Evaluation of contrast-enhanced ultrasound for diagnosis of dysplastic nodules with a focus of hepatocellular carcinoma in liver cirrhosis patients

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Objective: To compare the enhancement features of dysplastic nodules with a focus of hepatocellular carcinoma (DN-HCC) versus HCC and regenerative nodules (RN) in cirrhotic patients.

Methods: One hundred and ninety-three cirrhotic patients were enrolled in this study; they had 215 focal liver lesions, 1.0-3.5 cm in size, which were examined using contrast-enhanced ultrasound (CEUS) with SonoVue® and diagnosed as HCC, RN or DN-HCC by biopsy. Samples were obtained using 18-gauge needles in the different enhanced areas. The enhancement features of DN-HCC, HCC and RN were evaluated.

Results: There were 86 HCC lesions, 102 RN lesions, and 27 DN-HCC lesions diagnosed by biopsy. Of 86 HCC lesions, 87.2% (75/86) showed complete enhancement during the arterial phase, and 12.8% (11/86) had inhomogeneous enhancement, with no enhancement in the central area during the arterial phase; 100% (86/86) exhibited washout during the late phase. Of 102 RN lesions, 95.1% (97/102) had delayed or simultaneous enhancement during the arterial phase, and 4.9% (5/102) displayed slight enhancement during the arterial phase; 26.5% (27/102) exhibited washout and 73.5% (75/102) exhibited no washout during the late phase. In 27 DN-HCC lesions, only part of the lesions enhanced during the arterial phase and washed out during the late phase; the other areas had delayed or simultaneous enhancement during the arterial phase, and 29.6% (8/27) exhibited slight washout in the late phase. In 86 HCCs, the pathological feature was HCC in the enhanced area of 75 lesions, hepatocellular fatty degeneration in the slightly enhanced area of 7 lesions, and hepatocellular necrosis in the unenhanced area and HCC in the enhanced area of 4 lesions. In 102 RNs, the pathological diagnosis was hepatocyte proliferation with or without fatty degeneration. In 27 DN-HCCs, the pathological feature was HCC in the enhanced area and hepatocyte regeneration in the unenhanced area.

Conclusions: CEUS is useful for the diagnosis of focal liver lesions in cirrhotic patients. CEUS can help determine the progression from RN to DN-HCC to HCC by analyzing the hemodynamics. CEUS can promote the diagnostic accuracy of a biopsy by providing more accurate information on the site of the biopsy.

Keywords: Ultrasound (US); contrast agent; hepatocellular carcinoma (HCC); regenerative nodules (RN)

Introduction

Since chronic liver disease is one of the most important factors in carcinogenesis, hepatocellular carcinoma (HCC) often occurs in association with liver cirrhosis (1). Some reports have proposed that the stepwise carcinogenesis of HCC progresses from regenerative nodules (RN), to low-grade dysplastic nodules (DNs) or high-grade DNs, to DNs with a focus of HCC (DN-HCC), and finally to HCC (2-4). To distinguish nodules in patients at high risk for HCC is very important for doctors who perform ultrasonography.

Because of the complicated liver texture, it is difficult to differentiate focal liver lesions within a cirrhotic background by conventional ultrasound (US) or even by color Doppler imaging (3,5-9). A contrast-enhanced US (CEUS) can define the microcirculation characteristics in liver nodules, so it is helpful for diagnosis of benign and malignant lesions. In recent years, some clinical research and applications of various kinds of contrast agents and US signal detection techniques have been focused on the evaluation of liver tumors, but very few studies have focused on the enhancement patterns of DN-HCC (10-14). In the present study, the characteristics of DN-HCC in cirrhotic livers were observed on CEUS by using SonoVue® (Bracco SpA, Milan, Italy) to explore the value of CEUS in the diagnosis of DN-HCC and the guidance of the biopsy.

Material and methods

Materials

During the time period from March 2004 to December 2013, 193 cirrhotic patients with 215 focal liver lesions, ranging in size from 1.0 to 3.5 cm on conventional US, were enrolled in our study. There were 141 males and 52 females, and their ages ranged from 32 to 77 years. All patients underwent biopsy immediately after CEUS. The biopsies were performed in the different areas of enhancement.

