In the underlying patient population, the positive predictive value (PPV) of CEUS was 86,7 % with a negative predictive value (NPV) of 88,5 %. Kappa coefficient between CEUS and the pathology showed a value of 0,659 with a significance (p) of < 0,001.

4. Discussion

The aim of the present study was to evaluate the diagnostic value of CEUS in comparison with corresponding histopathological results. Besides CE-CT and CE-MRI, few studies had already described CEUS as an effective and efficient non-invasive diagnostic tool for the detection and evaluation of the intra-tumoral microperfusion [9, 26–28]. As a result US is being implemented by leading professional societies, including EASL or AASLD, as the diagnostic tool of choice in screening and surveillance of patients at high risk for developing HCC [20–22, 29].

While sonography can make a statistically significant statement regarding the differentiation between HCC, focal nodular hyperplasia (FNH), hepatic adenoma (HA) or metastatic liver cancer (MLC) [30–32], it has some limitations for example in differentiating HCC from intrahepatic cholangiocarcinoma (ICC) due to overlapping sono-morphological features [33–35], in the context of hemodynamic changes in cirrhotic patients, in NAFLD or non-alcoholic steatohepatitis (NASH) patients, in the lack of a large field of view or for assessing liver lesions that are located at great depths [13, 36–39].

By comparing the morphological findings of CEUS with the histopathological results, Cohen's kappa coefficient was 0,659 ($p > 0{,}001$) which indicates a strong inter-modality reliability and underlines the effectiveness of CEUS in detecting HCC. Additional studies had previously shown that CEUS may help to differentiate between poor, moderate and well differentiated HCC subtypes [9, 40–42]. Furthermore, CEUS showed a diagnostic sensitivity of 96,6%, a specificity of 63,9%, a PPV of 86,7% and a NPV of 88,5%. In comparison to our results a previous study described that CEUS showed high sensitivity of 93,5% for assessing even small HCC lesions of less than 2,0 cm size [43]. Besides the good correlation between CEUS and the histopathological results it has also a huge benefit in the examination of children where the use of US contrast agent has recently been approved by the U.S. Food and Drugs Administration (FDA) [44, 45]. While the patient is exposed to radiation during CT-scans and the use of CT or MRI contrast medium with its associated potential risks to the kidney function and the thyroid gland is almost indispensable to achieve sufficient diagnostic results, contrast media used for CEUS feature an excellent safety profile. First studies in small cohorts could already demonstrate safe and feasible application of CEUS during pregnancy for assessing unknown hepatic lesions [46, 47].

5. Conclusion

In addition to the excellent safety profile, CEUS offers a strong inter-modality reliability with histopathology and should therefore be included as a non-invasive examination method in the diagnostic clarification of unclear liver lesions.

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6. Publication II

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Long-term study analysis of contrast-enhanced ultrasound in the diagnosis of focal nodular hyperplasia

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Abstract.

BACKGROUND: Focal nodular hyperplasia (FNH) is a hyperplastic mass of vascular abnormality and the second most common benign liver lesion. It can be discovered incidentally or during a surveillance examination in patients at risk for hepatic malignancy, mostly by conventional ultrasound. CEUS has been used as an additional alternative method for the rapid diagnosis of FNH. However, none of the previous studies compared the diagnostic performance of CEUS to MRI retrospectively in a 10-year observation.

OBJECTIVE: The aim of this long-term retrospective study is to assess the diagnostic performance of CEUS in the imaging of FNH and compare the results to MRI.

MATERIAL AND METHODS: A single experienced physician performed CEUS examinations in 244 patients between 2009 and 2019 with suspected focal nodular hyperplasia after conventional ultrasound. A second-generation blood pool agent (SonoVue®, Bracco, Milan, Italy) was administered. Additional dynamic MRI with contrast agent was performed in a subgroup of 95 patients.

RESULTS: Out of 244 patients, FNH could be displayed in 221 patients on CEUS. A subgroup of 95 patients had CEUS examinations and CEMRI for diagnosis comparison. In comparison with CEMRI, CEUS presented a sensitivity of 97%, a specificity of 76%, a positive predictive value of 93% and a negative predictive value of 89%.

CONCLUSION: CEUS is a safe and feasible approach that assess the diagnosis of focal nodular hyperplasia equally to MRI. The focal lesion enhancement can be depicted in real-time in the arterial, venous and late phase facilitating the prompt diagnosis.

Keywords: CEUS, focal nodular hyperplasia, MRI, long-term study

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1. Introduction

Focal nodular hyperplasia (FNH) is a hyperplastic mass of variable size that results from a vascular abnormality and represents the second most common benign hepatic lesion [1–5]. It can be revealed incidentally by conventional ultrasonography (most frequently detected in women between 30 and 50 years) as it is typically an asymptomatic lesion or during a surveillance examination in patients at risk for hepatic malignancy [1, 3, 4]. FNH is pathologically composed of a large central fibrous scar with radiating septae containing arterial structures which are responsible for a specific contrast enhancement pattern [5], different than other liver lesions [8–10].

The differentiation of FNH from other focal liver lesions (FLLs) such hepatic cyst, hemangioma, adenoma and hepatocellular carcinoma is clinically important due to different clinical managements and outcomes of the patients [4, 5, 11, 12]. For example, focal nodular hyperplasias are normally treated conservatively instead of hepatic adenomas which require regular follow-ups or even surgical removal because of the risk of tumor hemorrhage or transformation into hepatocellular carcinoma [11, 13].

