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Contrast-Enhanced Ultrasound: a Simple and Effective Tool in Defining a Rapid Diagnostic Work-up for Small Nodules Detected in Cirrhotic Patients during Surveillance

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ABSTRACT

Background & Aims: Disappearance of portal blood flow and arterial vascularization is the hallmark of hepatocarcinogenesis. The capability of a dynamic imaging modality detecting arterial hypervascularization of small nodules is crucial to promote a rapid diagnostic and therapeutic work-up improving survival. We aimed to evaluate the capability of CEUS to detect arterial vascularization of ≤ 2 cm HCC nodules arising during surveillance so as to shorten the diagnostic and therapeutic work-up.

Methods: From October 2009 to September 2014, among 1757 consecutive cirrhotic patients under surveillance with ultrasound (US), 243 patients had new single nodules 7-20 mm; 229/243 had a conclusive histologic diagnosis and comprised the study group. All patients underwent CEUS followed by enhanced MRI and US guided percutaneous 18G needle core biopsy of the nodules. Of the 229 nodules, 27 were hyperechoic, 171 hypoechoic and 31 isoechoic lesions.

Results: The histology results revealed that 199/229 nodules were HCC and 30 were benign. Of 199 HCC, CEUS evidenced arterial hypervascularity in 190 nodules (95.5%) (sensitivity 94.48 %, specificity 100%, PPV 100%, NPV 76.92 %). Of the 39 CEUS arterial-unenhanced nodules, 30 were benign and 9 (23%) were well-differentiated HCC. eMRI showed arterial hypervascularity in 199 nodules (86,9%). Of these, only 193 (97%) were histologically HCCs while 6 were benign (sensitivity: 97%, specificity: 80%, PPV: 97%, NPV: 80%).

Conclusions: CEUS has a great capability to detect arterial hypervascularity of small HCC. Because only 4.5% of new nodules escape the demonstration of arterial hyervascularity, CEUS must be performed immediately after conventional US to contrast the malignant fate of small lesions arising in a cirrhotic liver.

Key words: hepatocellular carcinoma – cirrhosis – contrast enhanced ultrasound.

Abbreviations: CEUS: contrast-enhanced ultrasound; CT: computed tomography; HCC: hepatocellular carcinoma; MRI: magnetic resonance; NPV: negative predictive value; PPV: positive predictive value; US: ultrasonography.

INTRODUCTION

Hepatocellular carcinoma (HCC) is an epithelial tumor originating in the liver and composed of cells with characteristics similar to those of normal hepatocytes [1].

Hepatocellular carcinoma is the 5th most frequent cancer in the world and the first cause of death in cirrhotic patients and its incidence is increasing, especially in Western nations [2, 3]. Most HCCs develop in patients with underlying chronic hepatitis or cirrhosis via a multistep process of carcinogenesis, ranging from regenerative nodules to classic HCC [1–4].

The prognosis of HCC depends largely on the stage at which the tumor is detected. Patients who present with symptoms generally have a dismal prognosis, as HCC usually does not produce symptoms beyond those of the underlying liver disease until it has become incurable; in such patients, median survival is less than 1 year and the 5-year survival is less than 10% [4]. By comparison, patients in whom HCC is detected at an early stage may benefit from life-prolonging, potentially curative treatments.

Therefore the detection of HCC early in its development is critical to improve the survival of affected patients. To this end, scientific societies have released clinical management guidelines that advocate surveillance of patients at risk due to cirrhosis or chronic viral hepatitis [5-7]. While the surveillance strategies incorporated by the various guidelines differ, all

current guidelines recommend ultrasonography (US) as the primary imaging test for surveillance, and two guidelines advocate the ancillary use of serum biomarkers [5-7]. In general, neither computed tomography (CT) nor magnetic resonance (MR) imaging are advocated for surveillance, although three guidelines permit these modalities for surveillance of patients in whom US is limited by obesity or other factors [5, 6] and for those at very high risk for HCC development [4].

Once a surveillance test is positive (i.e. an abnormality that may represent HCC is detected), a more definitive imaging examination is recommended for noninvasive diagnosis and staging of HCC.

