

The Role of Sonazoid-contrast-enhanced Ultrasound In Precision Diagnosis and Guidance for Radiofrequency Ablation Therapy of Hepatocellular Carcinoma: a Literature Review

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Abstract: Hepatocellular carcinoma (HCC), constituting 75-85% of primary liver cancer cases, ranks as the third leading cause of global cancer-related mortality. The early diagnosis of HCC is critical for determining optimal clinical therapeutic strategies. Radiofrequency ablation (RFA) has emerged as a preferred for managing early-stage HCC cases, primarily due to its less invasive nature, simplicity and safety. Advanced imaging for pre-operative evaluation of HCC has been increasingly used. Contrast-enhanced ultrasound (CEUS) has improved the clinical application of ultrasound (US) in HCC. It also provide more accurate informations for guiding RFA procedures. In the future, CEUS, through its convergence with emerging artificial intelligence (AI)-driven technologies, will play an even greater role in HCC management. This systematic review evaluates the utility of Sonazoid-enhanced contrast ultrasound (Sonazoid-CEUS), particularly its unique Kupffer phase imaging, in improving HCC diagnostic and optimizing RFA precision via real-time monitoring capabilities and prolonged imaging windows (up to 60 minutes).

Key words: Hepatocellular carcinoma; Kupffer cell; Sonazoid-enhanced contrast ultrasound; Radiofrequency ablation

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Primary liver cancer is the sixth most common cancer globally and the third leading cause of cancer-related mortality worldwide, with Hepatocellular carcinoma (HCC) accounting for 75-85% of cases [1-2]. HCC is typified by rapid disease progression, adverse prognosis, and high mortality rates, with early diagnosis is crucial for therapeutic strategies. Advances in diagnostic and therapeutic strategies have enabled the

development of various non-surgical treatment options for HCC, including radiofrequency ablation (RFA), transarterial chemoembolization (TACE), and percutaneous ethanol injection therapy [3-4]. Among those, RFA has emerged as a preferred for managing early-stage HCC cases, due to its simplicity and safety [5].

According to the guidelines, RFA is recommended for patients with highly elderly status, comorbid condi-

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tions, severe cirrhosis, and tumors located deep within the hepatic parenchyma [6]. Furthermore, randomized clinical trials have demonstrated that, for small HCC cases, the overall treatment efficacy of RFA is comparable to that of surgical resection [7–9]. However, the success of RFA relies on accurate lesion targeting, using imaging guidance to locate the tumor, assess its size and number, determine the optimal needle angle, plan the ablation zone, and evaluate treatment effectiveness [10].

Advanced imaging modalities, such as conventional ultrasound (US) techniques, echo-induced nanobubble US (ceRNA-based US), three-dimensional (3D) US, computed tomography (CT), contrast-enhanced computed tomography (CECT), and three-dimensional computed tomography (3D CT), have been developed to enhance the accuracy and effectiveness of RFA procedures [11–14]. Magnetic resonance imaging (MRI) has been established as robust platform for provide more accurate informations of guiding RFA procedures [15]. In contrast, contrast-enhanced ultrasound (CEUS) imaging offers advantages over MRI, including real-time imaging capabilities, suitability for serial examinations, greater accessibility and more affordability.

CEUS operates by utilizing microbubbles of contrast agents to enhance echo signals, thereby enabling visualization of blood perfusion within abnormal tissues [16]. The Sonazoid-enhanced contrast US (Sonazoid-CEUS) system exhibits a distinctive advantage through its long-term imaging endurance and robust stability, providing comprehensive diagnostic insights [17]. Evidence also indicates that CEUS can enhance the success rates of tumor ablation by providing real-time guidance for therapeutic interventions [18]. This systematic review seeks to evaluate the role of Sonazoid-CEUS, particularly its Kupffer phase, in guiding RFA procedures and assessing its diagnostic utility in patients with HCC.

