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Case Report

Discrepancy of contrast-enhanced ultrasonographic pattern with two contrast agents in steatohepatic subtype hepatocellular carcinoma: A case report ☆☆☆★

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ABSTRACT

Contrast-enhanced ultrasound (CEUS) is one of the important imaging modalities for diagnosis of hepatocellular carcinoma (HCC). Sonovue and Sonazoid are the third-generation of ultrasound contrast agents that have been commercialized and widely used in clinical applications. This study introduces the imaging differences between these two agents in vascular phases for the first time. A 54-year-old man clinical suspected liver cancer. He had chronic hepatitis B for more than 20 years. The result of alpha-fetoprotein was 36.45 μg/L (normal < 20 μg/L). The imaging pattern of CEUS with Sonovue was “fast-in and fast-out” performance, while the pattern of “fast-out” was absent after portal phase with Sonazoid, even in Kupffer phase. The lesion was diagnosed as lipid-rich HCC by contrast-enhanced MRI. After liver resection, pathology revealed that it was hepatocellular carcinoma contained poor-differentiated steatohepatitis subtype and moderate-differentiated microtrabecular subtype. The imaging difference mainly existed in the part of steatohepatitis subtype. Steatohepatitis subtype HCC can be showed as “fast-in and no wash-out” characteristic in Sonazoid CEUS. Though the mechanism remains not fully clarified, this different enhanc-

Abbreviations: HCC, hepatocellular carcinoma; CEUS, Contrast-enhanced ultrasound; UCA, ultrasound contrast agents; SH-HCC, Steatohepatic hepatocellular carcinoma.

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ing pattern may provide a potential for the supplement of the guidelines and differential of steatohepatitis subtype HCC.

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Introduction

Hepatocellular carcinoma (HCC) is the most common type of liver cancer, accounting for 75%-85% of liver malignant tumor [1]. The clinical diagnosis of HCC mainly depends on serum biomarkers alpha-fetoprotein and contrast-enhanced imaging, including contrast-enhanced ultrasound (CEUS), computed tomography and magnetic resonance imaging. As a highly portable, time-saving and inexpensive modality, CEUS has an accuracy close to that of contrast-enhanced MRI in the diagnosis of HCC, especially in small HCC [2]. Currently, CEUS is recommended as a first-line modality for HCC diagnosis in China and Japan [3],[4].

The third-generation ultrasound contrast agents (UCA) have been developed and widely used in the diagnosis of various organs, and its main representative products are Sonovue (Bracco, Italy) and Sonazoid (GE, USA). Both of UCA consist of fluorocarbon gases coated with phospholipid membrane, Sonovue consists of sulfur hexafluoride, while Sonazoid consists of perfluorobutane. In the liver, Sonazoid can be phagocytosed by Kupffer cells [5,6], which means that Sonazoid has a post-vascular phase (10 minutes after injection) that Sonovue does not have. Therefore, it is generally believed that the imaging differences between Sonovue and Sonazoid are concentrated in the Kupffer phase, and it is not obvious in vascular phase [7,8].

To our knowledge, our case is the first to report differences between Sonovue and Sonazoid imaging, especially in the vascular phase. Though this occasion is uncommon, we think it is of great significant, not only for warning of the misdiagnosis of liver cancer, but also for the possibility of providing a new idea to explain the mechanism of UCA.

Case presentation

A 54-year-old man was referred to our hospital for a physical examination revealed a liver lesion. He did not have any symptoms. The patient said he had chronic hepatitis B for more than 20 years without standard treatment. The patient had abstained from smoking and drinking for 3 years. A physical examination found no abnormalities. Blood test results were almost normal except that alpha-fetoprotein was 36.45 μ g/L (normal < 20 μ g/L), CA19-9 89.89U/ml (normal < 37 U/ml), and hepatitis B surface antigen, core antigen and E antigen positive.