Focal liver lesions are usually first detected by conventional ultrasound (US) in routine examinations. However, despite its importance, the characterization of these lesions is not always certain in the native B-mode imaging. Additional imaging with contrast media such as multiphase CT and contrast-enhanced magnetic resonance imaging (CEMRI) are normally necessary in order to characterize a hepatic lesion with a high degree of confidence [3]. Because of the high radiation in CT and limited accessibility to MRI, contrast-enhanced ultrasound (CEUS) has been used in specific centers for the certain prompt diagnosis of FNH [14, 15]. CEUS has been considered as a safe, fast, noninvasive and easy to perform option with a real-time approach that display the enhancement characteristics of a hepatic lesion [4, 16–19].

Although some previous studies concerning the CEUS findings of FNH have taken CECT and CEMRI to be the gold standard [20–22] and others already reported CEUS reliability for the diagnosis of FNH [14, 20, 23–25], none of these studies compared the diagnostic performance of CEUS to MRI retrospectively in a 10-year observation.

The aim of this long-term retrospective study is to assess the diagnostic performance of CEUS in the imaging of focal nodular hyperplasia and compare the results to MRI.

2. Materials and methods

This retrospective study analysis was carried out according to the ethical principles expressed in the Declaration of Helsinki 2002 and waived by the local institutional ethical committee of the institutional review board (17-087). The use of contrast-enhanced ultrasound with the aim to investigate hepatic lesions at our institution is routine practice and does not deviate from the normal departmental protocol. Informed oral and written consent was obtained from all patients prior to each CEUS and MRI examination.

2.1. Study population

Between 2009 and 2019, 1936 patients were referred for CEUS in our department, in order to evaluate uncertain focal liver lesions after incidental finding by conventional ultrasound. From this database, a group of 244 patients were referred for CEUS as a suspected FNH after the conventional ultrasound. A subgroup with 95 patients underwent additional MRI examinations for diagnosis comparison. Patients with more than one lesion had only the larger lesion added to the study.

Patients with suspected focal liver lesions were included in this study if:

(1) they had undergone a conventional ultrasound examination of the liver between 2009 and 2019 as first imaging method and conventional ultrasound suspected an FNH
(2) they had undergone CEUS examination of the liver between 2009 and 2019 as second imaging method
(3) they had undergone MRI examination of the liver as an additional imaging method between 2009 and 2019
(4) all archived images could be retrieved from PACS-System
(5) all archived reports could be retrieved from RIS-System

The exclusion criteria of this study were:

(1) patients with suspected but undetectable hepatic lesions by conventional ultrasound
(2) patients without additional MRI due to several factors such as: cardiac insufficiency, history of anaphylactoid or anaphylactic reaction to contrast media, severe renal impairment
(3) pregnant women
(5) insufficient quality of images with artifacts such as: gas bowel for CEUS, movement or metal artifacts for MRI

2.2. Ultrasonography and CEUS

All ultrasound examinations were performed on high-end systems with CEUS specific protocols (Siemens Acuson Sequoia and Siemens S2000, EPIQ 7, Philips Ultrasound). Siemens ultrasound systems provided C4-1 and C6-1 HD transducers for the examinations and Philips ultrasound system provided C9-2 transducer. Each high-end sonographic system was constituted by a low mechanical index (always < 0.2) in order to avoid early destruction of microbubbles from the contrast medium.

At first, all patients undergone a baseline B-mode ultrasound and color Doppler in our Department using standard inter- and subcostal approach. Then, after the conventional evaluation of the lesion, a second-generation blood pool agent (SonoVue®, Bracco, Milan, Italy) was applied. The blood pool agent consists of phospholipid-stabilized shell microbubbles filled with sulfur hexafluoride gas. SonoVue® was applied as a bolus injection with an individual dose of 1.2 to 2.4 mL through a 20 or 22-gauge cannula placed in the antecubital vein, followed by a flush-injection of 5-10 ml saline solution. Normally, a single dose of contrast media was sufficient. The target hepatic lesion was observed continuously for 6 min. Cine loops and still frames of all phases: arterial (0–30 s), portal (31–120 s) and late (121–360 s) phase were recorded and archived in the Picture Archiving and Communication System (PACS) of our institution. No adverse reactions were observed in all examinations.

All ultrasound examinations, baseline B-mode US and CEUS, were performed and interpreted by a single proficient radiologist with more than fifteen years' experience in conventional ultrasound and experience with CEUS since 2003.

2.3. Magnetic resonance imaging (MRI)

Dynamic MRI studies were performed additional to CEUS examinations of the liver in a subgroup of 95 patients. MRI examination was performed up to 4 weeks after CEUS. All MRI examinations were reported by senior radiologists on PACS workstations and they all had access to the clinical information of the patient. Senior radiologists used for hepatic lesion diagnosis the widely well-known criteria of hepatic tumor differentiation and specification on MRI (16,17). Due to the 10-year retrospective analysis and the ever-growing research and changes in MRI technology, not all exams had exactly the same protocols. However, all exams were dynamic with the application of contrast agent according to

the diagnosis guidelines of liver lesions at the time. MRI examinations and specific details of the MRI studies under these conditions were defined by the local radiologist performing the MRI according to standard protocols.

All examinations were performed in a high-field-strength (1.5 Tesla, Avanto and Aera - Siemens Healthcare) with phased array coils covering the whole liver for signal reception. Despite protocol deviations, all patients received a routine clinical imaging protocol of the liver with the important sequences: unenhanced T1 spoiled TE gradient sequence in phase and out of phase technique Breath-hold (5 – 8 mm slice thickness; axial), T2-weighted fast spin-echo/turbo spin-echo/singleshot turbo-spin-echo sequence fat suppression optional (5 – 8 mm slice thickness, breath-hold; axial and coronal), T2-weighted fast spin-echo fat-supressed, and T1-weighted gradient-recalled echo (GRE) with chemically selective fat suppression (FS) and without FS sequences (3 mm slice thickness) before and after the injection of contrast medium (10, 60, 120, 180, and 240 seconds) using an MR-compatible injector. DWI (diffusion-weighted imaging) was also included in the protocol. Most of the patients received a dose of 0.025 mmol/kg of gadoxetic acid (gadolinium-ethoxybenzyl-diethylenetriamine pentaacetic acid (GD-EOB-DTPA), Primovist®; Bayer-Schering Pharma, Germany) intravenously with of 2 mL/s, followed by 30 mL of 0.9% saline solution. Hepatobiliary phase was obtained at 10 to 20 minutes after contrast injection.