Part 1 Application of Sonazoid-CEUS in the Diagnosis of HCC

Contrast agent of Sonazoid-CEUS

Sonazoid (GE Healthcare, Oslo, Norway), a second-generation US contrast agent, comprises lipid-stabilized microbubbles encapsulating perfluorobutane gas [19]. Sonazoid's defining feature resides in hepatic Kupffer cell-mediated phagocytosis, enabling sustained parenchymal enhancement (60–120 minutes) through its specialized Kupffer phase imaging during the postvascular period [20]. Figure 1 demonstrates HCC time-intensity curve analysis via Sonazoid-CEUS, featuring Kupffer-phase imaging with prolonged contrast retention. Sonazoid-CEUS employs low mechanical index (MI) protocols to preserve microbubble integrity, facilitating reliable hemodynamic assessment of hepatic lesions during vascular phase imaging. This modality further enables comprehensive Kupffer-phase hepatic evaluation while augmenting the identification of HCC-pathognomonic perfusion defects.

Compared Sonazoid-CEUS with conventional B-mode US

Conventional US remains the primary imaging modality in hepatic lesion detection. However, this modality exhibits inherent limitations in detecting small and isoechoic lesions, particularly those localized in deep hepatic parenchyma or anatomically challenging regions. Sonazoid-CEUS demonstrates unique diagnostic capabilities in HCC detection across multiple clinical scenarios, particularly in surveillance protocols [21]. Kudo et al. [22] found that Sonazoid-CEUS provided an additional yield of 16 HCCs versus B-mode US in 292 patients. A prospective randomized controlled trial revealed that Sonazoid-CEUS detected smaller hepatic

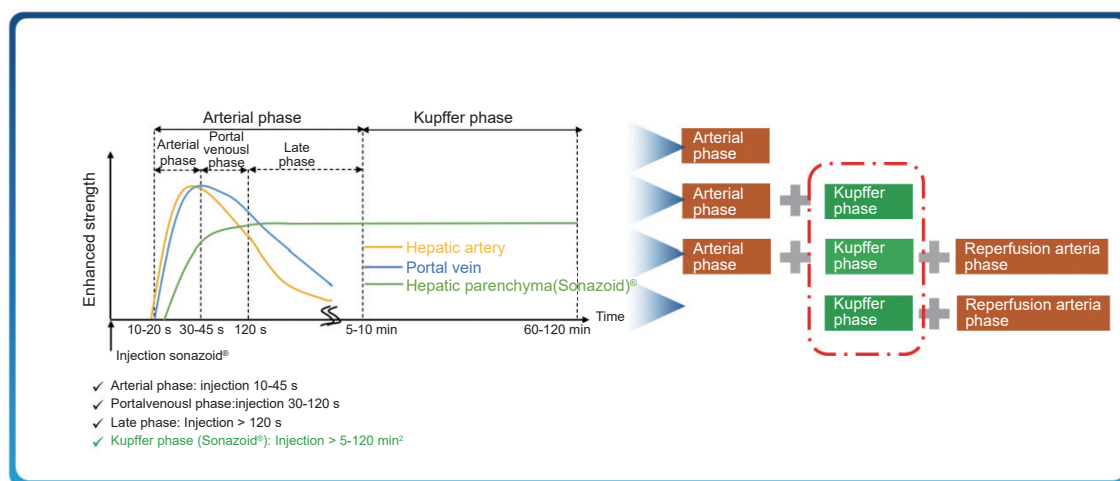


Figure 1 HCC time-intensity curve analysis via Sonazoid-CEUS, featuring Kupffer-phase imaging with prolonged contrast retention

lesions than conventional B-mode US [23]. Goto et al. [24] revealed that Sonazoid-CEUS demonstrated significantly higher sensitivity and specificity compared to conventional B-mode US, with values of 0.982 and 0.978, respectively, compared to 0.892 and 0.949, respectively.

Previous research demonstrated that while Sonazoid-CEUS did not significantly enhance diagnostic sensitivity for early-stage HCC, it substantially decreased false-positive referrals in surveillance populations [25]. This diagnostic challenge stems from cirrhotic parenchyma harboring regenerative/dysplastic nodules (DNs) that frequently mimic HCC characteristics on conventional B-mode US, consequently elevating false-positive probabilities [26]. Sonazoid-CEUS offers a notable diagnostic advantage by reducing false-positive rates. Figure 2 shows a case in which a lesion was misdiagnosed on conventional B-mode US but was correctly identified as early-stage HCC by Sonazoid-CEUS.