This study received institutional review board approval of Beijing Cancer Hospital, and patients gave informed consent.

Contrast agent and ultrasound (US) procedures

The US contrast agent, SonoVue, was administered intravenously as 1.5-2.4 mL boluses through the antecubital vein within 2-3 s, immediately followed by flushing with 5 mL 0.9% saline. CEUS was performed using the Technos DU8 US system (Esaote, Milan, Italy), GE LOGIQ E9 or 9 (Milwaukee, WI, USA) or Philips IU-22 (Bothell, WA, USA) with probe frequencies of 2.5 to 5.0 MHz.

The mechanical index (MI) was adjusted to about 0.06-0.10 based on the lesion depth. The perfusion and enhancement patterns of the target lesions were continuously observed throughout all phases of CEUS for about 4-6 min. To obtain all phases of the lesions, the probe remained fixed and respiration was controlled. If the first injection was not satisfactory or additional fields needed to be observed, a second contrast injection was given 10-15 min later. The CEUS diagnosis of the lesions was made based on the enhancement patterns during the arterial phase (0-30 s), the portal phase (31-120 s), and the late phase (after 120 s).

Imaging analysis

The surrounding liver parenchyma was used as a control. The enhancement of the lesion earlier than, later than, or equal to the surrounding parenchyma was defined as early enhancement, late enhancement, or equal enhancement, respectively. The washout was conducted similarly. Lesion enhancement greater than, less than, or equal to the surrounding parenchyma was defined as hyper-, hypo-, or iso-enhancement, respectively. If the lesion showed different degrees of enhancement, it was defined as heterogeneous enhancement. The enhancement patterns for all nodules were observed during the arterial phase (0-30 s), the portal phase (31-120 s), and the late phase (>120 s).

Diagnosis methods

All patients underwent biopsy immediately after CEUS. Usually the samples were obtained using 18-gauge needles (Bard, Crawley, UK) in the different enhanced areas. The shot length was set up to 1.5 or 2.2 cm depending on the nodule size. The number of puncture attempts was decided by the quantity of the specimen obtained. Repeat CEUS examinations were performed every 3-6 months for patients with negative biopsies. If a part of a nodule presented abnormal enhancement in the follow-up examination, a repeat biopsy was needed.

The pathological examination was performed by two pathologists with more than 20 years of experience. HCC and RN were diagnosed using standard diagnostic criteria of the International Working Party (1). DN-HCC was diagnosed as a focus of carcinoma in a DN.

Statistical analysis

The SPSS 10.0 software (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. The study data were analyzed with a Chi-squared test. The level of statistical significance was set at $P<0.05$.

Results

Characteristics of patients

The characteristics of patients are shown in *Table 1*. There

were 86 HCC lesions, 102 RN lesions, and 27 DN-HCC lesions diagnosed by biopsy. Patients with HCC, RN, and DN-HCC underwent two or three puncture attempts.

Enhancement patterns

The appearance of HCC, RN and DN-HCC on CEUS is shown in *Table 2* and *Figure 1*. There were statistically significant differences in the appearance of HCC, RN and DN-HCC on CEUS (P<0.001).

Of 86 HCC lesions, 87.2% (75/86) showed complete enhancement during the arterial phase or the portal phase, and 12.8% (11/86) had inhomogeneous enhancement, with no enhancement in the central area, during the arterial phase; 100% (86/86) exhibited washout during the late phase (*Figure 2*). Of 102 RN lesions, 95.1% (97/102) showed delayed or simultaneous enhancement during the arterial phase, and 4.9% (5/102) had only slight enhancement during the arterial phase; 26.5% (27/102) showed washout and 73.5% (75/102) showed no washout during the late phase (*Figure 3*). In 27 DN-HCC lesions, a

part of the lesions enhanced during the arterial phase and washed out during the late phase; the other areas exhibited delayed or simultaneous enhancement during the arterial phase and slight washout in the late phase (*Figures 4,5*).