CEUS with Sonovue and Sonazoid were firstly performed using a Sequoia ultrasound system (Siemens, Germany) with a C6-1 convex array transducer at 3.5-5.0Mz. The mechanical index (MI) was 0.08 in Sonovue, and 0.19 in Sonazoid. UCAs were configured according to the instructions, and the injection

dose of Sonovue was 1.2 mL, and that of Sonazoid was 0.6 ml. After injection of UCA, 5ml saline was used to flush the catheter. The time interval between CEUS with Sonovue and Sonazoid was 2 hours. CEUS with both of two UCAs showed rapid hyperenhancement in arterial phase. CEUS with Sonovue showed mild wash-out in late-portal and delayed phases, while CEUS with Sonazoid showed wash-out peripherally and persistent hyperenhancement centrally in delayed and Kupffer phases (Fig. 1). There was a significant discrepancy between CEUS imaging with two UCAs. No adverse events occurred during the process of CEUS.

After CEUS, liver contrast-enhanced MRI was performed. In and out-phase sequence of abdominal MRI revealed a central portion of the lesion that was rich in lipids, and the lesion was diagnosed as lipid-rich HCC by contrast-enhanced MRI (Fig. 2).

Finally, the patient was clinical diagnosed with HCC and underwent liver resection. After operation, the tumor was staged as T1bN0M0. The gross pathological examination revealed that the cut surface of the lesion was tan-yellow peripherally and yellow centrally with focal areas of hemorrhage (Fig. 3). HE staining showed moderate-differentiated microtrabecular subtype HCC peripherally and poor-differentiated steatohepatitic subtype HCC (SH-HCC) centrally. Immunohistochemistry showed expression of CD68 was slightly lower in the peripheral part of the lesion than that in the central part (Fig. 4).

Combined with pathology and CEUS, microtrabecular subtype HCC showed typical enhancement patterns when using both UCAs. Steatohepatitis subtype HCC showed a typical enhancement pattern on Sonovue, but did not develop “wash-out” after portal phase with Sonazoid.

The patient was in good physical condition after surgery, and MRI examination two months later showed no tumor recurrence or intrahepatic metastasis. The patient was very satisfied with the whole process of treatment.

Discussion and Conclusions

We report a case of hepatocellular carcinoma containing steatohepatitic subtype with significant differences of enhancement pattern between using Sonovue and Sonazoid in the vascular phase and Kupffer phase. Although this phenomenon is uncommon, its occurrence may have an important clinical value.

The diagnostic performance of CEUS was reported similar to MRI in the diagnosis of HCC [9]. In addition, CEUS had a high resolution for microcirculation observation in focal lesion [10]. Therefore, CEUS was conducive to the diagnosis of focal liver lesion and was recommended in several guidelines. As the most common liver cancer, HCC typically presents with rapid hyperenhancement in arterial phase and mild wash-out

