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Review

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Role of the contrast-enhanced ultrasoud in the diagnosis of HCC in cirrhotic liver

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Abstract

The development of second generation ultrasound (US) contrast-medium and specific imaging techniques with dedicated softwares, allows to observe the liver perfusion in real time, becoming an useful and less invasive method to describe precisely the vascularization of hepatic lesions. This significantly increased the ability of US to detect and characterize focal liver lesions. The aim of this review article is to evaluate the role of contrast enhancement US in the diagnosis of hepatocellular carcinoma in cirrhotic liver, with reference to the guidelines of American Association for the Study of Liver Diseases, European Association for the Study of the Liver and European Federation of Societies for Ultrasound in Medicine and Biology.

Keywords: Contrast-enhanced ultrasound, hepato-cellular carcinoma, cirrhotic liver

INTRODUCTION

Hepatocellular carcinoma (HCC) is the most common malignancy of the liver. Ultrasound (US) examination and measurement of serum levels of alpha-fetoprotein (AFP) represent the most common screening method for HCC[1].

However, the conventional grayscale US and Color-Power Doppler US show limited ability in characterizing

liver tumors[2-5] and the sensitivity of these biomarkers in the detection of early HCC or small lesions is limited. AFP levels may also be elevated in other malignancies, such as intrahepatic-cholangiocarcinoma (ICC) or colon cancer, as well as during follow-up of chronic viral hepatitis[6].

The study of vascularization within the nodule in focal liver lesions (FLLs) in a cirrhotic liver is considered to be useful in identification and characterization with various imaging techniques[7-16].

With the development of a second generation of US contrast-agent and real-time contrast-specific techniques, contrast-enhanced ultrasound (CEUS) has been widely used in clinical studies and has greatly improved the diagnostic ability of US in identification of FLLs[16-21].

CONSTRAST MEDIA

Currently, there are four US contrast agents for liver studies: (1) SonoVue (BraccoSpA, Milan Italy introduced in 2001) that consists of stabilized gaseous microbubbles (sulfur hexafluoride) equal to or smaller than red blood cells, with a diameter of less than 7 μm, stabilized inside a phospholipid shell; (2) Definity (Lantheus Medical, Billerica, MA, USA, introduced in 2001) consists of stabilized microbubbles of perflutren with a lipid shell; (3) Optison (GE Healthcare) consists of stabilized microbubbles of human serum albumin with octofluoropropane; and (4) Sonazoid (Daiichi-Sankyo, GE Tokio, Japan, introduced in 2007) that consists of stabilized gaseous microbubbles (perfluorobutane) with phospholipid shell (hydrogenated egg phosphatidyl serine)[22].

Definity and Optison have been authorized only in USA and Canada for cardiological imaging; in Canada Definity is used also for other body districts. Sonazoid is used only in Japan and SonoVue in Europe and China. In Europe only Optison is used for cardiological imaging.

In consideration of what previously said, in our article we will exclusively refer to SonoVue, the only US contrast medium authorized in Europe for the study of FLLs.

Basic of CEUS

The contrast media SonoVue consists of microbubbles of stabilized phospholipids containing sulphure-hexafluoride, with the same or inferior dimension of red blood cells (diameter inferior to 7 μm). Due to their small size the microbubbles act as an "blood pool agent" and allow the real time study of the macro- and microvascular circulation for several minutes[23-25].

The interaction between the microbubble blood pool and the incident US beam is the key to understand the mechanism of action of the US contrast agent and its clinical applications. When the microbubbles are hit by the US beam at low mechanical index (MI) (< 100 kPa - MI < 0.1), they are exposed to a low-level positive (compression) and negative (dilatation) sound pressure. In this case the microbubbles behave in a linear way as simple reflectors, without breaking. In this way a linear reflection phenomenon is generated which results in a wide reinforcement of the scattering coming from the circulating blood. Increasing the acoustic intensity of the incident beam (MI between 0.1 and 1), the oscillation becomes more intense and asymmetric and the physical behavior of the microbubbles becomes non-linear. Because of non-linear reflection, if the microbubbles are hit by an acoustic beam with this intensity, they generate a reinforcement of the fundamental signal and a harmonic energy.