2.4. Image analysis

For this retrospective analysis, archived images and documentation files of all patients were retrieved from the PACS of our department for the evaluation of liver lesions on CEUS and for comparison with MRI. Each hepatic lesion was documented according to its characteristics on conventional ultrasound, CEUS and MRI:

US:

1. Lesion echogenicity: documented as hyper-, iso- and hypoechoic
2. Central scar echogenicity: documented as hyper-, iso- and hypoechoic
3. Vasculature on Color Doppler examination

CEUS:

1. Enhancement according to the adjacent normal liver parenchyma: documented as hyper-, iso- or hypo-enhancing
2. Enhancement filling pattern Presence or abscence of additional features:
3. Spoke-wheel arteries
4. Feeding artery
5. Central scar

MRI:

1. Lesion intensity in native T1 and T2-weighted sequences: documented as hyper-, iso to moderately hypointense lesion
2. Presence or abscence of a central scar and its intensity in native T1 and T2-weighted sequences: documented as hyper-, iso to moderately hypointense lesion
3. Enhancement pattern after administration of contrast agent in arterial, portal venous, late and hepatobiliary phases

2.5. Statistics

All statistical analysis was performed by using SPSS software (SPSS Inc, Chicago, IL, USA). Continuous data were presented as mean ± standard deviation (SD) and percentage (%). Categorical data were given by frequency distribution tables. Indeterminate classifications were rated as incorrect classifications.

The diagnostic performance of CEUS was expressed by calculating sensitivity, specificity, accuracy, positive predictive value (PPV) and negative predictive value (NPV) for unenhanced ultrasound, CEUS (with SonoVue®) and additional MRI. Differences between CEUS and additional MRI were tested using the McNemar two-sided test. $P < 0.05$ was considered as statistically significant.

3. Results

Out of 1936 examined patients (1009 men and 927 women; mean age, 61 years ± 16 SD; range, 14–100 years) with a suspected focal liver lesion, 244 patients (62 men and 182 women; mean age, 49 years ± 15 SD; range, 20–93 years) with suspected FNH by conventional ultrasound underwent CEUS for further evaluation. Focal nodular hyperplasia could be displayed on CEUS in 221. Out of 221 FNH lesions, 5 lesions presented atypical imaging characteristics. Other lesions out of 244 such as hemangioma, adenoma, hepatocellular carcinoma and lymphoma were depicted in 19 patients at CEUS (4 adenomas, 8 hepatocellular carcinomas, 5 hemangiomas, 1 lymphoma, 1 liver with blood filled cysts). CEUS could not determinate the lesion in 3 patients and one lesion could be only further classified as a malign lesion.

However, due to inclusion criteria (additional MRI), diagnosis comparison between CEUS and MRI could be performed in a subgroup of 95 patients (20 men and 75 women; mean age, 50 years ± 16 SD; range, 20–90 years). Out of 95 patients, 74 patients were diagnosed with FNH on MRI. Atypical imaging characteristics of FNH on CEUS depicted in 2 patients (from the subgroup with 95 patients) were confirmed in MRI. Others MRI exams depict 7 adenomas, 5 hemangiomas, 7 hepatocellular carcinomas, 1 lymphoma and 1 indeterminate lesion.

In addition, histological diagnoses were available in 5 patients (1 man and 4 women; range, 33–80 years) for final results. FNH was diagnosed by CEUS and MRI in 3 patients and confirmed histopathologically. HCC was diagnosed by CEUS and MRI in 1 patient and also confirmed histopathologically. FNH was diagnosed in one patient by CEUS, diagnosed as adenoma by MRI and confirmed as adenoma histopathologically.

In comparison with CEMRI, CEUS presented a sensitivity of 97%, a specificity of 76%, a positive predictive value (PPV) of 93% and a negative predictive value (NPV) of 89%. All patients were successfully examined without any adverse reaction. The CEUS accuracy was presented with 92,6 %.

4. Discussion

The identification and characterization of and between diverse focal liver lesions such as FNH, hepatic cyst, hemangioma, adenoma and hepatocellular carcinoma is vital for the patient due to different clinical managements and outcomes [11,12]. The proper imaging classification requires the evaluation of morphological lesion characteristics as well as vascularity and enhancement patterns [26, 27]. Therefore, the application of contrast medium allows the assessment of essential additional information of the target lesion. The standard procedure with the administration of contrast agent is well-stablished in imaging methods such as CT and MRI [28, 29].

Ultrasonography is the first-line method for the evaluation of focal liver lesions and the standard approach includes B-mode imaging and Color Doppler. However, this method is not able to demonstrate dynamic enhancement of the lesion and presents a high number of uncertain diagnosis. The dynamic enhancement pattern of a tumor can be displayed with an appropriate ultrasound system (low-mechanical index) and with the application of a blood pool contrast agent (SonoVue®) in real time [30, 31].

When compared to conventional ultrasound and CT, CEUS examination of the liver provides considerably more information about quantity, differentiation and classification of lesions, as already showed in several studies [14]. However, CEUS have not been widely used in the daily routine for numerous reasons [32]. One of the reasons is that this kind of information can also be delivered by MRI. In fact, MRI is still the gold standard imaging method for the evaluation of benign liver lesions [29]. Other reason is that physicians are not familiar yet with CEUS and prefer to order a CT or MRI.