During hepatocarcinogenesis the density of portal triads diminishes while the density of unpaired arteries increases. The net effect is that intranodular arterial supply diminishes initially and then increases; advanced HCCs typically show arterial hypervascularity as compared to background liver [8].

As the nodule evolves from dysplastic to malignant, neoangiogenic unpaired arteries progressively supplant intratumoral portal tracts. Therefore, overt HCC is mainly fed by the hepatic artery, whereas normal liver parenchyma and premalignant nodules are mostly perfused by the portal branches [9]. This arterialization of the blood supply accounts for the hyperenhancement shown by malignant nodules during the arterial phase and for the wash-out during the portal and sinusoidal phases on dynamic imaging modalities such as contrast-enhanced US (CEUS), enhanced CT (eCT) and enhanced MR (eMR) [9].

Hence, non invasive diagnosis of HCC is based mainly on the assessment of vascularity. As a consequence, the presence of hypervascularization in the arterial phase is the hallmark of malignancy [8, 9].

We can assume that the use of CEUS soon after the conventional US – CEUS being a simple, easy-to-perform and immediately available dynamic imaging modality - is crucial to detect the arterial hypervascularization of small new nodules arising in a cirrhotic liver during surveillance, in order to promote a rapid work-up for final diagnosis, avoiding a late diagnosis, enabling an early treatment and therefore improving survival. CEUS substantially improves the accuracy of conventional US in the characterization of focal liver lesions by showing different vascular patterns between benign and malignant lesions during the arterial portal and sinusoidal phase [10].

Therefore, the aim of this prospective study was to evaluate the capability of CEUS to detect arterial vascularization of single, small (≤2cm) HCC nodules arising in cirrhotics during surveillance so as to shorten the diagnostic and therapeutic work-up of HCC patients.

MATERIAL AND METHODS

From October 2009 to September 2014, 1757 consecutive cirrhotics under surveillance with conventional US for development of HCC in their livers, were prospectively enrolled in the study. During this period, 243 new, single, clearly visible nodules, 7-20 mm in diameter, were detected. After conventional US all patients underwent CEUS within 3 days.

Subsequently, after a maximum period of 7 days all patients underwent eMRI.

After CEUS and e-MRI all patients underwent percutaneous US guided needle biopsy of the nodule. Of the 243 consecutively detected nodules, 229 had a conclusive histological diagnosis and composed the study group.

Percutaneous biopsy of the nodules was performed with an 18G cutting needle (Biomoll, HS Service, Rome, Italy) in all cases. Percutaneous biopsy of the nodules was performed under US guidance with hand free technique by the same operator (A.G. 30 years expertise in interventional US). Nodules in the left lobe of the liver were punctured with the patient in a supine position while nodules of the right lobe were mainly punctured in the intercostal space with the patient lying on the left flank, as routinely performed in our Unit for percutaneous biopsy of focal liver lesions larger than 6 mm [11]. When the specimen was judged sufficient for histological examination only one passage was performed; in all other cases 2-3 passages were performed (mean 1.4) until the specimen was judged sufficient. Histological diagnosis of HCC was made by an expert liver pathologist (P.G.) according to the histological criteria of the International Working Party [12]. The result of percutaneous biopsy was used as a gold standard for HCC diagnosis in all cases.

CEUS was performed using 2.4 ml of SonoVue (Bracco Imaging, Milano, Italy) according to our criteria [13-15], using last generation, high definition, commercially available machines (Ascendus-Hitachi, Japan; Aplio 500, Toshiba, Japan).

The whole vascular phase, consisting of the arterial phase (10 to 30 seconds following contrast injection), the portal phase (30 to 60 seconds) and the late phase (60 to 240 seconds), was accurately studied and recorded [13-15]. A second injection of further 2.4 ml of SonoVue was needed in 8 patients because of inadequate visualization of the overall vascular enhancement after the first injection.

