New Aspects Concerning Sulphur Hexafluoride Use As Contrast Agent for Ultrasonographyc Diagnosis

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Introduction of contrast agents as microbubbles to improve ultrasound examination techniques has increased the rate of detection and characterization of liver lesions. Contrast-enhanced ultrasonography (CEUS) is a relatively new method of exploring the liver and diagnosing focal liver lesions (FLL). The aim of this study is to determine the efficiency of microbubbles used for CEUS in managing patients detected in conventional ultrasonography with FLL. We examined 84 patients with FLL, detected on conventional ultrasonography (US), during October 2012-September 2015. We performed contrast-enhanced ultrasonography, using sulphur hexafluoride as a contrast agent, and contrast-enhanced MRI to set the diagnosis. For 37 patients (44.1%) CEUS showed a malignity pattern of enhancement (according to the EFSUMB - European Federation of Societies for Ultrasound in Medicine and Biology- Guidelines 2012), while for 54 (64.3%) patients MRI set the diagnosis of malignity. CEUS detected fewer malignant lesions than MRI, in 20.1% of the cases MRI set de diagnosis. We need further studies in order to improve the quality of CEUS images and technique.

Keywords: Sulphure hexafluoride, contrast agent, ultrasonography, focal liver lesions

Accurate characterization of focal liver lesions is essential for the use of new treatment strategies in the management of liver lesions. Early diagnosis of liver lesions increases the possibility of curative treatment [1-2]. The relationship between some tumors suspected to be of a liver origin is discussed in relationship with certain diagnostic problems or therapeutic methods represented by the surgical procedures, performed as both a diagnostic and therapeutic purpose. Such tumors are rare injuries, but make delicate problems of diagnosis and therapeutic attitude [3].

It is essential to underline that the liver has the anatomy feature of having a dual blood supply from both the portal vein and the hepatic artery, subsequently the liver parenchyma receives 2/3 of its necessary blood supply from the portal vein and 1/3 from the hepatic artery [4]. However, the vascularization of hepatocellular carcinoma (HCC) is mostly dependent on the hepatic artery [5], and this justifies both the diagnostic use of contrast enhanced imagistic methods, and also enables the method of transarterial therapy as an effective treatment of HCC [6].

Describing the liver topographic anatomy is considerably useful for explaining aspects of pathogenesis, diagnostic and therapy aspects of various diseases with potentially different locations [1, 2, 7, 8]. Especially when assessing segmental anatomy of the liver, vascular elements and subsequently their tributary parenchyma are to be carefully observed [9].

Compared to other means of diagnosis, ultrasonography has the great advantage of being non-invasive. On the other hand, digestive diagnostic endoscopy, for example, apart from its invasive character, offers the possibility of direct visualization of lesions, and, subsequently, leading to a better description and perform histology samples for anatomo-pathological diagnosis and staging [10, 11].

Ultrasonography is one of the first imaging investigations performed, since it does not require long time for measurements and does not provide radiations over the patient [12]. It is widely available, asses vascular invasion, it is suitable for screening programs, but it depends on operator's experience, so it has a low sensitivity, and may not differentiate all types of tumors.

Computed-tomography (CT) has an improved sensitivity in characterizing lesions, relatively fast to perform, but it has an increased cost, as well as an increased amount of radiation and risk of allergy to contrast agents. Magnetic resonance imaging (MRI) is a more sensitive imaging technique, especially for smaller lesions, with no radiation and a high resolution. It is an expensive technique, with a longer time of evaluation.

Introduction of contrast agents as microbubbles and improving ultrasound examination techniques have increased the rate of detection and characterization of liver lesions, offering new perspectives for clinical practice [13]. Contrast agents using a low mechanical index allow the formation of images based on nonlinear acoustic effects of microbubbles. Second generation microbubbles are characterized by a flexible shell that allows them to vibrate in response to the ultrasound beam generated at low acoustic power. Oscillations make them several times more reflective than normal tissues, so that enhance the gray scale images and Doppler signals in real time. In this way it is possible to evaluate the real-time focal liver lesions, setting the diagnosis without using other imaging techniques, such as computed-tomography (CT) or magnetic resonance imaging (MRI) [12].