The literature already showed that CEUS has several advantages in the routine clinical practice such as no renal, thyroid or cardiac toxicity and rare allergic reaction [17]. Moreover, CEUS is a safe and fast technique that, when done properly, can avoid additional examinations and reduce health costs. Furthermore, CEUS can display the enhancement pattern of the target lesion in real-time. Whilst CT exposes the patient to high radiation and MRI is a time-consuming and expensive technique which is not available in all centers.

In the patient's best interest, it is trivial to know which technique is most suitable for any given diagnostic query. Histology is the diagnostic gold standard for hepatic solid tumors while imaging methods such as CT and MRI have been accepted as the gold standard for hemangioma and FNH. Because of that, this study compared CEUS examinations of FNH to MRI.

A total of 244 patients with suspected FNH on conventional ultrasound underwent CEUS, 221 were diagnosed with FNH. A subgroup of 95 underwent additional MRI as a second imaging procedure after CEUS. MRI showed FNH in the majority of patients (78%) and displayed 21 (22%) other findings such as 7 adenomas, 5 hemangiomas, 7 hepatocellular carcinomas, 1 lymphoma and 1 indeterminate lesion.

Concordant FNH diagnoses obtained by CEUS and MRI were 72, 2 lesions were observed as FNH in MRI but not in CEUS. One of these lesions was too small to define as enhancement pattern was not recognizable. The other one lesion displayed an hyperenhancement in the arterial phase and an hypoenhancement in the portal and late phase, because of this enhancement pattern and patient´s characteristics such as liver cirrhosis, the lesion was classified by CEUS as probably malignant such as HCC and an additional MRI was recommended. The MRI exam depicted an atypical FNH.

A total of 16 lesions were not identified as FNH and their diagnosis on CEUS were concordant to MRI (hemangiomas, adenomas, 1 lymphoma and HCCs). In these cases, all lesions displayed distinct enhancement patterns that could be observed in both methods. Only 5 lesions were depicted as FNHs on CEUS but displayed as other lesions on MRI (hemangiomas, adenomas, HCC, and indeterminate as described in the results). These results achieved were due to several reasons: size of the lesion, non-characteristic enhancement pattern, difficulty in compare lesion enhancement with adjacent liver parenchyma enhancement because of liver disorder amongst others.

It is important to note that the results of this study might be biased in favour of CEUS because of these two factors of the study design: 1. only primary ultrasound-suspected FNHs were included in the comparison study 2. all the examinations were performed and interpreted by a single proficient radiologist. However, results have been concordant to some studies already discussed with even a bigger cohort than this one [30]. In addition, there is no other option for the recruitment of patients with hepatic lesions in the routine. Furthermore, exams interpretation requires a senior radiologist with long term experience and expertise in this narrow field is very limited, with no available other physicians inside our department.

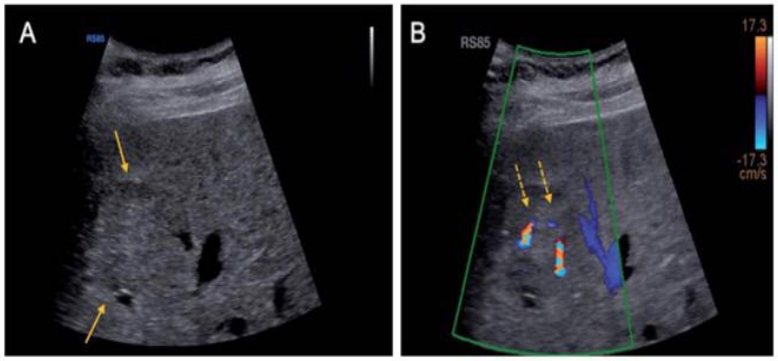

Fig. 1. 40-year-old female with no relevant medical history. A: 3 cm isoechoic lesion in segment 8 found in B-Mode sonography (arrows). B: Color Doppler image demonstrates prominent feeding vessels (arrows).

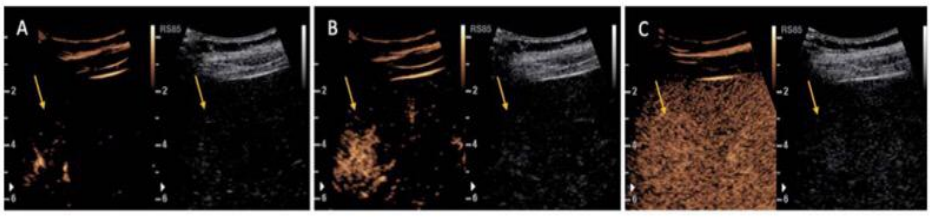

Fig. 2. Same patient as in Figure 1. Split-screen contrast-enhanced sonograms (left panels) and B-mode US (right panels) from intercostal views demonstrate a focal nodular hyperplasia (arrows). A and B: arterial phase shows the centrifugal filling of the lesion (10-14 seconds after administration of contrast agent). C: Sustained enhancement of the lesion was showed in the portal venous and late phase, the lesion appears slightly hyperechoic or isoechoic relative to the surrounding liver parenchyma.

This study presented another limitation, which is the lack of histology for the diagnosis as histology was added in only 5 patients. However, focal nodular hyperplasia and adenoma are lesions that can be diagnosed by an imaging method as already proved and because of that rarely require biopsy.

In order to identify and classify a liver lesion correctly, it is important to understand the typical and atypical enhancement pattern of each lesion [15, 16]. The typical enhancement characteristics of FNHs at CEUS are usually recognized as hyper-enhancement in the arterial phase and hyper-/iso-enhancement in the portal and late phases [4, 14, 19, 25] (Figs. 1, 2, 6 and 7), which fills the lesion from its center to its periphery in form of a centrifugal filling. Moreover, the presence of others features such as the "spoke-wheel sign", central scar and feeding artery also helps to achieve a confident diagnostic of FNH.