Compared Sonazoid-CEUS with SonoVue-CEUS

The two approved hepatic imaging modalities, SonoVue-CEUS and Sonazoid-CEUS, facilitate comprehensive HCC characterization by detailing not only tumor vascularity but also hemodynamic patterns [27,28]. A comparison of physical characteristics between Sonazoid-CEUS and SonoVue-CEUS, as demonstrated in table 1. SonoVue is used exclusively as an intravascular contrast agent. Bokor et al. [29] conducted a comparative safety evaluation of SonoVue and

demonstrates the comparable clinical safety profiles of SonoVue contrast agent in both healthy individuals and Chronic obstructive pulmonary disease (COPD) populations. Another studies on Sonazoid-CEUS demonstrated no clinically significant alterations in blood biochemistry, confirming its favorable safety profile with absence of adverse reactions [30,31]. Notably, caution is advised for patients with egg allergy due to the presence of hydrogenated phosphatidylserine in the lipid encapsulation components.

Comparative analyses of Sonazoid versus SonoVue diagnostic efficacy yield conflicting evidence, the results has not been unified [32-35]. However, Huang et al. [34] demonstrated comparable HCC detection accuracy between Sonazoid-CEUS and SonoVue-CEUS among high-risk patients. The Kupffer phase did not make a significant improvement in diagnostic efficacy. This disparity likely stems from differential washout kinetics between contrast agents, with Sonazoid-CEUS demonstrating prolonged clearance (101 s) versus SonoVue-CEUS (84 s), potentially enhancing late-phase HCC pattern recognition.

Comparison of Sonazoid-CEUS with MRI or CT

The detection of HCC has traditionally relied on cross-sectional imaging modalities, primarily CT and MRI, which serve as the cornerstone for diagnosis and staging [36-38]. Recent studies have shown excellent diagnostic concordance (96-100%) between Sonazoid-CEUS Kupffer phase imaging and superparamagnetic

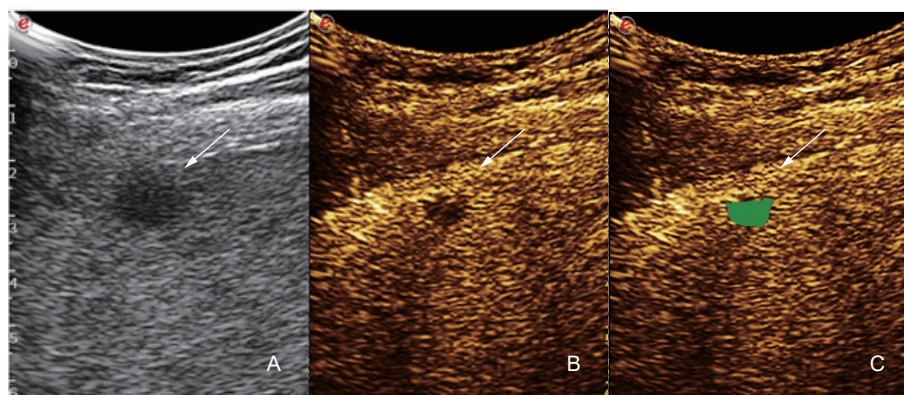


Figure 2 Case 1. Sonazoid-enhanced US images were obtained from a 48-year-old male patient diagnosed with chronic viral hepatitis B. B-mode US showed a 16×11 mm vaguely defined hypoechoic lesion (A, white arrow), while with Sonazoid-enhanced a part of the lesion showed hyper-enhancement (B, white arrow) and the rest lesion presented hypo-enhancement (C, green area) during the Kupffer phase. Its diagnosis was early HCC (High grade dysplastic nodules with a part of HCC). Finally, the lesion was confirmed to be early-stage HCC by contrast-enhanced MRI.

Table 1 Comparison of physical characteristics between Sonazoid-CEUS and SonoVue-CEUS.