In 86 HCC lesions, the pathological diagnosis was HCC in the enhanced area of 75 lesions, hepatocellular fatty degeneration in the slightly enhanced area of 7 lesions, and hepatocellular necrosis in the unenhanced area and HCC in the enhanced area of 4 lesions. In 102 RN lesions, the pathological diagnosis was hepatocyte proliferation with or without fatty degeneration. In 27 DN-HCC lesions, the pathological diagnosis was HCC in the enhanced area and hepatocyte proliferation in the unenhanced area.

Discussion

Early diagnosis of HCC is important because treatment

Table 1 Characteristics of HCC, RN and DN-HCC

Characteristics	HCC	RN	DN-HCC
No. of patients	86	80	27
No. of nodules	86	102	27
Mean age (year)	54.3	51.6	52.7
Gender			
Male	59	58	23
Female	27	22	4
Size of nodules (cm)	1.0-3.0	1.0-2.9	1.2-3.5

HCC, hepatocellular carcinoma; RN, regenerative nodules; DN-HCC, dysplastic nodules with a focus of HCC.
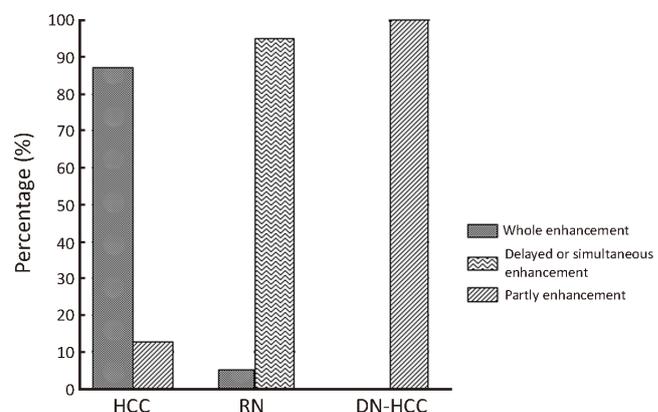
Figure 1 The appearances of HCC, RN and DN-HCC on CEUS in arterial/portal phase. HCC, hepatocellular carcinoma; RN, regenerative nodules; DN-HCC, dysplastic nodules with a focus of HCC; CEUS, contrast-enhanced ultrasound.

Table 2 Appearances of HCC, RN and DN-HCC on CEUS

Pathology	No. of lesions	Arterial/portal phase enhancement			Late phase wash out		
		Partly*	Whole	Simultaneous or delayed	Partly**	Whole	No.
HCC	86	11	75	0	0	86	0
RN	102	0	5	97	0	27	75
DN-HCC	27	27	0	0	27	0	0