The "spoke-wheel sign" have been already discussed in other studies [22] and is referred as the radial arterial vascularity that enables the enhancement a centrifugal pattern. It was observed more commonly in smaller lesions that measure 3 cm or less [22]. The central scar is a feature that was showed to be detected only in relatively large FNHs (>3 cm) and normally does not show an enhancement [22].

The feeding artery is the most common sign of an FNH but it also showed the lowest odds ratio for characterization, being also observed in other focal liver lesions. Some studies also suggested that the diagnosis of FNH is size dependent because of pattern observation, but conflicting data is presented in the literature. In this study, CEUS was not able to classify the lesion in 3 patients because the lesion was too small (<3 cm).

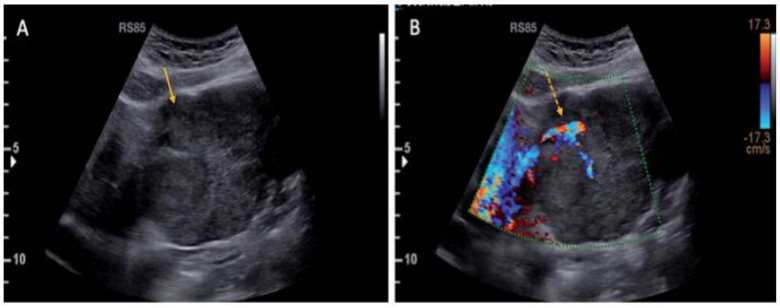

Fig. 3. 39-year-old female with no relevant medical history. A: 7.8 cm isoechoic lesion in the left liver lobe found in B-Mode sonography (arrow). B: Color Doppler image demonstrates prominent vessels (arrow).

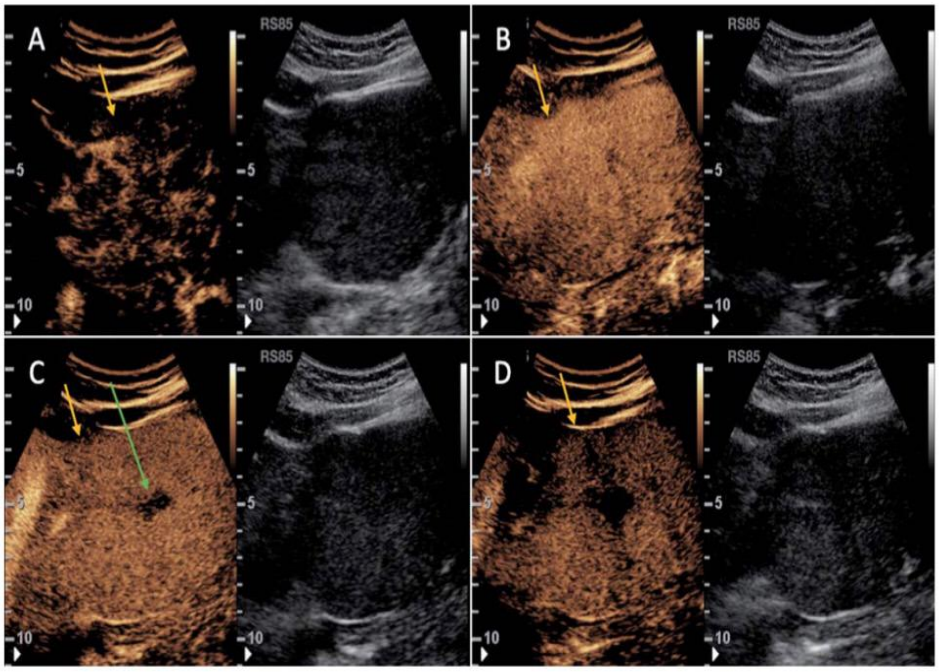

Fig. 4. Same patient as in Figure 3. Split-screen contrast-enhanced sonograms (left panels) and B-mode US (right panels) from subcostal views demonstrate a focal nodular hyperplasia (yellow arrows) with atypical pattern. A and B: arterial phase (7-15 seconds) displays a spoke-wheel-like centrifugal filling of the lesion. C: Sustained enhancement of the lesion in the portal venous phase (90 seconds); isoechoic relative to the surrounding liver parenchyma. Hypoenhanced stellated scar in the center of the lesion during the portal phase (green arrow). D: Hypoenhancing of the lesion in the late phase (3 minutes after administration of contrast agent) showing an atypical pattern of the lesion.

Atypical findings as contrast agent washout in portal and late phase may occur, but as already reported only in few percentages of the cases [23, 24] (Figs. 3–5). Moreover, it might even display malignant characteristics due to a hyperenhancement in the arterial phase and hypoenhancement in the portal and late phases [19]. This might be an uncommon feature but is a very important concern as it complicates the distinction between FNH and a malignant lesion. Supplementary methods or even histology is often necessary in these cases. In this study 97.7 % of 221 patients displayed the typical