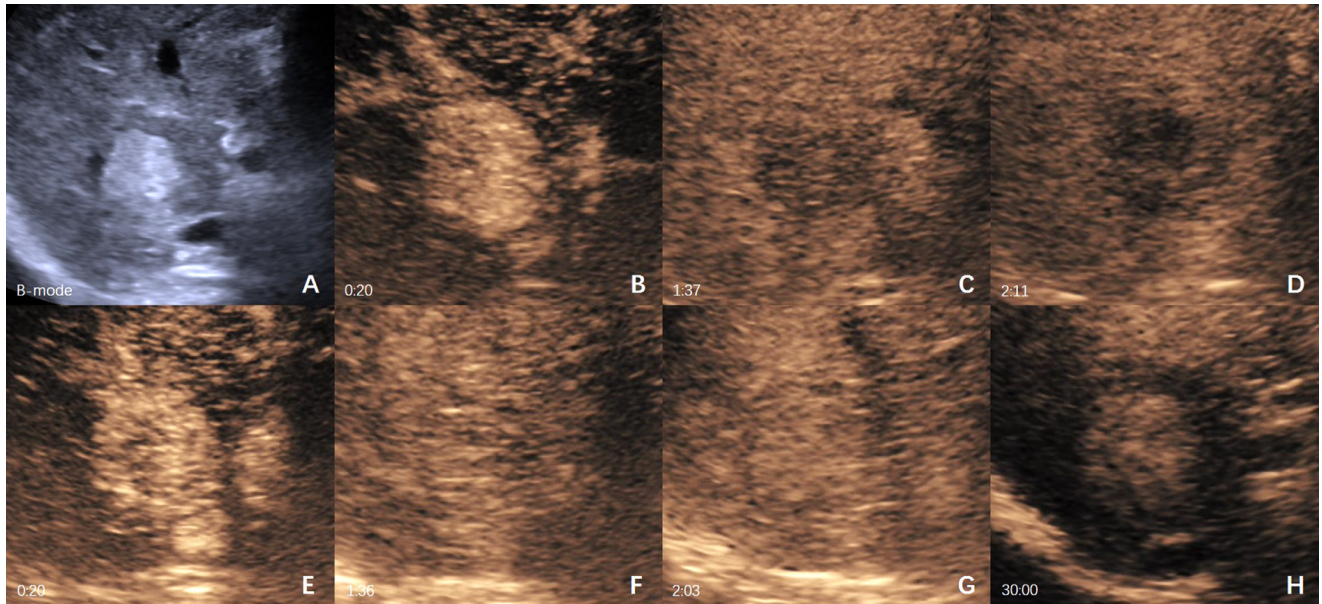


Fig. 1 – Ultrasound and CEUS features. A. B-mode ultrasound showed hyperecho in the center and isoecho in the periphery; B. & E. Hyperenhancement lesion was showed in CEUS with Sonovue and Sonazoid in arterial phase (20s); C. There was slight wash-out at the center of the lesion in the portal phase (97s) in CEUS with Sonovue; D. The lesions continued wash-out during the delay phase (131s) in CEUS with Sonovue; F. Isoenhancement of the lesion was showed in the portal phase (96s) in CEUS with Sonazoid; G. There was slight wash-out of the peripheral lesion in the delay phase (123s) in CEUS with Sonazoid; H. The center of the lesion did not show wash-out, while significant hypoenhancement was showed in the periphery in Kupffer phase (30min) in CEUS with Sonazoid. CEUS: contrast-enhanced ultrasound.