MR imaging was performed by a radiologist blinded of CEUS results by using a 1.5-T system (MagnetomAvanto - Siemens, Erlangen, Germany) equipped with a 72-channel system, which provided a maximum gradient strength of 45 mT/m, with a peak slew rate of 200 mT/m/msec. All images were obtained in the transverse plane using phased-array coils, with a rectangular field of view of 22-24 x 35-40 cm, which was adjusted for each patient. The baseline MR imaging examination consisted of a T1-weighted gradient echo sequence followed by a T2 Fast Spin Echo, both during end-expiratory breath hold. For contrast-enhanced dynamic MR imaging, 0.1 mmol/kg of body weight dose of gadobenatedimeglumine (MultiHance; Bracco Imaging - 0.2 mL/kg of body weight) was administered through antecubital vein at 3 mL/sec by an 18-22 gauge intravenous catheter by means of a power injector (Spectris; Medrad), followed by 20mL saline flush with the same injection rate. T1-weighted three dimensional (3D) spoiled gradient-echo volumetric interpolated breath-hold examination (VIBE) images were acquired at 25, 40 and 150 seconds after the start of the contrast material administration during the hepatic arterial dominant, portal venous, and equilibrium phases respectively, and, if necessary, during the delayed hepatobiliary phase at approximately 120 minutes after the injection. The study was

approved by our Institutional Review Board and all patients gave their informed written consent .

Statistical analysis

Pearson's chi-square test was used to evaluate the significance in the differences of the frequencies among the populations. Cohen's Kappa was used to evaluate the concordance between CEUS and eMRI vs the gold standard (i.e. liver biopsy) and between CEUS and eMRI, respectively. Data processing and analysis were performed with IBM SPSS Statistical analysis 22.0 2013.

RESULTS

The study group included 137 males and 92 females, median age 69 (range 51-86 years). Cirrhosis was due to HCV in 145 patients, to HBV in 39 patients, to alcohol abuse in 12 patients and mixed etiology in 33 patients. Table I summarizes the clinical features of the patients of our series. On conventional US of the 229 nodules, 27 were hyperechoic, 171 hypoechoic and 31 were isoechoic.

Histology examination revealed that 199 of 229 nodules were HCC and 30 were benign lesions. Table II shows the histological diagnosis of the 229 nodules compared with the native ecogenicity patterns. Tables III and IV show the native echogenicity findings and sizes of the 199 HCC and 30 benign lesions, respectively.

CEUS showed arterial hypervascularity in 190 nodules (94.5%) and all these arterial-hyperenhanced nodules were HCC (sensitivity: 95.5%, specificity: 100%, PPV: 100%; NPV: 76.9%). On CEUS, 105 (55.3 %) hyperenhanced nodules, after the initial arterial hyperhenacement (10-30 seconds), became isovascular with liver parenchyma either in the portal or late phases. Of the remaining 85 nodules (44.7%), none showed hypovasculariry in the portal phase (30-60 seconds) and all became hypovascular in the very late phase (after 180 seconds). Of the 39 CEUS arterial-unenhanced nodules, 30 were benign and 9 were well-differentiated HCC.

Table V reports the native US patterns and the size of the nodules of our series according to the corresponding vascular findings on CEUS. There was a statistically significant difference between the frequency of the arterial enhancement of the nodules ≤ 10 mm and the nodules 11-15 mm in diameter (p=0.0001) and between the frequency

of arterial enhancement of the nodules < 1 cm and nodules 16-20 mm in diameter (p=0.005). There was no statistically significant difference between the frequency of arterial hyperenhancement in the nodules 11-15 mm and nodules 16-20 mm (p=0.96). When considering the echogenicity of the nodules, there was a statistically significant difference in the enhancement between the groups with tumor diameters of < 1, 1-1.5 and 1.6-2 cm and only for hypoechoic nodules [P=0.001 among the three groups (<1, 1-1.5 and 1.6-2 cm, respectively)].

Of note, 17 out of 21 (81%) hyperechoic angioma-like lesions which had a final diagnosis of HCC evidenced arterial hypervascularity and all of these were histologically HCCs. eMRI showed arterial hypervascularity in 199 nodules (86.9%). Of these, only 193 (97%) were histologically HCCs while 6 were benign (4 where dysplastic nodules and 2 were regenerative nodules) (sensitivity: 97%, specificity: 80%, PPV: 97%, NPV: 80%).

In table VI the comparison between CEUS and eMRI sensitivity and specificity is reported. The concordance between CEUS and eMRI versus the gold standard, i.e. liver biopsy, was 0.96 and 0.95 respectively. Cohen's kappa was 0.85 for the CEUS and 0.78 for the MRI. The concordance between CEUS and MRI was 0.98.