The ultrasound contrast agents currently used in diagnostic of liver lesions are micro-bubbles consisting of gas stabilized by a shell. The contrast agents that are in use nowadays are:

- SonoVue® (sulfur hexafluoride with a phospholipid shell, produced by Bracco SpA, Milan, Italy),
- Definity®/Luminity® (octafluoropropane [perflutren] with a lipid shell) Lantheus Medical, Billerica, MA, USA.
- Sonazoid® (perfluorobutane with a phospholipid shell: hydrogenated egg phosphatidyl serine), Daiichi-Sankyo, GE Tokyo, Japan.

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There are other contrast agents which may be useful in studying liver lesions, but they are either not licensed for the liver in any country or, in the case of Levovist®(Bayer Schering AG, Germany), production has ceased [13].

Even if all contrast agents have a different physico-chemical composition, their behaviour for CEUS is similar, enhancing the vascular pool after intravenous injection, with slow dissipation over about 5 min. An exception is Sonazoid®, which has an extended late phase, the postvascular phase or *the Kupffer phase* [14], which can last up to several hours in the liver and spleen, long after it has disappeared from the detectable vascular pool, due to the fact that the contrast agent is phagocytozed by Kupffer cells.

The microbubbles SonoVue have a mean diameter of about 2.5 μm, with 90% having a diameter less than 6 μm and 99% having a diameter less than 11 μm. Each millilitre of SonoVue contains 8 μL of the microbubbles. The interface between the sulphur hexafluoride bubble and the aqueous medium acts as a reflector of the ultrasound beam thus enhancing blood echogenicity and increasing contrast between the blood and the surrounding tissues [13].

The intensity of the reflected signal is dependent on concentration of the microbubbles and frequency of the ultrasound beam. At the proposed clinical doses, SonoVue has been shown to provide marked increase in signal intensity of more than 2 min for B-mode imaging in echocardiography and of 3 to 8 min for Doppler imaging of the macrovasculature and microvasculature.

Sulphur hexafluoride is an inert, innocuous gas, poorly soluble in aqueous solutions. There are literature reports of the use of the gas in the study of respiratory physiology and in pneumatic retinopexy. The total amount of sulphur hexafluoride administered in a clinical dose is extremely small, (in a 2 mL dose the microbubbles contain 16 μL of gas). The sulphur hexafluoride dissolves in the blood and is subsequently exhaled [13].

After a single intravenous injection of 0.03 or 0.3 mL of SonoVue/kg (approximately 1 and 10 times the maximum clinical dose) to human volunteers, the sulphur hexafluoride was cleared rapidly. The mean terminal half-life was 12 min (range 2 to 33 min). More than 80% of the administered sulphur hexafluoride was recovered in exhaled air within 2 min after injection and almost 100% after 15 min.

In patients with diffuse interstitial pulmonary fibrosis, the percent of dose recovered in expired air averaged 100% and the terminal half-life was similar to that measured in healthy volunteers. Non-clinical data reveal no special hazard for humans based on conventional studies of safety pharmacology, genotoxicity and toxicity to reproduction. Caecal lesions observed in some repeat-dose studies with rats, but not in monkeys, are not relevant for humans under normal conditions of administration [15].

The aim of this study is to determine the efficiency of sulphur hexafluoride used in contrast-enhanced ultrasonography (CEUS) for diagnosing focal liver lesions (FLL) detected on abdominal ultrasound examination (US), avoiding other imaging methods (contrast MRI).

Experimental part

Material and method

We performed a prospective study during October 2012 - September 2015. In the study were included 84 patients with FLL and, after US examination, which was not sufficient for diagnosis, we performed CEUS according to the EFSUMB Guidelines [13]. We divided our patients into patients without chronic liver disease (excluded using clinical, biological, US criteria) and patients with chronic liver disease (chronic hepatitis or liver cirrhosis).