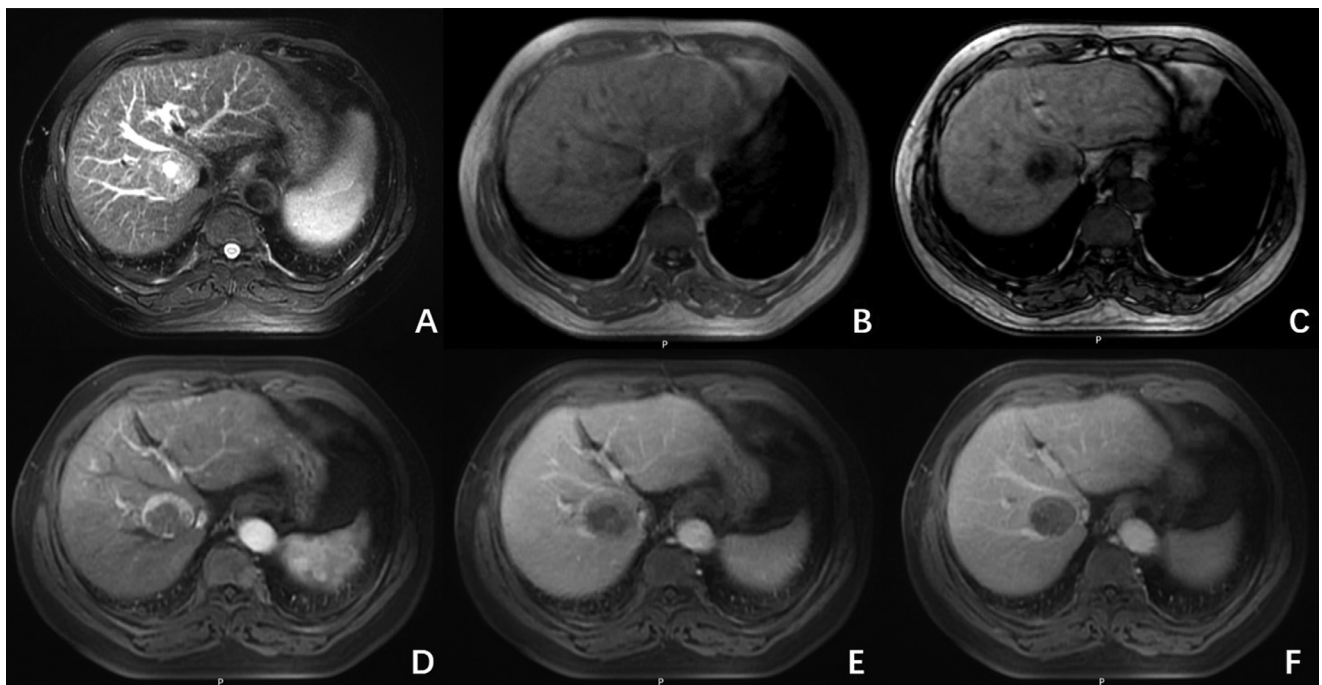


Fig. 2 – Contrast-enhanced liver MRI features. A. The T2-weighted sequence demonstrates a hypersignal lesion between the middle and right hepatic veins; B. & C. The signal of out-phase sequence is lower than that of in-phase sequence, indicating that the lesion was rich in lipid; D. Heterogeneous hyperenhancement of the lesion was shown in the arterial phase in contrast-enhanced MRI; E. wash-out can be seen inside the lesion in portal vein phase; F. Peripheral hypersignal was shown in delay phase.

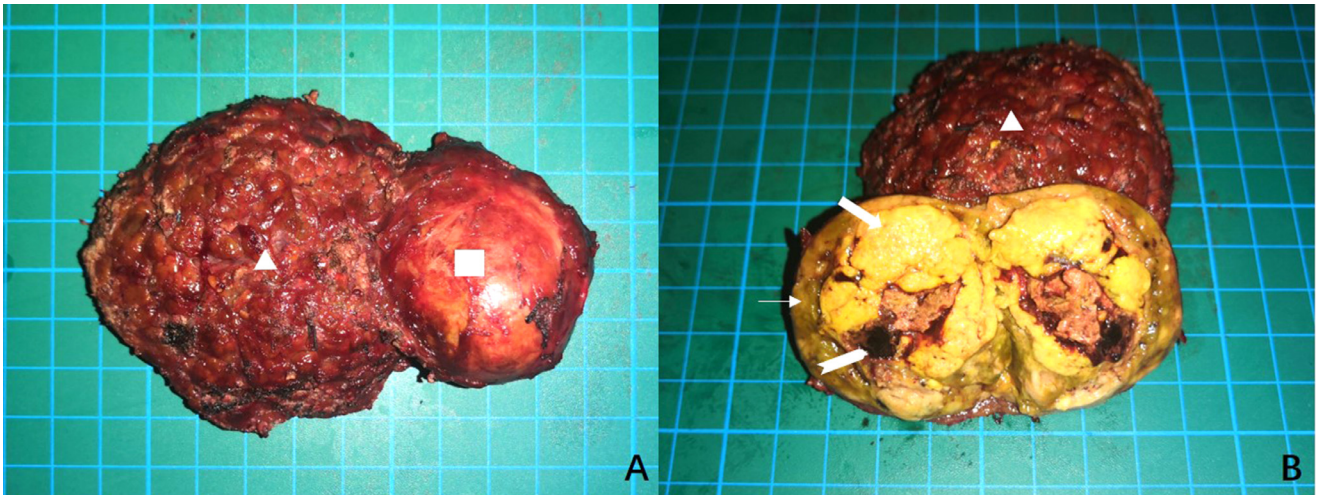


Fig. 3 – Gross pathology manifestation. A. whole resected tissue, tumor (rectangular) and peripheral liver (triangular); **B.** The gross specimen of the tumor cut open, including peripheral tan-yellow (thin arrow), central yellow (thick arrow, and hemorrhage area (swallowtail arrow).