Characteristics	Sonazoid	SonoVue
Gas	Perfluorobutane	Sulfur hexafluoride
Diameter	2 μ m	2.5 μ m
Shell	Sodium hydrolecithinylserine	Distearoyl hosphatidylcholine
Characteristic	Transpulmonary circulation blood vessel + organ specific imaging	Transpulmonary circulation angiography

iron oxide-enhanced MRI (SPIO-MRI) [39,40]. Additionally, using Sonazoid-CEUS offers the advantages of being more straightforward to perform and at a lower cost compared to SPIO-MRI. In addition, Numata et al. [41] evaluated 108 hypervascular HCC nodules that were initially identified on contrast-enhanced CT. Among these, conventional US detected 90 nodules (83%), while Sonazoid-CEUS identified an additional 15 nodules (14%) during the Kupffer phase. According to another study, Sonazoid-CEUS in the Kupffer phase offered greater sensitivity (95.4% vs. 85.2%) and accuracy (94.7% vs. 82.3%) than contrast-enhanced CT in the detection of HCC [36]. Moreover, Sonazoid-CEUS leverages its unique respiratory elimination pathway. It is thus a safe diagnostic alternative for patients with renal impairment, who are unsuitable for contrast-enhanced CT that relies on renal excretion.

The diagnostic value of the Kupffer phase in Sonazoid-CEUS for HCC

Kupffer cells, which are specialized macrophages residing in the liver, account for roughly 30% of all sinusoidal cells [42]. Kupffer phase imaging reflects the density of Kupffer cells, with HCCs typically exhibiting low intra-tumoral Kupffer cell density due to changes in the tumor's vascular supply [43]. The case in figure 3 demonstrates that HCC exhibits an enhancement deficiency in the Kupffer phase. Previous study has shown that approximately 87-96% of HCCs exhibit hypo-enhancement during the Kupffer phase [44]. Multiple

studies have investigated the optimal application of Kupffer phase imaging for HCC diagnosis [33,28]. Beyond tumor detection, Kupffer phase imaging is also used for evaluating histological grading in HCC [38,45-46]. A study by Korenaga et al. [40] established a Kupffer phase ratio cutoff value of 0.6 for differentiating well-differentiated HCC from other HCC types, achieving both high sensitivity (0.917) and specificity (0.920).

With the advancement of artificial intelligence (AI) models, AI-based approaches have become increasingly prevalent in detection and diagnosis of HCC. CEUS images offer critical data in the development of AI models. Recent studies have established that Kupffer phase imaging with Sonazoid-CEUS possesses significant predictive value for the differentiation of HCC [47-49]. Kondo et al. [48] shown that a pretrained support vector machine (SVM), when applied to CEUS images, achieved accuracies, sensitivities, and specificities of 90.4%, 93.6%, and 86.9%, respectively, in differentiating between benign and malignant liver cancers. The Kupffer phase is essential in Sonazoid-CEUS for distinguishing HCC from other liver lesions, as diagnostic specificity is significantly reduced without it. A recent study by Zhen et al. [50] exhibited that a combined model, utilizing radiomic features from both tumor and peritumoral regions in the Kupffer phase of Sonazoid-enhanced US, showed promising diagnostic performance in distinguishing HCC from atypical benign focal liver lesions (FLLs).