*, there were statistically significant differences in the appearances of arterial/portal phase enhancement among HCC, RN and DN-HCC on CEUS (χ^2=326.624, P<0.001); **, there were statistically significant differences in the appearances of late phase wash out among HCC, RN and DN-HCC on CEUS (χ^2=335.315, P<0.001). HCC, hepatocellular carcinoma; RN, regenerative nodules; DN-HCC, dysplastic nodules with a focus of HCC; CEUS, contrast-enhanced ultrasound.
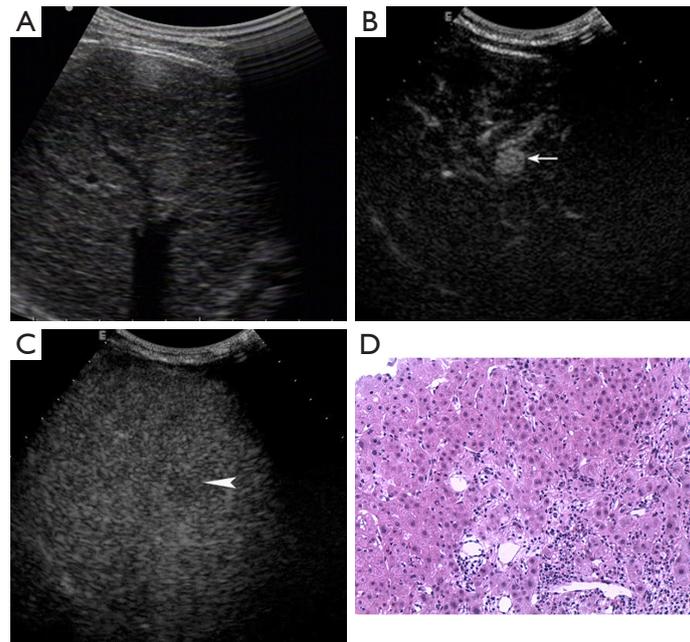
Figure 2 A 50-year-old man with hepatitis B related cirrhosis for 11 years. (A) US shows a small isoechoic nodule with surrounding dark ring in right lobe; (B) CEUS shows enhancement in arterial phase (←); (C) the lesion washes out as hypoechoic in late phase (Δ); (D) the pathology shows well differentiated HCC (HE, 20×). US, ultrasound; CEUS, contrast-enhanced ultrasound; HCC, hepatocellular carcinoma; HE, hematoxylin-eosin.
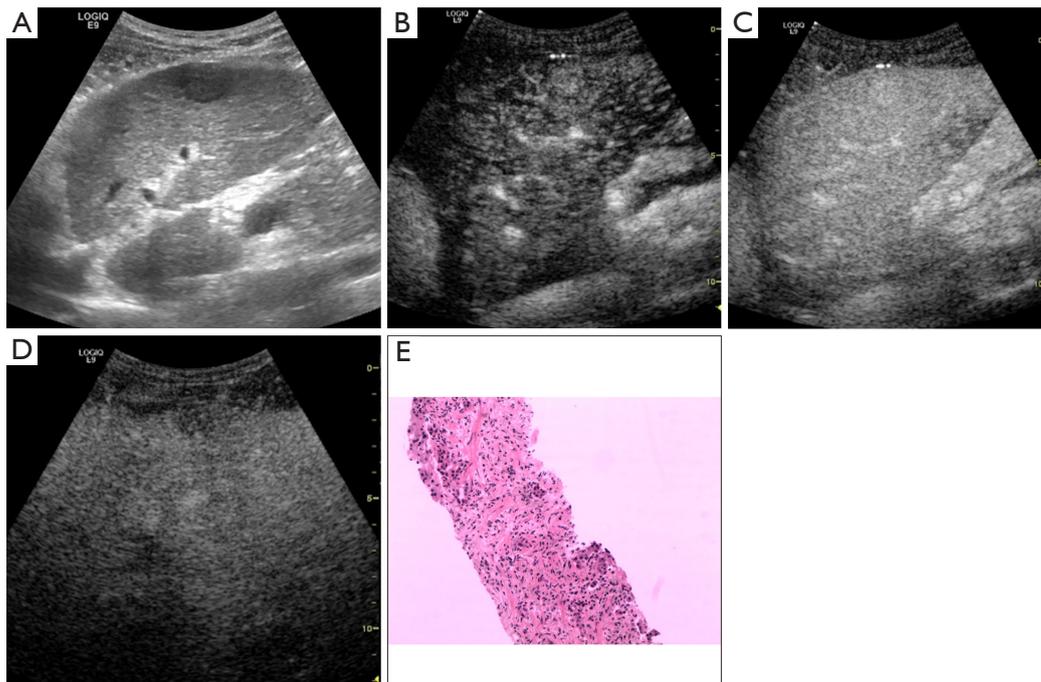
Figure 3 A 42-year-old man with hepatitis B for 14 years. (A) US shows a small hypoechoic nodule in the right lobe; (B) CEUS shows slight enhancement in the arterial phase; (C) the lesion shows iso-enhancement during the portal phase; (D) the lesion washes out as hypoechoic in late phase; (E) the pathology shows regenerative hepatocytes and fibrous tissue hyperplasia (HE, 4×). US, ultrasound; CEUS, contrast-enhanced ultrasound; HE, hematoxylin-eosin.