Table I. Clinical characteristics of the 229 patients of our series

Age (years), n (range)	69 (51-86)
Males, n	137
Anti-HCV positive, n	145
HBsAg positive, n	39
Alcohol abuse, n	12
Mixed etiology of cirrhosis, n	33
Child-Pugh A, n	199
Child-Pugh B, n	30
Platelet count (10^9/l)	93 (37-189)
Total bilirubin (mg/dl)	0.89 (0.5-2.7)
Albumin (g/dl)	3.3 (2.5-3.8)
AFP (ng/ml)	12 (0.7-56)
Nodule size (mm)	14 (7-20)

Continuous variables are reported as median and range. AFP, Alpha-fetoprotein; Anti-HCV, antibody against hepatitis C virus; HBsAg, hepatitis B virus surface antigen.

Table II. Histological diagnosis of the 229 nodules of our series compared with the native echogenicity on US

		Conventional US pattern		
	Total	Hyperechoic	Hypoechoic	Isoechoic
Hepatocellular carcinomas n (%)	199	21 (10.5%)	154 (77.4%)	24 (12.1%)
Poorly differentiated	62	18 (29%)	41 (66%)	3 (5%)
Moderately differentiated	100	3 (3%)	90 (90%)	7 (7%)
Well differentiated	37	6 (16.2%)	20 (54.1%)	11 (29.7%)
Benign lesions n (%)	30	6 (20%)	17 (56.6%)	7 (23.3%)
Focal steatosis	12	6 (50%)	6 (50%)	0
High grade dysplastic nodules	15	0	11 (73%)	4 (27%)
Regenerative nodules	3	0	0	3 (100%)

Table III. Native echogenicity findings and size of the 199 HCC nodules of our series

		Nodules size			
		7-10 mm (median 9 mm)	11-15 mm (median 12 mm)	16-20 (median 17 mm)	Total
Native echogenicity US pattern	Hyperechoic	6 (29%)	13 (62%)	2 (9%)	21
	Hypoechoic	14 (9%)	102 (66%)	38 (25%)	154
	Isoechoic	2 (8.3%)	16 (66.7%)	6 (25%)	24
	Total	22 (11%)	131 (69%)	46 (23%)	199

Table IV. Native echogenicity of US findings and size of the 30 benign nodules of our series

		Nodules size			
		7-10 mm (median 9 mm)	11-15 mm (median 12 mm)	16-20 (median 17 mm)	Total
Conventional US pattern	Hyperechoic	0	2 (33%)	4 (77%)	6
	Hypoechoic	4 (24.5%)	7 (41%)	6 (35.3%)	17
	Isoechoic	2 (28.5%)	3 (43%)	2 (28.6%)	7
	Overall	6 (20%)	12 (40%)	12 (40%)	30

DISCUSSION

Our results show a high capability of CEUS in demonstrating the arterial hypervascularity of small (≤ 2 cm) nodules arising in cirrhotic patients during conventional US surveillance, nodules later demonstrated as HCC on histological examination. In fact, in our study only 4.5% of HCC nodules did not show the typical arterial hyperenhancement at CEUS, leaving CEUS as inconclusive for their characterization. The capability of CEUS in detecting arterial hypervascularity even in such small nodules is not surprising because of the high quality of the new, commercially available, machines used in the study, as shown in Fig. 1. A limit of our study should be the absence of intrahepatic cholangiocarcinomas (ICC) in our series in order to obtain data for a differential diagnosis. On the other hand the rapid wash out of ICC in cirrhosis, which is the main characteristic of ICC [17], was never observed in our study (more than 50% failed to show hypovascularity either in the portal or late phase). Furthermore, in our series, the typical wash out (i.e. appearance of hypovascularity) was never present in the portal phase but in some nodules only in the very late phase (over 180 seconds), and this findings are not typical of ICC .

Therefore CEUS appears to be a highly effective tool in shortening the diagnostic workup of liver nodules. Subsequently CEUS can be considered highly effective in reducing also the therapeutic work up in patients at risk of developing HCC during surveillance, when CEUS is compared with conventional US.