The non linear behavior of the microbubbles shows itself in a way not dissimilar to stationary tissue. The main advantage that derives from the use of US contrast media is that the amount of the signal coming from the second harmonic, which originates from the microbubbles, is of a length greater than that coming from stationary tissues.

Therefore, thanks to the use of specific software, the linear signals are deleted from the tissues and the images are formed only thanks to the non-linear signals coming from the microbubbles. The use of these more powerful acoustic waves, however, causes the breaking of part of the micro-bubbles. To minimize this phenomenon, we have chosen to work at low mechanical indices. This study technique allows to cancel the signal coming from the tissues and to have pure images coming exclusively from the microbubbles[25-29].

Although the correct setting of the US scanner and the scanning techniques are important for avoiding artifacts[30], MI and inadequate gain are the two main causes of error in the visualization of the signals coming from the tissues.

PROTOCOL OF SURVEILLANCE OF HCC

In our institute, we use a HCC surveillance protocol in patients with cirrhosis, based on the six-monthly dosing of alpha-fetus protein serum levels and on the execution of a six-monthly hepatic US examination in patients in the Child Pugh class A and B. In patients in the Child Pugh class C, the US can be also performed every three months.

DIAGNOSIS OF HCC

Baseline us

HCC typically appear as hypoechoic compared to the surrounding hepatic parenchyma. It can also appear as isoechoic, hyperechoic or with mixed echogenicity, with a typical characteristic of nodule in nodule. About 50% of HCC can appear as a nodule with peripheral hypoechoic halo[22]. Both the conventional Color-Doppler and the Power-Doppler US have a limited ability to describe intralesional vascularization, because they are insensitive to slow and deep blood flows[31,32]. Generally the Doppler HCC pattern is characterized by an arterial vascularization with a basket pattern due to thin blood vessels that surrounds the nodule[11,22,33].

CEUS procedures

Before starting the CEUS evaluation, it is mandatory to perform an evaluation in B-mode; in particular it is necessary to analyze the site, the size, dimensions, echogenicity of the lesion and its relationship with the other structures. An evaluation of the vascular pattern of the lesion in Color-Doppler is useful to define the eventual presence of central or peripheral vascular vessels. Once the target lesion has been identified, the specific mode of imaging must be selected for the contrast with a low MI. SonoVue is injected into the antecubital vein with a bolus, followed by a bolus flash of a solution of 10 mL of sodium chloride. To avoid destroying the microbubbles during the injection, the calibre of the needle must not be less than 20 gauge[22]. The target lesion and the surrounding parenchyma are observed for 5-10 min in real time and registered in a video clip. The arterial phase is defined as 0-30 s from the injection, the portal phase 31-75 and the late phase from 75-180 s up to 10 min[31].

CEUS

The most common appearance in cirrhotic liver of HCC is an hyper-arterial enhancement compared to the surrounding hepatic tissue [Figure 1], which is found in 93.5%-97% of cases[31,33-38] and generally appear homogeneous and intense. In the nodules that have diameters larger than 2 cm, hyper-enhancement can also be non-homogenous because of the area of necrosis within the lesion [Figure 2]. A slight peripheral enhancement is found in 5 (34.6%) of cases of HCC; it can represent the tumor capsule [Figure 3] or blood vessel around the lesion[31,33-39]. In the majority of cases HCC shows a precocious enhancement compared to the surrounding tissue, in particular, the rates of detection of the hyper-enhancement in lesions < 1.0 cm, 1.0-2.0 cm and 2.0-3.0 cm are respectively 67%, 83%-88% and 92%-100%[3,31,36-40] [Table 1]. Furthermore other lesions like dysplastic nodules and hyper-vascularized hemangioma can have the same contrast enhancement pattern[41].