Indication for CEUS were represented by characterization of focal lesions detected incidentally in patients without known chronic liver disease and characterization of focal lesions detected in surveillance programs of chronic liver diseases [13, 16].

Exclusion criteria for performing CEUS were: pregnant women, patients with acute cardiac infarction or with class III/IV cardiac failure or arrhythmias.

After obtaining informed consent, CEUS was performed and all patients were monitored for adverse events, during the next four hours after the procedure. The clinical status, blood pressure and heart rate were followed-up for each patient.

One experienced ultrasonographist, who was not aware of the patients' history, performed US scanning using a Hitachi Ultrasound System with a 3.5 MHz convex array probe. An initial examination, including a color-power Doppler analysis, was performed. The US scan parameters - focal zone and time gain compensation - were not changed during the examination. A low frame rate (5 Hz) and a low mechanical index (MI), bellow 0.08, were used for real-time imaging. One focus was positioned below the level of the lesion. Each examination lasted about 5 min after bolus injection of the contrast.

The US contrast agent used in the study was SonoVue® (Bracco, Italy), a perfluoro gas containing agent, provided as a sterile, lyophilized powder contained in a septum-sealed vial. The low-solubility gas microbubbles of sulphur hexafluoride are surrounded by a flexible phospholipid shell for stability.

A white, milky suspension of sulphur hexafluoride microbubbles was obtained by adding 5 mL of physiological saline (0.9% sodium chloride) to the powder (25 mg), followed by hand agitation. Each patient received an i.v. bolus of SonoVue® for each lesion to be characterized (usually 2.4 mL) via a 20-gauge i.v. catheter placed in the ante-cubital vein, followed by 10 mL saline flush. The hemodynamic behavior of SonoVue® enhancement during the arterial phase (15-30 s), portal venous (30-120 s) and late vascular phases (120-300 s) was evaluated. All sonographic examinations were digitally recorded.

The location and size of the lesion were assessed on unenhanced and CEUS scans. In addition, the vascularity and pattern of SonoVue® enhancement of the lesion (hypo-, hyper-, iso-enhancing), as compared with the adjacent liver parenchyma during the arterial, portal venous and late phases were evaluated. The spatial and temporal pattern of each lesion's filling was assessed in the arterial phase.

Due to their size - equal to or smaller than red blood cells, the contrast agent acts as blood pool agent and allows description of macrovascularization and micro-vascularization (fig.1), having also a slow dissipation over about 5 min. A low MI is chosen for continuous real-time imaging, and for minimizing microbubble destruction.

Ultrasound diagnosis, in terms of the nature (malignant or benign) and type of the lesion (hemangiomas, HCC or metastases) was based on SonoVue® enhanced US. The number, location, size and characteristics of the lesions were recorded.

Characterization of liver lesions through CEUS is based on a comparison between the contrast of a lesion to the normal parenchyma during the three phases – vascular (starts at 10-20 s after injecting a contrast agent into a peripheral vein and takes 10-15 s), portal (takes about 2 min after injection of contrast), and the late phase (takes up to 4-6 min after administration of contrast enhancement) [13]. Washout in most malignant breasted-

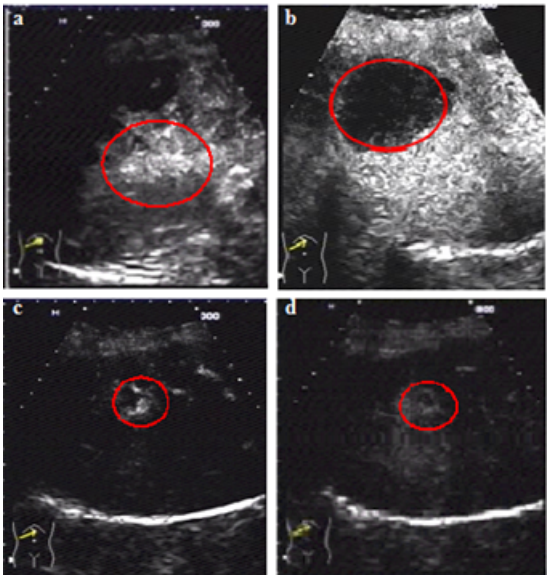

Fig. 1. Imaging based on SonoVue® enhanced US the contrast agent acts as blood pool agent and allows description of macrovascularization and microvascularization: a -Hyper-enhancement in arterial phase (00:19); b - *Washout* in portal phase (01:07min); c - Hyperenhancement in arterial phase (0:24 m); d - Hyperenhancement in portal phase - hemangioma (00:56 min)

up formations is much faster in comparison with normal liver parenchyma. Benign lesions are often best characterized during the pressure, because it can disappear in the next phases [17].