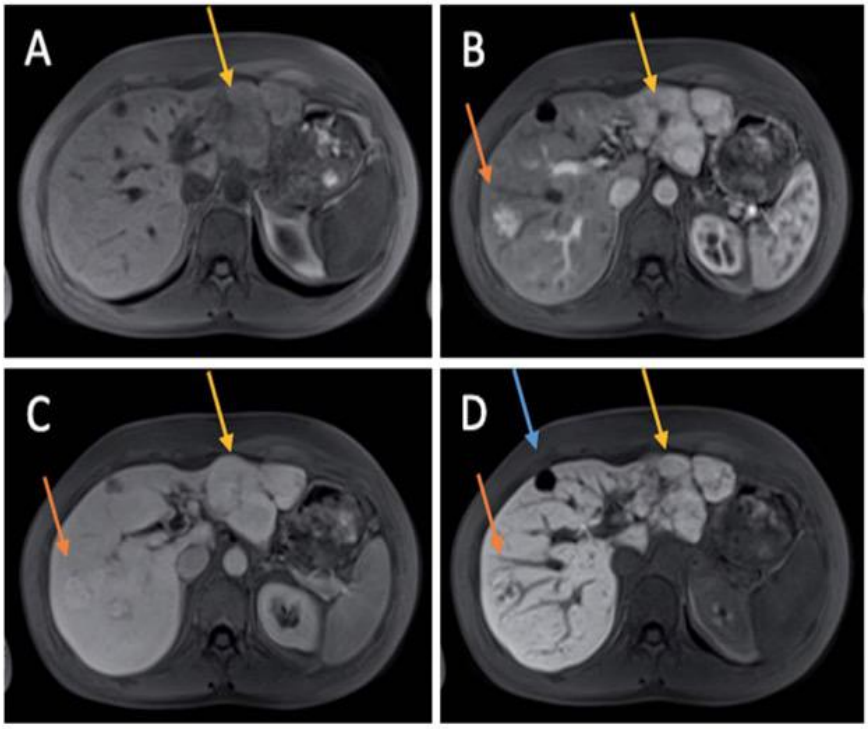

Fig. 5. Same patient as in Figures 3 and 4. MRI of two FNH lesions, one on the left liver lobe (yellow arrows; 7.8 cm, showed in Figures 3 and 4) and the other one in segment 8 (orange arrows; 3 cm) with the same pattern characteristics. Sequences are fat saturated. T1-weighted sequences without (A) and after (B-D) contrast agent. A: Moderately hypointense lesions in native T1 weighted sequence. B: Intense enhancement of the lesions in the late arterial phase. Hypointense central scar of the lesion on the left liver lobe (yellow arrow). No central scar in the second lesion (orange arrow). C: Isointense enhancement of the lesions on portal venous phase relative to the liver parenchyma. D: FNH lesions fade toward background liver intensity on the delayed hepatobiliary phase and show an homogeneous appearance with enhancement remaining; hypeintense enhancement if compared to the liver parenchyma. Except for the central scar. Liver cyst (blue arrow).

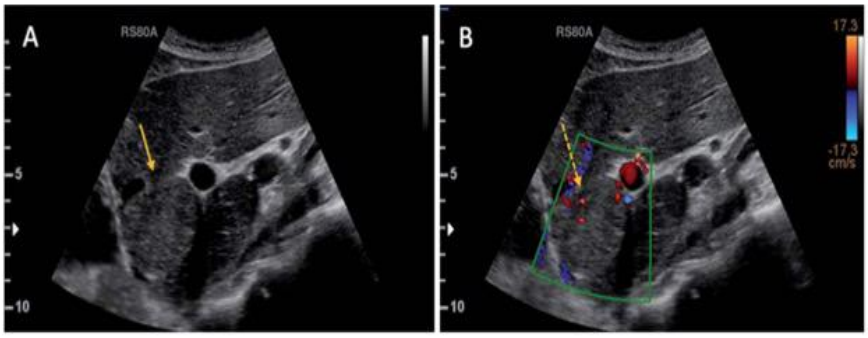

Fig. 6. 49-year-old male with no relevant medical history. A: 3,5 cm isoechoic lesion in segment 4 found in B-Mode sonography. B: Color Doppler image demonstrates vasculature in the periphery.

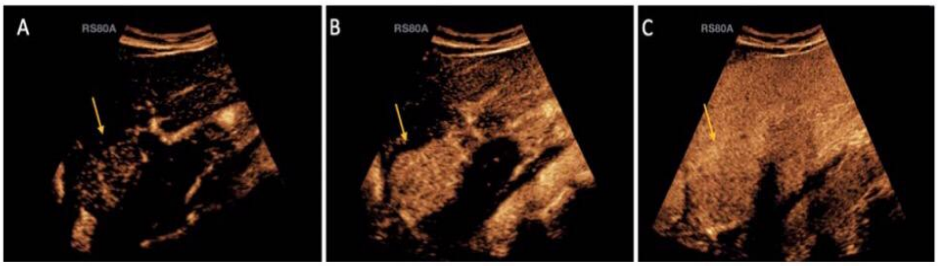

Fig. 7. Same patient as in Figure 6. Contrast-enhanced sonograms from subcostal views demonstrate a focal nodular hyperplasia (arrows) in segment 4. A and B: arterial phase shows the centrifugal filling of the lesion (7-10 seconds after administration of contrast agent). C: Sustained enhancement of the lesion in the portal venous and late phase (90 seconds to 2 minutes after injection of contrast agent), the lesion appears isoechoic relative to the surrounding liver parenchyma.