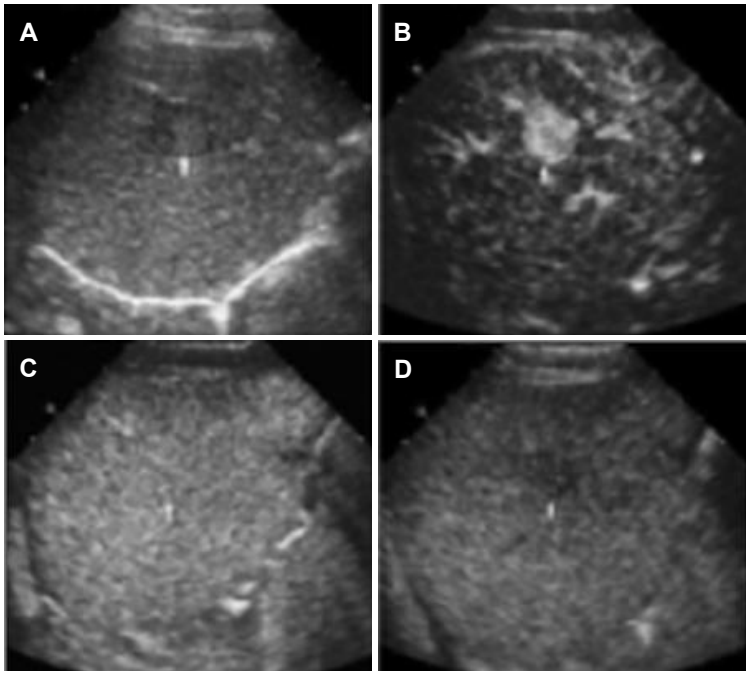

Figure 1. A: US shows a hypoechoic hepatocellular carcinoma (HCC); B: arterial phase (19 s) shows a homogeneous hyper-enhancement of the lesion; C: portal phase image (82 s): the nodule is isoechoic; D: late portal phase (190 s): the HCC is slightly hypoechoic with respect to surrounding liver

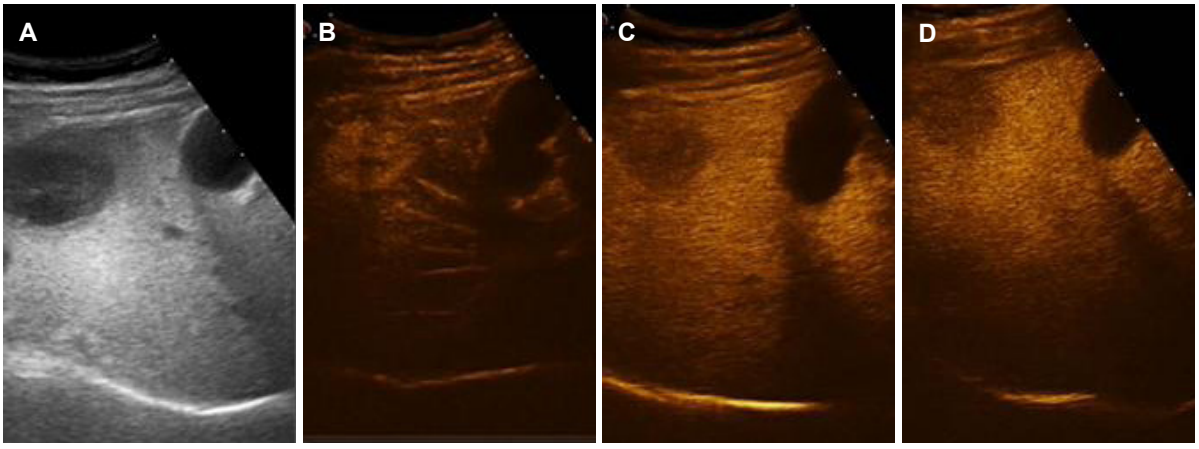

Figure 2. A: US shows a hypoechoic hepatocellular carcinoma (HCC) (> 2 cm); B: arterial phase (26 s) shows an inhomogeneous hyper-enhancement of the lesion; C: portal phase image (70 s) shows wash-out of contrast medium; D: late phase image (95 s): the HCC is hypoechoic with respect to surrounding liver