The main difference between malignant and benign lesions is that in late phase all benign lesions, except cysts (non-enhancing) and thrombosed haemangiomas, present iso-enhancement or slight hyper-enhancement compared to the surrounding liver parenchyma, while malignant lesions are hypo-enhancing. This criterion of specificity characterization of focal liver lesions varies from 95% to 100% [18,19].

In HCC, ultrasound contrast agents have a specific behavior in more than 90% of cases, hyper-enhancement in arterial phase, followed by rapid wash-out [20-22]. During the late phase, HCC are usually poorly captured compared to the surrounding parenchyma, except well-differentiated lesions that remain iso-enhanced. Differential diagnosis between regenerative nodules and HCC can be made, because the former have a synchronous enhancement to the surrounding parenchyma, remain iso-enhanced during the portal and late phase. Well-differentiated HCC sometimes may not have arterial hyper-enhancement and late hypo-enhancement [23].

A CEUS examination was considered conclusive if, after contrast administration, the FLL had a typical enhancement pattern according to the EFSUMB guidelines [13]. Afterwards, the same patients were assessed by contrast-MRI and the results were compared to the ones in CEUS.

Results and discussions

From 84 patients, for 37 patients (44.1%) CEUS showed a malignity pattern of enhancement (according to the EFSUMB Guidelines 2012, fig.1), while for 54 (64.3%) patients MRI set the diagnosis of malignity.

The repartition of patients according to gender and diagnosis in CEUS and MRI is shown in table 1.

In 20.1% of cases, MRI settled the diagnosis, CEUS being inconclusive.

The diagnosis in CEUS and MRI were settled to patients with or without chronic liver disease, as shown in table 2.

McNemar tests for significance between CEUS and MRI, in the 2 subgroups of patients- with and without chronic liver disease were performed using SPSS software. The value of p was non-significant for all the diagnosis considered (adenoma, cyst, hepatocellular carcinoma, hemangioma, metastasis, dysplastic nodule, regeneration nodule) for the patients without chronic liver disease, as shown in table 3.

Procedure	Diagnosis	Number of cases	Women	Men
CEUS	Adenoma	7 (8.3%)	5	2
	Cyst	8 (9.5%)	5	3
	HCC	26 (31%)	12	14
	Hemangioma	25 (29.8%)	14	11
	MTS	10 (11.9%)	4	6
	Dyplastic nodule	1 (1.2%)	1	-
	Regeneration nodule	12 (14.3%)	7	5
MRI	Adenoma	3 (3.6%)	3	-
	Cyst	6 (7.1%)	3	3
	HCC	39 (46.4%)	19	20
	Hemangioma	24 (28.6%)	13	11
	MTS	13 (15.5%)	7	6
	Dyplastic nodule	2 (2.4%)	2	-
	Regeneraton nodule	1 (1.2%)	1	-

Table 1
DIAGNOSIS IN CEUS AND MRI CONSIDERING GENDER

Procedure	Diagnosis	Cases with chronic liver disease	Cases without chronic liver disease
CEUS	Adenoma	2 (4.3%)	5 (13.5%)
	Cyst	4 (8.5%)	4 (10.8%)
	HCC	24 (51.1%)	2 (5.4%)
	Hemangioma	9 (19.1%)	16 (43.2%)
	MTS	-	10 (27%)
	Dyplastic nodule	1 (2.1%)	-
	Regeneration nodule	12 (25.5%)	-
MRI	Adenoma	-	3 (8.1%)
	Cyst	3 (6.4%)	3 (8.1%)
	HCC	36 (76.6%)	3 (8.1%)
	Hemangioma	8 (17.0%)	16 (43.2%)
	MTS	-	13 (35.1%)
	Dyplastic nodule	2 (4.3%)	-
	Regeneration nodule	1 (2.1%)	-