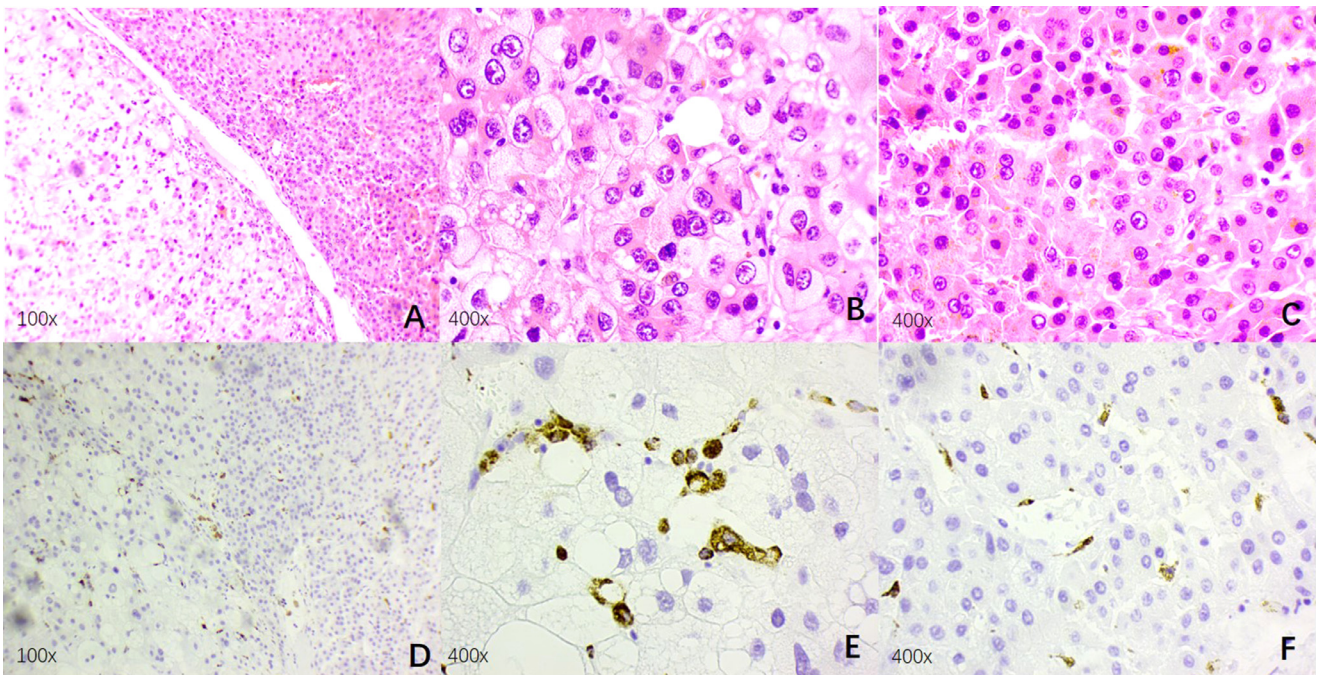


Fig. 4 – Pathology result. A. hematoxylin-eosin staining showed the lesion was HCC with two regions, the central (triangle) region and peripheral region (rectangular) of the lesion (100x magnification); **B.** The central region of the lesion was moderate-differentiated steatohepatic subtype HCC (400x magnification); **C.** The peripheral region of the lesion was poor-differentiated microtrabecular subtype HCC (100x magnification); **D.** Immunohistochemical staining of CD68 (mononuclear macrophage system) showed the distribution of Kupffer cells in the central (triangle) and peripheral regions (rectangular) of the lesion (100x magnification); **E.** & **F.** The number of Kupffer cells in the central region of the lesion was slightly larger than that in the peripheral region (400x magnification). HCC: hepatocellular carcinoma.