To increase the specificity of CEUS on the basis of these findings, a demonstration of the washout-phase is decisive and its presence also depends on the size of the nodule: the wash-out is described only in 20%-30% of nodules with diameters of 1-2 cm and in 40%-60% of nodules with diameters of 2-3 cm[22,38,42-59].

The speed of the wash-out can define the level of differentiation of HCC: poorly differentiated show rapid wash-out, while the well differentiated HCC tends to be iso- or hypo-enhanced compared to parenchyma in the portal or late venous phase[21,31,60-62] [Figure 4].

Table 1. Typical enhancement of hepatocellular carcinoma in the arterial phase based on the size of lesion

Size lesion (cm)	rate of detection of the hyper-enhancement in lesion
< 1.0 cm	67%
1-2 cm	83%-88%
2-3 cm	92%-100%

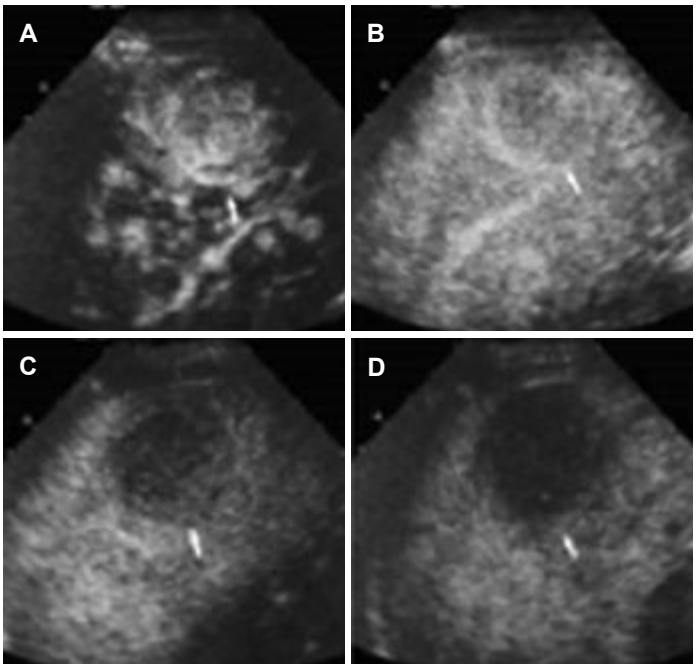

Figure 3. A: Arterial phase (18 s) shows a heterogeneous hyper-enhancement of the lesion; B: portal phase (32 s): the nodule is slightly hypoechoic; C: portal phase (90 s): the nodule is hypoechoic; D: late portal phase (180 s): the nodule is remarkably hypoechoic with respect to the surrounding liver. Capsule of the lesion is well represented (arrows) more evident in A and B

In order to increase the sensitivity of the diagnosis of HCC, in the cirrhotic liver it is useful to observe for more than 4 min, in fact in these cases the wash-out tends to start later, generally not before 60 s after the injection, and in a quarter of cases it appears after only 180 s[40]. For this reason the presence of precocious wash-out (< 60 s) has been described in HCC poorly differentiated and in cases of ICC[22,40,61-62].

In conclusion, a hyper-enhancement in the arterial phase, followed by a washout in the late phase is a typical CEUS pattern in HCC in cirrhotic livers[63]. Usually regenerative/dysplastic nodule doesn't show this kind of pattern contrast enhancement that appears similar to the parenchyma.