CEUS should be considered as a complementary examination to conventional US. It is easy to perform immediately after US. It is cheap since the cost of last generation US-contrast agents is relatively low. CEUS is a highly effective tool especially because it allows an immediate and specific diagnosis to be given to a new liver nodule arising from cirrhosis, permitting the initiation of the appropriate therapy of the cirrhotic patients. All those aspects are of key importance from a clinical perspective, during the daily practice in a hepatology department where CEUS should be the technique of choice in the diagnostic work-up of HCC, after conventional US.

Table V. Vascular patterns of arterial enhancement of the HCC nodules according to their size

Nodules size		7-10 mm (median 9)		11-15 mm (median 12)		16-20 (median 17)	
		n = 22		n = 131		n = 46	
CEUS arterial enhancement		arterial hyperhnancement		arterial hyperhnancement		arterial hyperhnancement	
		YES	NO	YES	NO	YES	NO
		17 (77%)	5 (23%)	128 (98%)	3 (2%)	45 (98%)	1 (2%)
Native echogenicity US pattern	Hyperechoic	4 (18%)	2 (9%)	12 (9%)	1 (7%)	1 (2%)	1 (2%)
	Hypoechoic	12 (54.5%)	2 (9%)	100 (76%)	2 (1%)	38 (83%)	0
	Isoechoic	1 (4.5%)	1 (4.5%)	16 (12%)	0	6 (13%)	0

Table VI. Comparison between CEUS and eMRI sensitivity and specificity

	Hepatocellular carcinoma		Sensitivity % (95% CI)	Specificity % (95% CI)	Predictive Value (95% CI)
	Yes	No			
	n = 199	n = 30			
CEUS arterial hypernhancement			95.5 (81.7-90.8)	100 (86-100)	
YES	190	0			100 (97.5-100)
NO	9	30			76,9 (60.3-88.3)
MRI arterial hyperenhancement			97 (93.2-98.8)	80 (60.9-91.6)	
YES	193	6			97 (93.2-98.8)
NO	6	24			80 (60.9-91.6)

Our results are very similar to those reported by Sugimoto et al. [16]. These authors compared CEUS using Sonazoid (a new US contrast agent with phagocytosis by Kuppfer's cells, available in Japan) with gadoxetate disodium-enhanced MRI in the assessment of arterial hypervascularity of 57 histologically confirmed HCCs, mainly using a 21G cutting needle percutaneous biopsy, or with partial hepatectomy. They found that CEUS yields a significantly higher value than gadoxetate disodium-enhanced MRI in the assessment of arterial hypervascularity of HCC and concluded that the accuracy of CEUS is superior to that of Gd-EOB-DTPA MRI in the assessment of intratumoral vascularity.

Contraversely, our results are in contrast with those recently reported by Forner and coworkers [17]. In their study, aiming to assess CEUS capability in demonstrating the arterial hypervascularity of single, small, <2 cm nodules in a series of cirrhotic patients under US surveillance as hallmark of HCC, CEUS failed to demonstrate arterial hyperenhancement in 15.1% of their 119 HCC lesions [16, 17]. They concluded that "absence of hyperenhancement during the arterial phase at CEUS in nodules < 2 cm in a cirrhotic liver does not predict

a less malignant profile" because of the high percentage of false negative results of CEUS, and therefore "since contrast hyperenhancement at CEUS should not prompt a different diagnostic work up, the use of CEUS in the diagnostic setting has a limited role". CEUS in Sugimoto's and in our experience has a very different sensitivity and specificity than those reported by Forner et al.

Many reasons can explain this difference. Firstly, in the study period between 2003 and 2011, Forner et al. used as gold standard fine needle biopsy (FNB) for HCC diagnosis only for the first 7 years and then substituted FNB with eMRI for the successive 5 years as the gold standard for the diagnosis of HCC [17]. Hence, a significant difference exists in the methodology between ours, Sugimoto's and Forner's study. Moreover, in the period of HCC proven by biopsy, biopsy was performed mainly with a fine needle (21G cytologic needle) [17] and not with a 21 or 18G cutting needle as performed in Sugimotos's study [16] and our study, respectively.