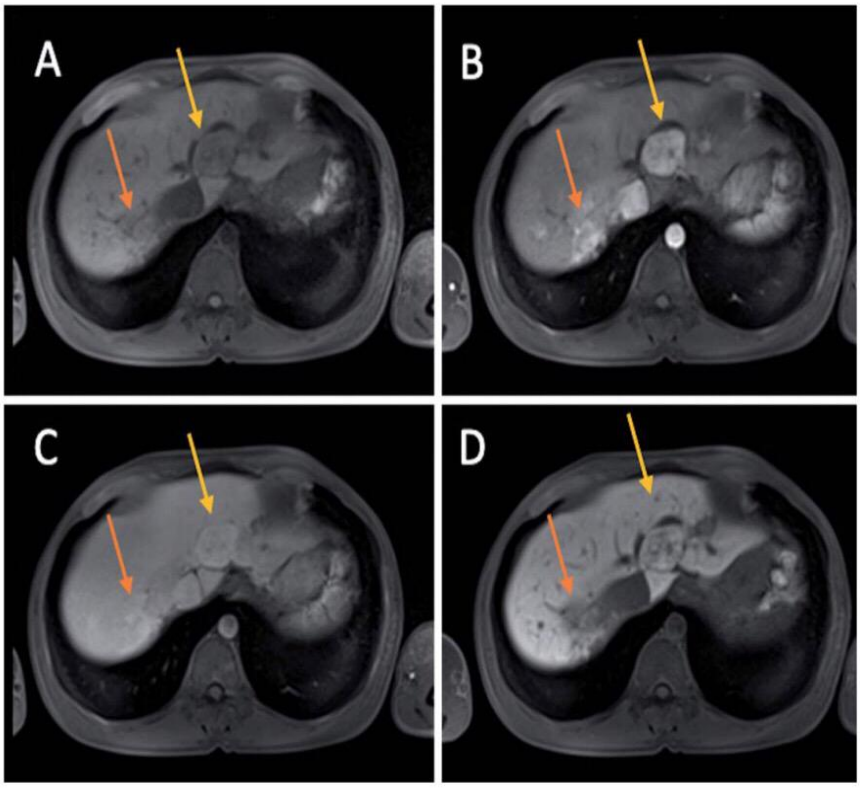

Fig. 8. Same patient as in Figure 6 and 7. MRI of the FNH (3,5 cm) lesion in segment 4 (yellow arrows). Hemangioma in the right liver lobe (orange arrows; 14 cm). Sequences are fat saturated. T1 weighted sequences without (A) and with (B-D) contrast agent. A: Hypointense lesion in native T1 weighted sequence (yellow arrow). B: Intense enhancement of the lesion in the late arterial phase (yellow arrow). C: Isointense enhancement of the lesion to liver on portal venous phase relative to the liver parenchyma (yellow arrow). D: FNH lesion fades toward background liver intensity on the delayed hepatobiliary phase and show a homogeneous appearance with enhancement remaining (yellow arrow); hypeintense enhancement if compared to the liver parenchyma.

enhancement pattern for FNH and only 5 patients (2.3%) presented an atypical enhancement of the FNH.

Although the diagnostic performance of CEUS on liver lesions have already been reported as equal to the CT and MRI with contrast agent [10, 25, 33], none of other studies were able to reproduce the diagnostic of CEUS in a 10-year observation.

In conclusion, with a sensitivity of 97%, a specificity of 76%, a positive predictive value (PPV) of 93% and a negative predictive value (NPV) of 89% this long-term study confirms that compared to MRI, CEUS showed equal diagnostic performance. Considering the absence of radiation and other benefits such as flexibility, cost effectiveness and rare allergic reaction, CEUS is definitely an equal alternative imaging diagnostic of liver lesions, specially FNH to MRI. Moreover, CEUS should be employed as the first-line imaging method for the diagnosis of FNH.

5. Conclusion

CEUS is a feasible and fast alternative tool to MRI in order to assess the diagnosis of focal nodular hyperplasia equally in everyday clinical practice. The additional application of contrast agent in ultrasound allows the depiction of the focal lesion enhancement in real-time in the arterial, venous and late venous phase facilitating the prompt diagnosis of newly discovered solid liver tumors, primarily detected by US.

Typical enhancement characteristics of FNH on CEUS are sufficient for the final diagnosis avoiding histological confirmation and their complications such as post-puncture bleeding. Atypical findings on CEUS may require an additional examination like MRI or even biopsy for histology in order to obtain a definitive diagnosis. However, indeterminate findings assessed by imaging techniques are mainly associated to rare liver lesions.

In addition, CEUS is a safe technique as the contrast agent has no renal, thyroid or cardiac toxicity and the occurrence of an allergic reaction is very rare (1 of 10.000 cases). Moreover, when performed by an experienced physician, it can substantially reduce the costs by avoiding supplementary examinations.

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7. Summary

Globally, ultrasound is the preferred method of examination for FLL. However, for atypical or complex liver lesions, the definitive diagnostic accuracy of ultrasound may be limited. CEUS is an imaging method that has been widely applied in Europe and Asia for more than two decades because of its real-time imaging, lack of radiation and low cost. Through continuous real-time imaging with low mechanical index, CEUS can obtain the blood supply and microcirculation perfusion of the tumor and can thus increase the detection rate of the tumor and even demonstrate the tiny tumors of unknown CT and MRI. The following is a summary of the publication I and II.

Publication I The diagnostic value of CEUS for assessing hepatocellular carcinoma compared to histopathology; a retrospective single-center analysis of 119 patients

Liver cancer is the sixth commonest tumor. Due to current lifestyle, especially in Western countries, the prevalence is rising, causing widespread clinical concern. CEUS is a real-time, low-risk and non-radiation imaging approach which can be used for screening and dynamic observation of microvascular perfusion.

The purpose is to retrospectively analyze the diagnostic value of CEUS in HCC. All patients received CEUS. After that, they would be treated with liver biopsy for multi-modality comparison. Taking pathological results as the gold standard, CEUS showed 96.6% sensitivity, 63.9% specificity, 86.7% PPV and 88.5% NPV in finding liver lesions suspected of HCC. Based on Cohen's Kappa coefficient (k=0,659), CEUS showed strong cross-modal consistency compared with histopathological findings. Therefore, CEUS contains much value for HCC diagnosis.