DISCUSSION

In 90% of cases the development of hepatocarcinoma occurs through a multi-step path in which the lesion passes from a benign to a malignant lesion following an order summarized in Table 2. During this long process, a reduction in the normal arterial blood supply and the contemporary and progressive increase in newly formed tumor vessels (neo-angiogenesis) were detected. The development of second generation contrast-medium and specific imaging techniques with dedicated softwares, allows to observe the perfusion of the lesion in real time, becoming an useful and less invasive method, in describing precisely blood supply of nodule[31]. However, in clinical practice, non invasive diagnosis of HCC is relatively recent. Until 2000 the diagnosis of HCC occurred through invasive biopic studies and successive histologic diagnosis[22].

Table 2. Development of hepatocellular carcinoma (HCC)

Large regenerative nodule
Low dysplastic nodule
High dysplastic nodule
Nodule of HCC
well differentiated - moderately differentiated - poorly differentiated

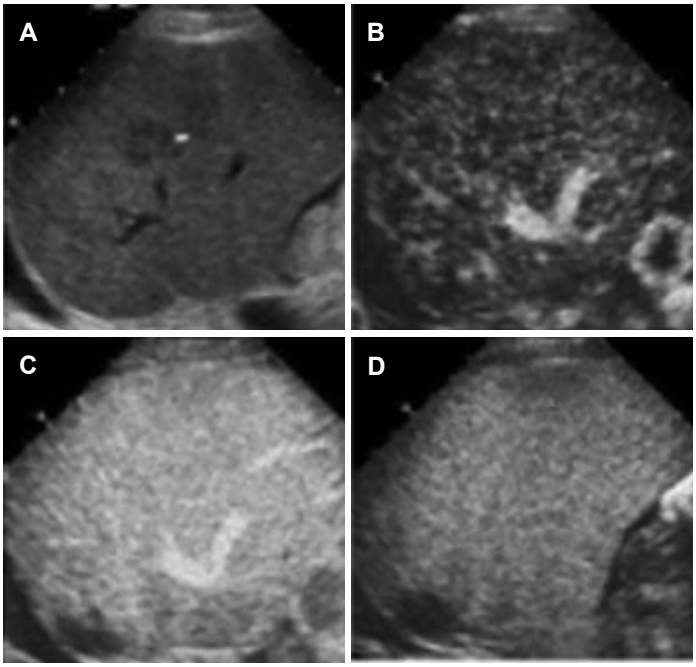

Figure 4. A: US shows a hypoechoic nodule; B: portal phase (32 s): arterial phase (23 s) shows a homogeneous isoenhancement of the lesion; C: portal phase (52 s): the nodule is isoechoic with respect to the surrounding liver; D: late portal phase (280 s): the hepatocellular carcinoma (HCC) is isoechoic with respect to the surrounding liver

In 2001 a group of experts European Association for the Study of the Liver (EASL) on HCC in Barcelona reported, for the first time, the criteria for a non invasive diagnosis[64]. These criteria required only the presence of a certain dynamic contrast enhancing behavior: the uptake of a contrast medium during the arterial phase documented through CT, angiography magnetic resonance imaging (MRI) or US. Therefore, in a cirrhotic liver, were considered HCC the nodule lesions with a diameter bigger than 2 cm that showed this uptake of contrast medium in 2 different imaging modalities or showed this contrast enhancing impregnation in a single imaging modality but with serum levels of AFP bigger than 400 ng/mL. In all other cases a biopsy was necessary[22,64].