Table 2
DIAGNOSIS IN CEUS AND MRI CONSIDERING PRESENCE/ ABSENCE OF CHRONIC LIVER DISEASE

Table 3

McNEMAR SIGNIFICANCE TEST- CEUS AND MRI FOR PATIENTS WITHOUT CHRONIC LIVER DISEASE (37 patients)

Diagnosis	P
Adenoma	0.5
Cyst	1.0
HCC	1.0
Hemangioma	1.0
MTS	0.250
Dysplastic nodules	0
Regeneration nodules	0

Table 4

McNEMAR SIGNIFICANCE TEST- CEUS AND MRI FOR PATIENTS WITH CHRONIC LIVER DISEASE (47 patients)

Diagnosis	P
Adenoma	0
Cyst	1.0
HCC	0.219
Hemangioma	1
MTS	0
Dysplastic nodules	0.375
Regeneration nodules	0.5

The same situation was observed in the group of patients with chronic liver disease, as shown in table 4.

CEUS became popular because of its simplicity, making it easy to learn and interpret. In the hands of a good sonographer, a diagnostic accuracy can be increased from 50% for conventional ultrasound up to 88% according to some studies [13], having a substantial importance in detection and characterization of liver lesions.

CEUS demonstrated greater sensitivity in comparison to power Doppler in evaluation of arterial vascularization of HCC [23]. Therefore, CEUS is an effective way of characterizing focal liver lesions and in particular of HCC, with a sensitivity ranging between 92 and 94% and a specificity of 87-96% [24-26].

The German study [25] included 1,349 patients with FLL discovered in standard US and in which CEUS had a 90.3% diagnostic accuracy for the diagnosis of FLL. CEUS correctly characterized 723/755 of the malignant lesions and 476/573 of the benign lesions, with 95.8% sensitivity and 83.1% specificity for differentiating benign versus malignant lesions. CEUS correctly diagnosed 84.9% of the HCCs and 91.4% of the metastases, so CEUS proved to be a sensitive method for the diagnosis of liver metastases and HCCs.

The multicentre French study (STIC) [26] included 874 patients with 1034 FLL. CEUS was compared to contrast spiral CT, contrast MRI or liver biopsy, considered to be the gold standard. Standard US correctly diagnosed 62.4% of the cases, CEUS increased the diagnostic performance to 86.1%. The diagnostic concordance between CEUS and the gold standard method was (71.8%, kappa = 0.4273.5%) in nodules on cirrhotic liver. For differentiating between benign vs. malignant, CEUS had 79% sensitivity and 88% specificity.

CEUS has some limitations: the acoustic window for liver visualization must be very good, thus the hepatic lesion must be well seen in standard US in order to be able to perform CEUS evaluation and to characterise the lesion. If more than one lesion is present in the liver, an injection of contrast agent is needed for each lesion, in order to characterize it in all vascular phases (especially on a cirrhotic liver). Therefore, not all FLL can be evaluated by CEUS, but only those that are well seen in standard ultrasound [27].

The results may be influenced by the use of different protocols for diagnosis, the quality of the ultrasound machine and the experience of the center or of the examiner, but also by the small number of patients included in the study.

Conclusions

Sulfur hexafluoride used as contrast medium SonoVue is a well tolerated, less expensive and largely available substance used in CEUS for the detection of suspect FLL. As the results of our study show, CEUS as a workup method was not conclusive for the diagnosis of 20.1% of FLL, when compared to MRI in an uniform population of patients. Potential causes of this discrepancy, as suggested by the literature are not substance-dependent, but reside in the highly operator dependence of the procedure. Nevertheless, further research is needed and improvement of technique in order to increase experience in diagnosing FLL with sulfur hexafluoride in CEUS.

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