Publication II Long-term study analysis of contrast-enhanced ultrasound in the diagnosis of focal nodular hyperplasia

FNH is the second most common benign FLL and is caused by hyperplastic vascular abnormality. It can be found by routine ultrasound in occasional or surveillance tests in patients at risk of liver tumors. CEUS has been used as a diagnostic method for

rapid diagnosis of FNH. CEUS is a fast, non-invasive and easy-to-implement option that can show the enhanced characteristics of liver lesions.

The purpose of this long-term retrospective study is to evaluate the diagnostic performance of CEUS in FNH imaging and compare the results with CEMRI. A subgroup of 95 patients underwent CEUS examination and CEMRI for diagnostic comparison. CEUS had a sensitivity of 97%, a specificity of 76%, a PPV of 93%, and a NPV of 89%, taking the result of CEMRI as the standard. CEUS is a safe and effective method that can evaluate the diagnosis of FNH like MRI. CEUS can identify FNH in real time in the arterial phase, portal phase, and delayed phase, as well as typical spoke-wheel enhancement pattern, which can help rapid diagnosis.

In short, in addition to the advantages of safety and real-time imaging, CEUS also has a very reliable histopathological correlation, so it should be used as the first non-invasive examination method when diagnosing unclear liver lesions.CEUS is a viable and rapid alternative to MRI, for purpose of quickly assessing and diagnosing FNH in daily clinical practice.

8. Zusammenfassung

Ultraschall ist weltweit die bevorzugte Untersuchungsmethode bei fokalen Lebererkrankungen. Bei atypischen oder komplexen Leberläsionen kann die definitive diagnostische Genauigkeit der Ustraschall jedoch eingeschränkt sein. CEUS ist eine bildgebende Methode, die in Europa und Asien seit mehr als zwei Jahrzehnten aufgrund ihrer Echtzeit, der fehlenden Bestrahlung und der geringen Kosten weit verbreitet ist. Durch die kontinuierliche Echtzeit-Bildgebung mit niedrigem mechanischem Index kann CEUS die Blutversorgung und Mikrozirkulationsdurchblutung des Tumors erhalten, was die Erkennungsrate des Tumors erhöhen und sogar die winzigen Tumore unbekannter CT und MRT darstellen kann. Das Folgende ist eine Zusammenfassung der beiden Veröffentlichungen.

Publikation I Der diagnostische Wert des kontrastmittelverstärkten Ultraschall (CEUS) zur Beurteilung des Leberzellkarzinoms im Vergleich zur Histopathologie; eine retrospektive Single-Center-Analyse von 119 Patienten.

Leberkrebs ist der sechsthäufigste Tumor. Aufgrund des derzeitigen Lebensstils, insbesondere in den westlichen Ländern, steigt die Prävalenz an, was zu weit verbreiteter klinischer Besorgnis führt. CEUS beschreibt ein risikoarmes und strahlungsfreies Echtzeit-Bildgebungsverfahren, das hauptsächlich für das Screening und die dynamische Beobachtung der mikrovaskulären Perfusion eingesetzt wird.

Der Zweck besteht darin, den diagnostischen Wert von CEUS Ergebnisse beim HCC retrospektiv zu bewerten. Alle 119 Patienten erhielten ein CEUS, gefolgt von einer Leberbiopsie zum Multimodalitätsvergleich. Nimmt man die pathologischen Ergebnisse als Goldstandard, so zeigte das CEUS eine diagnostische Sensitivität von 96,6%, eine Spezifität von 63,9%, ein PPV von 86,7% und ein NPV von 88,5% bei Nachweis von Leberläsionen, bei denen der Verdacht auf ein HCC besteht. Gemäss dem Kappa-Koeffizienten nach Cohen (k=0,659) zeigte CEUS im Vergleich zu histopathologischen Befunden eine starke cross-modale Daher ist das CEUS für die Diagnose des HCC äusserst wertvoll.

Publikation II Langzeitstudienanalyse des kontrastmittelverstärkten Ultraschall bei der Diagnose der fokalen nodulären Hyperplasie

FNHS ist die zweithäufigste gutartige FLL, die durch eine hyperplastische Gefäßanomalie verursacht wird. Sie kann durch Routine-Ultraschall bei gelegentlichen oder Überwachungstests bei Patienten mit einem Risiko für Leberkrebs festgestellt werden. CEUS wurde als diagnostische Methode zur schnellen Diagnose von FNH eingesetzt. CEUS gilt als eine schnelle, nicht-invasive und einfach zu implementierende Echtzeit-Option, die die verbesserten Merkmale von Leberläsionen zeigen kann.

Zweck dieser retrospektiven Langzeitstudie ist es, die diagnostische Leistung von CEUS in der FNH-Bildgebung zu bewerten und die Ergebnisse mit der MRT zu vergleichen. Eine Untergruppe von 95 Patienten unterzog sich einer CEUS-Untersuchung und einer CEMRI zum diagnostischen Vergleich. Im Vergleich zur CEMRI hat CEUS eine Sensitivität von 97%, eine Spezifität von 76%, einen positiven Vorhersagewert von 93% und einen negativen Vorhersagewert von 89%. CEUS ist eine sichere und effektive Methode, die die Diagnose von FNH wie die MRT bewerten kann. CEUS kann FNH in Echtzeit in der arteriellen Phase, der Portalphase und der verzögerten Phase sowie die typischen Radspeichen von FNH beschreiben, was eine schnelle Diagnose erleichtern kann.

Kurz gesagt, CEUS weist neben den Vorteilen der Sicherheit und der Echtzeit-Bildgebung auch eine sehr zuverlässige histopathologische Korrelation auf. Daher sollte es als erste nicht-invasive Untersuchungsmethode bei der Diagnose unklarer Leberläsionen verwendet werden. CEUS ist eine praktikable und schnelle Alternative zur MRT, FNH in der täglichen klinischen Praxis schnell zu beurteilen und zu diagnostizieren.

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