In 2005 EASL and American Association for the Study of Liver Diseases (AASLD) reached a new radiological signal to further distinguish HCC: wash-out in the venous/late phase[5,22]. So the non invasive diagnosis of HCC was based both on the presence of uptake of the contrast medium in the arterial phase and on the wash-out in the venous/late phase. For nodules larger than 2 cm these radiological criteria should have been present in just one imaging modality; for nodules of the dimensions of 1-2 cm these radiological signs should have been shown in at least two imaging modalities (CT, MRI and CEUS). The AFP was eliminated from the diagnostic algorithm due to some limitations[5,22]. Due to the ability to visualize in real time the perfusion of hepatic lesions, CEUS can have a foremost role in the diagnosis of HCC; however it is currently accepted in variable ways in national and international guidelines. At the moment, CEUS is recommended by European Federation of Societies for Ultrasound in Medicine and Biology (EFSUMB) and is part of the Japanese guide-

Table 3. Recommendations of European Federation of Societies for Ultrasound in Medicine and Biology for the use of contrast-enhanced ultrasoud

The characterization of the nodules
To make a rapid diagnosis (however, CT or MR remain necessary, if not contraindicated, for the stadiation
When CT and MR are inconclusive especially in nodules that can't be submitted to biopsy
To contribute to selecting a nodule when they are many or have different contrast patterns
To monitor the changes in the nodule
After an inconclusive histology

lines for HCC[22,23,65,66], but it has been removed from American and EASL guidelines[48,53]. The main reason for this exclusion lies in the possibility of a mistaken diagnosis between ICC and HCC using only CEUS[67,68]. Furthermore this exclusion from AASLD guidelines is also related to the fact that, in the United States, contrast enhancing agents are not authorized for the study of the liver and so CEUS is not available. However, in clinical practice, the probability of mistaken diagnosis is minimal when CEUS is carried out by an expert physician[69], because the ICC shows a rapid wash-out. Apart from this, in recent years a significant variability has been described, that has made the use of CEUS still more controversial[69]. In 2010 AASLD recommended that, for nodules bigger than 1 cm, the non invasive diagnosis for HCC can be determined with a single means of imaging (CT multidetector or MRI with dynamic contrast)[53], if the typical contrast enhancement pattern is present; however when typical radiological aspects are not present and the behavior of the nodule is not characteristic, it is necessary to evaluate the nodule through a second imaging technique or with a biopsy[53]. This change is based on the conclusion of several studies that have demonstrated that the use of a single contrast technique causes a reduction in the positive predictive value that remains higher than 90%[42,59], they highlight a higher specificity than the typical radiological sigh[41,70]. AASLD guidelines suggest the necessity of adhering closely to imaging protocol and carrying out non invasive diagnosis of HCC in expert centers[2,53].

Recent EASL guidelines are similar to those of AASLD, suggesting the use of multiphase imaging CT and up to date MRI for non invasive diagnosis of HCC[48]; in particular for nodules between 1-2 cm, a single imaging technique is advised when carried out exclusively in excellent centers and with high grade radiological equipment or 2 imaging techniques when these criteria are not present and are carried out in inferior contexts. Such prudent recommendations of EASL guidelines are based on evidence of equivocal data concerning non invasive diagnosis of nodules 1-2 cm[22,48,53]. EFSUMB suggests a very different role for CEUS, describing it separately in two patients subgroups, with and without cirrhosis; this because of the great difference between types of hepatic nodules in cirrhotic and non cirrhotic livers[22-23]. In cirrhotic livers, among the recommendations of EFSUMB for the use of CEUS[23] are summarized in Table 3. The multicenter German Society for Ultrasound in Medicine (DEGUM) included 1349 patients with FLLs diagnosed on US; CEUS was compared to the biopsy in 75% of cases and in 25% with contrast enhancement (CE) CT or CE-MRI. The accuracy of CEUS was 90.3%[71-75].