There are recent international studies that disagree with the results reported by Forner et al. In a recent meta-analysis comparing CEUS and enhanced MRI for diagnosis of HCC
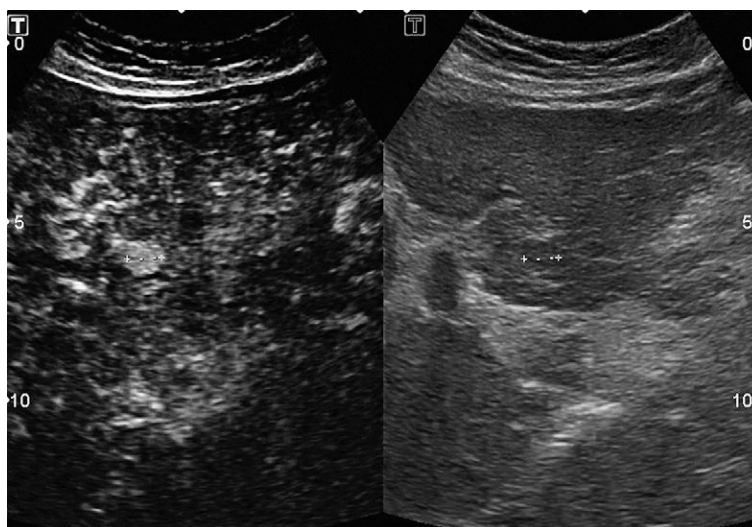

Fig. 1. Example of high capability of CEUS in demonstrating arterial hypervascularity of small (< 2 cm) HCC nodules. On the right: conventional US shows a small, new, hypoechoic nodule (9.6 mm) [calipers]. On the left: CEUS arterial phase: an hyperechoic, hypervascular nodule [calipers] is clearly seen at 7 sec. after 2.4 ml of Sono-Vue injection.

[18], including only patients diagnosed by percutaneous biopsy [13, 19], CEUS showed a statistically better specificity than MRI in the pair-wise comparisons (0.86 vs. 0.78; p = 0.014), and a statistically better sensitivity than computed tomography (CT) (0.88 vs. 0.78; p = 0.030) [19] .

In addition, in a very recent study, the specificity of CEUS + CT and/or MRI was significantly higher than the specificity of CT and/or MRI, CEUS, or intra-operative ultrasound (P = 0.004, P = 0.002, and P = 0.002, respectively). The diagnostic accuracy of CEUS + CT/MRI was higher than that of CT/MRI (P = 0.001) [20].

Again, a very recent report by Di Martino et al. [21] evaluating gadoxetic acid MR imaging in the characterization of the "Grey Zone of hepatocarcinogenesis" aiming at reporting radiological findings and diagnostic accuracy of gadoxetic acid MRI in the evaluation of small (≤2 cm) regenerative nodules, dysplastic nodules and well-differentiated HCC, found that "according to the AASLD radiological diagnosis the mean sensitivity, specificity and diagnostic accuracy in the diagnosis of HCC were, respectively,76.4%, 80%, 0.84 for regenerative nodules, dysplastic nodules and HCC" [21]

This last point is very important in the setting of the non invasive diagnosis of HCC, because both last AASLD and EASL guidelines for the management of HCC in cirrhosis [5, 7] indicate only eCT and eMRI for the noninvasive diagnosis of HCC, removing CEUS, formerly included from 2005 until July 2010 in the same guidelines.

CONCLUSION

CEUS has a high capability of demonstrating the main specific finding of HCC as arterial hypervascularity in HCC nodules ≤ 2 cm discovered during US surveillance.

Because CEUS can easily demonstrate the malignant profile of new small nodules arising in a cirrhotic liver in the majority of cases of HCC due to its arterial hypervascular pattern, CEUS should be included in the diagnostic management of HCC in order to avoid a late diagnosis, enable an early treatment and improve survival.

Conflicts of interest: All authors declare that they have no conflict of interest.

Authors' contribution: A.G. designed the research and drafted the manuscript. L.M., M.G.M., F.M. contributed to data analysis; P.G., F.A., C.C., P.M. and B.S. contributed to data acquisition; A.G. and V.G. contributed to data interpretation.

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