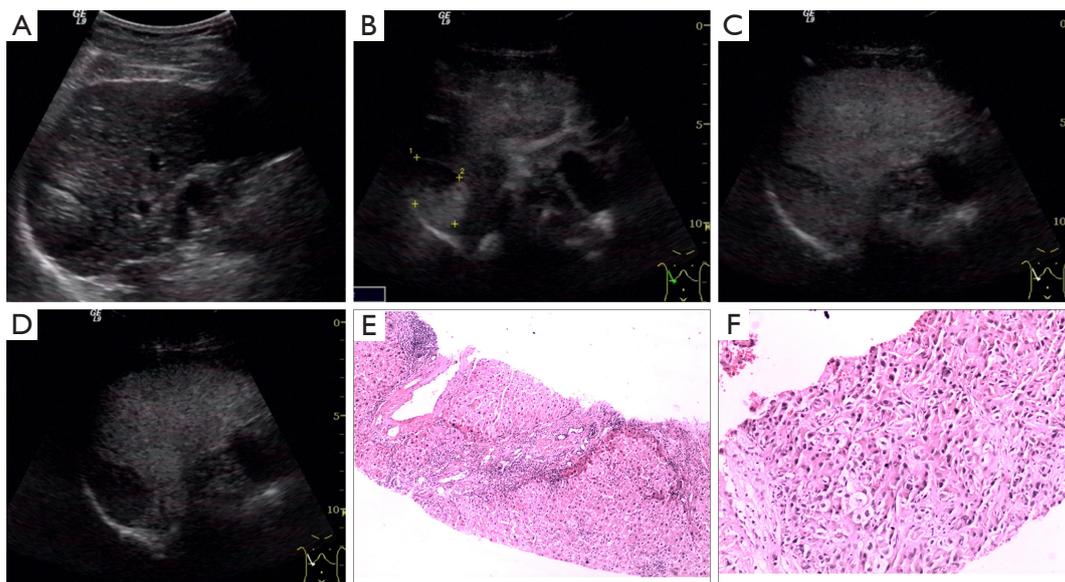
Figure 4 A 65-year-old man with hepatitis B for 20 years. (A) US shows a hyperechoic nodule with the size of 3.5 cm × 2.5 cm; (B) the deep part of the nodule presents fast enhancement in arterial phase, and the anterior part shows hypoechoic enhancement; (C) the anterior part enhances slightly, and the deep part of the nodule is simultaneous to the liver in portal phase; (D) the lesion washes out as hypoechoic in late phase; (E) biopsy at un-enhanced area in arterial phase is regenerative hepatocytes (HE, 4×); (F) biopsy at the enhanced area in arterial phase is well differentiated HCC (HE, 10×). US, ultrasound; HE, hematoxylin-eosin.
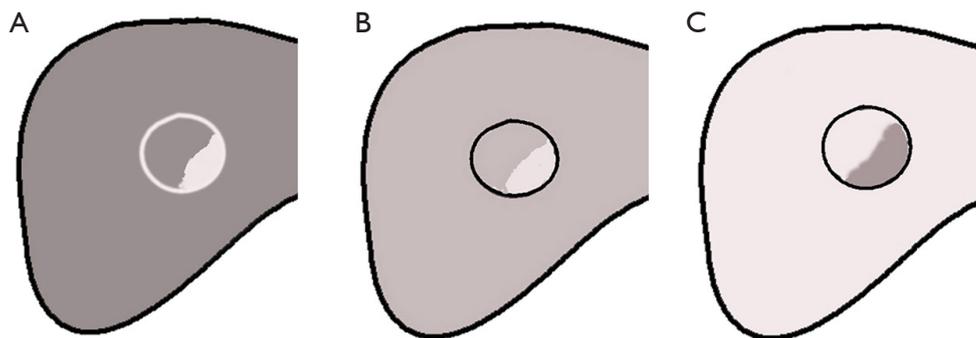
Figure 5 The enhancement patterns of DN-HCC on CEUS. (A) Part of the lesions shows hyper-enhancement during the arterial phase, and the other areas shows simultaneous enhancement during the arterial phase; (B) part of the lesions shows hyper- or iso-enhancement during the portal phase, and the other areas shows iso-enhancement during the portal phase; (C) part of the lesions washes out during the late phase, and the other areas shows iso-enhancement during the late phase. DN-HCC, dysplastic nodules with a focus of HCC; CEUS, contrast-enhanced ultrasound.