Another two DEGUM studies evaluated the capacity of CEUS in the characterization of FLL, comparing CEUS in the first study with CE-CT and in the second with CE-MR. In both cases there were no statistically significant differences[75-77]. In 2012, Goto *et al.*[78] reported a major sensibility and sensitivity of baseline US in comparison with CEUS, using Sonazoid, in the detection of HCC during the post-vascular phase. In the differential diagnosis between HCC and ICC there is some controversy about the role of washout: in the late phase the wash-out of HCC seems to be less marked than the other liver neoplasms like ICC and metastasis[23,38,69,79]. Reanalyzing the data of the studies, Guo and Xu[80], found that the clinical consequences that come from this risk do not seem to justify the complete removal of CEUS as an imaging technique in the characterization of FLL. With regard to this, further positive evidence is being gathered: Li *et al.*[81] evaluated in the first place the usefulness of CEUS in differentiating ICC from HCC in cirrhotic patients through a detailed analysis of the characteristics of temporal enhancement. Therefore, in a cirrhotic liver if a nodule shows a hyper-enhancement

in the arterial phase followed by a precocious and marked washout in the portal phase, the nodule is highly suspected of ICC; HCC, however, shows a moderate washout in the portal phase and, sometimes, can show iso-enhancing compared to surrounding parenchyma. These results have provided the last evidence to reprove the opinion of AASLD[80].

The meta-analysis with evidence from 1998 to 2016 of Zhang *et al.*[82] showed that CEUS was a useful diagnostic instrument for distinguishing HCC from other FLLs and, in conclusion, could also become a front line imaging instrument in the future. Masuzaky *et al.*[83] and Chan *et al.*[84] reported that CEUS has an important role in patient candidates to the treatment with radiofrequency ablation (RFA), increasing the detection of HCC that are not seen or poor seen on B-mode US and provides real-time guidance of RFA with good short-term treatment responses. Intrinsic limitations of CEUS vary in relation to patient characteristics (cooperation, obesity, meteorism), characteristics of lesion (site-dimesions-depth) and the CEUS experienced operator.

Another important limitation of CEUS compared to cross sectional image formation is that only one FLL can be evaluated at a time and the repeated administration in bolus of SonoVue is necessary to evaluate other FLLs. However, in clinical practice, only 2 and 3 FLLs situated in the same segment lobe can be simultaneously and easily examined with CEUS[85]. On CEUS, the evaluation of enhancement is statistically significant in relation to the depth; in particular, at a depth greater than 9 cm from abdominal wall, only 58% of FLL present the same arterial enhancement compared to the corresponding phase in multi-slice CT; this contrasts with 95% of the lesions situated more superficially[86].

Some studies have demonstrated that a number of lesions, varying 5%-25%, remain unterminated after CEUS, because they do not present a characteristic pattern[86]. Contrast-enhancing agents until today have not demonstrated cardio-, hepatic- or nephro-toxic effects. It is not necessary to carry out laboratory tests to evaluate hepatic or renal function before their administration. There is limited data about use during pregnancy, breast-feeding or in pediatrics. In a retrospective study[87] of 23,188 investigations with SonoVue the rate of serious adverse events was only 0.0086% (29 cases), including a pseudo- anaphylactic shock and a bronchospasm, but there were no fatalities.

CONCLUSION

CEUS is a non invasive, rapid, economical and accurate method for the diagnosis and management of HCC in cirrhotic patients; moreover it is repeatable, less stressful and less invasive for the patients and doesn't require exposure to radiation. CEUS is not nephro-toxic and is non allergenic. When the nodular lesions are controlled in the cirrhotic liver, CEUS allows a rapid characterization with good precision when carried out by a medical expert.

DECLARATIONS

Authors' contributions

Designed the work, collection and data analysis, critical revision of the article and final approval of the version to be published: Loria F, Parlati A, Loria G

Contributed to the execution of the ultrasound examination and to the interpretation of the results and to the bibliographic research: Loria F, Parlati A, Loria G, Frosina L, Crea G, Basile S, Alessio C, Di Leo G, De Caridi A, Maschio V, Zizzi N, Trapuzzano O, Galea SG

Contribuited also to revision of the article: Loria F, Parlati A, Loria G, Frosina L, Crea G, Basile S, Alessio C, Di Leo G, De Caridi A

Contributed also to drafting the article: Loria F, Parlati A, Loria G, Maschio V, Zizzi N, Trapuzzano O, Galea SG

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All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate
Not applicable.

Consent for publication
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