On the other hand, AI models have demonstrated sig-

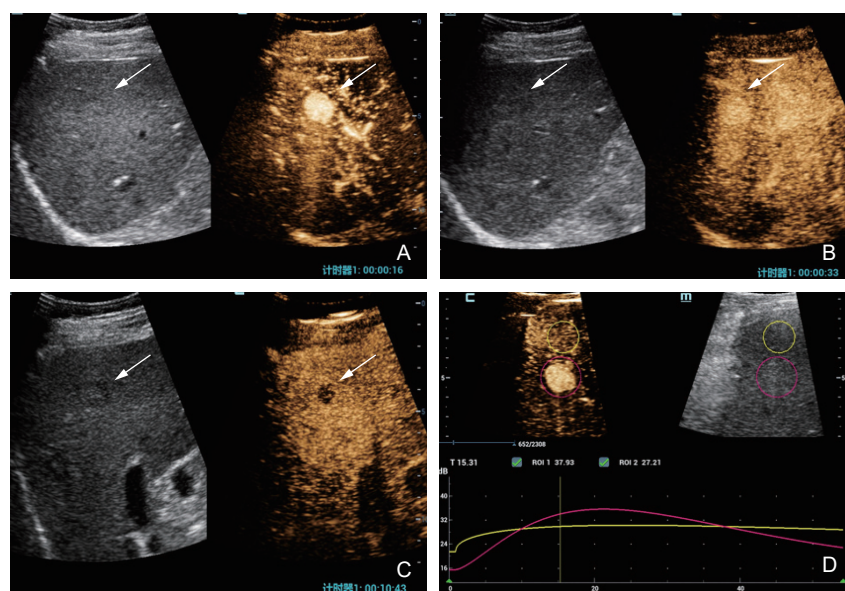


Figure 3 Case 2. Sonazoid-enhanced US images were obtained from a 32-year-old male patient diagnosed with chronic viral hepatitis B. The lesion demonstrated nonrim arterial phase hyperenhancement (APHE) (A, white arrow) and early washout (B, white arrow) in the portal venous phase and manifested an enhancement defect in the subsequent Kupffer phase (C, white arrow). Time-intensity Curve Analysis of HCC using Sonazoid-CEUS (D, yellow line presents HCC after reperfusion Sonazoid-enhanced, red line presents Hepatic parenchyma after reperfusion Sonazoid-enhanced). The lesion confirmed HCC by contrast-enhanced MRI.

nificant potential in the management of HCC, particularly in predicting microvascular invasion (MVI) and evaluating pathological grading. The research by Zuo et al. [51] established that a Kupffer phase Sonazoid-CEUS radiomics model provides a robust and reliable means to preoperatively predict MVI in HCC lesions, with an area under the receiver-operating-characteristics curve (AUC) of 0.834. Recent studies have developed deep learning models using Kupffer phase images to predict MVI and prognostic outcomes in HCC patients, achieving an AUC ranging from 0.865 to 0.895 [52–53].

To maximize the diagnostic utility of imaging data acquired during the Kupffer phase, Sugimoto et al. [54] developed a modified CEUS liver imaging reporting and data system (CEUS LI-RADS) for Sonazoid, incorporating Kupffer phase defects instead of late and mild washout. Multiple studies involving high-risk patients have demonstrated that the revised CEUS LI-RADS protocol offers high sensitivity for HCC diagnosis, ranging from 67.6% to 89.6% [28,55–57].

Part 2 Application of Sonazoid-CEUS in the Therapy of RFA for HCC

Percutaneous ablation is widely recognized as a gold-standard treatment for hepatic malignancies and has been incorporated into both national and international clinical guidelines [58]. RFA for HCC, guided by Son-

azoid-CEUS, represents a minimally invasive and safe alternative for treatment, owing to its rapid imaging capabilities, low contrast agent volume, and minimal risk of parenchymal damage to the liver and kidneys [59]. Additionally, reducing treatment duration significantly contributes to improved patient tolerance, prolonged ablation time, decreased complication rates, and enhanced therapeutic efficacy. Furthermore, these improvements collectively enhance the overall effectiveness and safety of the ablation process [60].

The unique features of Sonazoid-CEUS during the Kupffer phase, incorporating real-time dynamic imaging, high sensitivity, and quantitative analytical capabilities, demonstrate its effectiveness as a modality for guiding RFA in HCC treatment. Its applications can be divided into three stages:

Preoperative stage

Sonazoid-CEUS enables Kupffer-phase imaging, which is marked by the "Black hole sign". This phenomenon is commonly observed in HCC, primarily due to reduced or absent intratumoral Kupffer cell density, which is characterized by the presence of contrast defects during the Kupffer phase. High-resolution imaging techniques and precise delineation of lesion boundaries are essential prerequisites for RFA, ensuring reliable identification of focal hepatic lesions. As shown in figure 4, the HCC exhibited hypo-enhancement during