is most effective when the tumor is small (2,15,16). DNs may develop into carcinoma (4). Early detection of DNs with small areas of HCC is helpful for effective treatment. US is usually used for following hepatic cirrhosis, and it has sensitivity for small nodules with hepatic cirrhosis. However, it is of limited utility for differentiating benign from malignant lesions. For HCC, a stepwise process of

carcinogenesis has been proposed, involving changes in the blood supply and perfusion of the nodules (2,17-19). The characteristics of the blood supply in hepatic nodules are the pathophysiological basis of early detection, diagnosis and prognosis of lesions. SonoVue, a blood-pool marker used in CEUS, is helpful for defining the characteristics of the blood supply in hepatic nodules, which play an important

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role in carcinogenesis and HCC tumor growth. Thus, CEUS is helpful for the early diagnosis of RN and small HCC in a background of hepatic cirrhosis with multiple nodules (10-13). However, very few studies have focused on the enhancement patterns of DN-HCC in cirrhotic patients.

In this study, we focused on the perfusion and echo-change of DN-HCC and examined the value of CEUS in the diagnosis of DN-HCC and biopsy guidance compared with HCC and RN.

Diagnosis and differential diagnosis of DN-HCC in CEUS

The intranodular blood supply of RN is similar to the surrounding parenchyma, within which there is no obviously abnormal blood supply. However, the intranodular portal venous supply gradually decreases, whereas the intranodular arterial supply first decreases and then increases in accordance with an increase in the grade of malignancy of the hepatocellular nodule (1,17-19). As most studies have described, no enhancement during the arterial phase and isoechoic staining in the portal and late phases were detected in RN with CEUS, which was histologically in accordance with no obviously abnormal blood supply in RN (20,21). Recently, many studies have shown that CEUS was helpful for the detection of HCC and the differential diagnosis with RN. In the arterial phase, a hypervascular HCC nodule exhibited enhancement while RN had no enhancement (22-24). Moreover, in the late phase, most HCC nodules exhibited washout while RN was usually similar to the surrounding hepatic parenchyma. Thus, RN or HCC could be diagnosed correctly depending on different enhancement patterns (5,25). DN-HCC nodules are composed of two different cells, high grade differential HCC and atypical hepatic cells. Therefore, different manifestations were found in the same nodule on CEUS. In this study, a part of the 27 lesions of DN-HCC enhanced during the arterial or the portal phase and washed out during the late phase, which was similar to HCC; the other areas exhibited delayed or simultaneous enhancement during the arterial phase and simultaneous or earlier washout during the late phase, which was similar to RN. Therefore, the nodule in a cirrhotic liver might be DN-HCC if it is partly like HCC and partly like RN.

CEUS before biopsy improves diagnostic accuracy

The information yielded by CEUS performed before biopsy procedures using conventional US guidance can significantly decrease the false-negative rate for malignant lesions. CEUS can be used to localize the site for biopsy more accurately by differentiating areas of viable tumor from denaturalization or necrosis, which reduces the number of puncture attempts and significantly increases the success rate of biopsy. In our study, the pathological diagnosis was HCC in the enhanced area and hepatocyte regeneration in the unenhanced area. A biopsy of DN-HCC might give false-negative results without CEUS guidance, which might affect the early detection and treatment of tumors.

Conclusions

It is important to distinguish among RN, HCC and DN-HCC in cirrhosis with small intrahepatic lesions. CEUS can be used to localize the site for biopsy more accurately by differentiating areas of DN-HCC from HCC or RN. CEUS may be used as a routine surveillance tool for cirrhosis patients with small nodules based on its simple performance, minor side effects, and high diagnostic specificity.

Acknowledgements

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