in late-portal phase (after 60s) and hypo-/non-enhancement in delay phase (after 120s) in CEUS imaging [11]. In this case of SH-HCC, performance of CEUS with Sonovue was basically consistent with the above typical characteristics, while CEUS with Sonazoid did not show overall wash-out after one minute, nor did in the Kupffer phase. This different Sonazoid

enhancing pattern may provide a possible condition for the supplement of the guidelines.

SH-HCC is a morphological variation of hepatocellular carcinoma, accounting for 13.5%-35.5% of HCC [12]. It is characterized by steatosis, swelling of tumor cells, inflammation, Mallory-Denk bodies, and intercellular fibrosis [13]. The study

of Matthew M. showed that SH-HCC can occur not in the background of fatty liver or metabolic syndrome, it is more likely to be caused by genetic alterations in shared genes or metabolic pathways within tumors [14]. SH-HCC often presents as a hyperechoic lesion on B-mode ultrasound and decrease in the MR out-phase signal due to the presence of a large amount of lipids. Multi-sequence MRI imaging makes it possible to identify SH-HCC, whereas conventional CEUS cannot do it. Now, using of two UCAs and their different enhancing pattern may provide a potential for differential of SH-HCC.

Sonovue and Sonazoid, 2 UCAs have been permitted in China, mainly show difference in the Kupffer phase. The current theory is that Sonazoid can be phagocytosed by the mononuclear macrophage system (Kupffer cells in the liver stained with CD68) and enhancing for a relatively long time. Kang compared the diagnostic performance of CEUS between 2 agents and concluded that Sonazoid had higher diagnostic performance than Sonovue [15]. Another article reported by He compared 2 contrast agents in focal nodular hyperplasia, and revealed Sonazoid may have a better performance at depicting central scar [8]. In this case with a pathologic diagnosis of HCC, there was a significant difference in both vascular phase and Kupffer phase. Therefore, differences in CD68 expression did not totally explain this. According to the present condition of this patient, firstly, we speculate that this may partly be related to the mechanical index. SonoVue used low-MI setting (0.08) and in contrast Sonazoid used medium-MI setting (0.19). As the MI increase, tissue harmonic signals also increase, leading to the difference on washout. However, the difference of MI also can not totally explain the phenomenon. Noritaka had reported a case of hypoechoic HCC containing lipid also showed similar "no-washout" manifestations in Sonazoid CEUS [16]. Secondly, we speculate that this may be related to physical properties of UCA. Sonazoid has a stable lipid shell that is negatively-charged and mimics liposomes on the surface of cell membranes, while Sonovue has a neutral lipid shell that is polyethylene glycol [17]. In addition, steatosis in SH-HCC leads to a substantial inhibition of Ca²⁺ influx into cells through a PKC-dependent mechanism [18,19]. Therefore, in SH-HCC, extracellular Ca²⁺ accumulation making the tissue positively-charged and may attract the negatively-charged agents. While, electrically neutral Sonovue may not attracted. This conjecture has not been fully confirmed by rigorously designed experiments, but it may be a promising hypothesized mechanism.

In this paper, we only reported one case, a large sample study was needed to prove the objectivity of this phenomenon. However, according to this phenomenon, doctors using Sonazoid alone should be aware of the potential pit-fall of SH-HCC, and this different Sonazoid enhancing pattern may provide a potential for the supplement of the guidelines and differential of SH-HCC.

Availability of data and materials

The data can be requested from the corresponding author on reasonable request.

Ethics approval and consent to participate

The patient in this case report was included in our prospective study which was approved by the ethics committee of the Chinese PLA general hospital (approval NO. S2021-270-01). The patient has signed informed consent to perform all procedures and publish articles using his medical records.

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