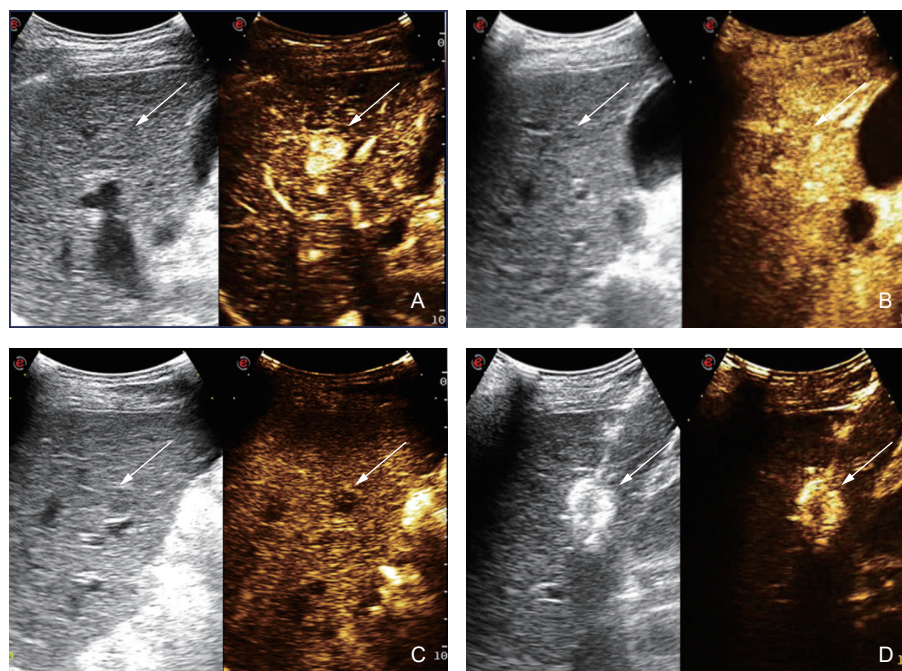


Figure 4 Case 3. Sonazoid-enhanced US images were obtained from a 67-year-old male patient diagnosed with chronic viral hepatitis B. On Sonazoid-enhanced US, the lesion demonstrated to have APHE (A, white arrow) and mild hyperenhancement in one minute (B, white arrow), while manifested hypo-enhancement from 10 minutes in the Kupffer phase (C, white arrow). The lesion was under RFA therapy in the Kupffer phase (D, white arrow). The lesion was ultimately confirmed to be HCC by contrast-enhanced MRI.

the Kupffer phase, which is beneficial for RFA. Park et al. [61] evaluated the utility of real-time Kupffer-phase imaging for guiding RFA in patients with HCC. Kupffer-phase imaging significantly improves liver lesion visibility over conventional B-mode US and offers valuable guidance for real-time RFA. What's more, Moriyasu et al. [62] exhibited that Kupffer-phase imaging exhibits high sensitivity and specificity in detecting focal liver lesions, particularly in small HCC. It changed the treatment strategy for 6.8% of patients.

Intropeative stage

Sonazoid-CEUS exhibits a distinctive Kupffer-phase imaging feature characterized by an ultra-long contrast agent display [63]. High-phase contrast images are generated with sustained stability for several hours, enabling comprehensive liver imaging [64]. Sonazoid-CEUS enables real-time procedural guidance during RFA, allowing clinicians to systematically optimize access routes, precisely evaluate needle placement, electrode positioning, and thermal lesion formation through sustained contrast visualization. Previous research found that Kupffer phase enhances lesion conspicuity while maintaining sustained visibility throughout the procedure [61]. Recent study has shown that the integration of contrast agents with intraoperative US has significantly modified the surgical approach in approximately 25% of patients, and facilitating real-time assessment of ablation completeness [65].

Postoperative stage

Sonazoid-CEUS demonstrates a reperfusion pattern during the Kupffer phase. Defect reperfusion imaging is

an advanced method to assess the presence of arterial blood flow within HCC that exhibits perfusion defects. A positive defect reinjection sign may suggest the presence of a residual nodule or recurrence. Furthermore, Kupffer phase is useful for evaluating the effectiveness of RFA therapy. This approach enables a more accurate assessment of the ablative margin, consequently lowering the probability of local tumor recurrence. Inoue et al. [14] found that the post-ablation site is easily recognizable as a perfusion defect on Kupffer phase imaging. If residual tumor tissue is visualized within the ablation zone during the Kupffer phase, defect reperfusion imaging enables precise delineation of the safety margins. Just as exhibited in figure 5, the HCC lesion underwent RFA. However, six months later, additional recurrence was identified. A study by Nishigaki et al. [66] shown that Sonazoid-CEUS performed three hours after the RFA procedure and achieved a successful assessment of treatment outcomes in most patients, with a significantly reduced risk of local tumor recurrence. Additionally, prolonged follow-up assessments achieved an overall local recurrence rate of 2.3% after CEUS at five years post-treatment, with a five-year cumulative survival rate of 58.4%.

Part 3 Challenges and Future Perspectives

Sonazoid-CEUS, particularly its Kupffer phase, is highly effective for diagnosing HCC, determining optimal RFA surgical decision, and enhancing personal intra- or post-operative management.

Recent advancements in CEUS technology, particularly in 3D imaging and virtual navigation, are anticipat-

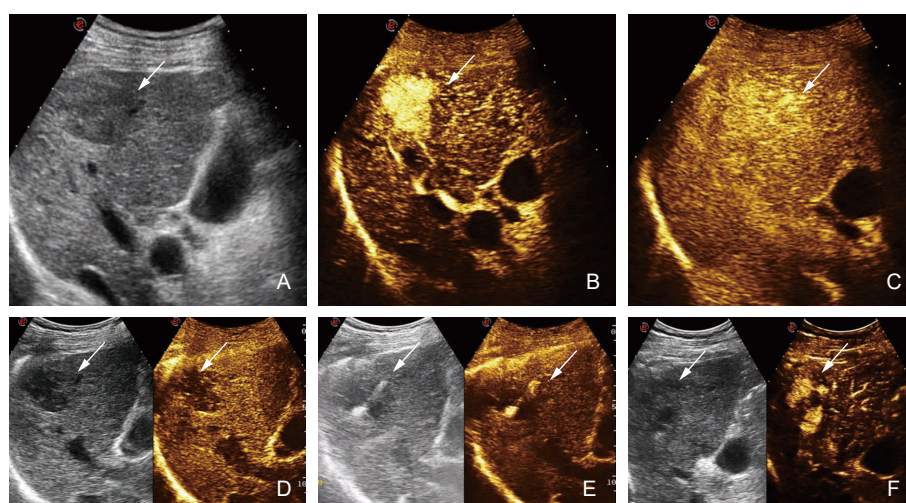


Figure 5 Case 4. Sonazoid-enhanced US images were obtained from a 61-year-old male patient diagnosed with chronic viral hepatitis B. B-mode US image showed a 39mm hypoecho liver lesion (A, white arrow) in hepatic segment VII, the lesion manifested hyper-enhancement during the arterial phase (B, white arrow) and showed subsequent iso-enhancement (26 seconds after contrast agent administration) and was no wash-out in the late phase to 5 minutes on CEUS (C, white arrow), then demonstrated an enhancement defect in the subsequent Kupffer phase (D, white arrow). The lesion was under RFA (E, white arrow). After six months the recurrence besides the lesion could be diagnosed (F, white triangle). The lesion was confirmed to be HCC by contrast-enhanced MRI.

ed to further enhance the refinement of RFA techniques for the treatment of HCC. Parallel advancements in the integration of AI with clinical and imaging data demonstrate significant potential for the development of predictive models. These models could accurately predict recurrence and metastasis of HCC, thereby offering robust, evidence-based recommendations for choosing between ablation and surgical treatments. Future research should prioritize standardizing Sonazoid - CEUS protocols, validating its application through larger multicenter studies, and exploring its integration with emerging technologies.

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Authors' Contributions

RS and HN conceived of and designed the study; TL drafted the manuscript; DW and HW performed case design and analysis; YZ, SL and YG performed the research. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

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Data Availability

Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

Declarations

Ethics approval and consent to participate

The patients/participants provided their written informed consent to participate in this study.

Conflict of interest

The authors have no conflict of interest to declare.

Consent for publication

Not applicable.

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