Review

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Contrast-enhanced ultrasound demonstrates greater adjuvant potential: past, current status, and future applications in hepatocellular carcinoma early diagnosis

Nana Li, Fang Nie, Yao Wang, Wenjing Pang, Wenzhu Wang, Yunpeng Zhao, Yingying Jia

Ultrasound Medical Center, Lanzhou University Second Hospital, Lanzhou, China

Abstract

Hepatocellular carcinoma (HCC) has an atypical onset of clinical symptoms and a rapid tumor progression. The majority of patients with HCC are already in the late stages of the disease when they are diagnosed, limiting them to the best available treatments. Contrast-enhanced ultrasound (CEUS) has achieved significant advances in the diagnosis of HCC, including the detection of small lesions, the investigation of more beneficial contrast agents, and the use of CEUS-based radiomics. The purpose of this review is to discuss the relevant research and future challenges of CEUS in the early detection of HCC, to advise more accurate therapy.

Keywords: Contrast-enhanced ultrasound; hepatocellular carcinoma; early diagnosis; radiomics

Introduction

Primary liver cancer (PLC) is the most common malignancy in global cancer. Although the prevalence of PLC has fallen by around 2% annually in recent years, it still ranks fifth among malignant tumors worldwide in terms of mortality rate, with a 5-year survival rate of about 20% [1]. Hepatocellular carcinoma (HCC) accounts for 90% of PLC, and its etiology varies greatly in the general population, while HBV infection is the most common cause of HCC [2]. Nonalcoholic steatohepatitis has been reported to be the fastest-growing cause of HCC in developed countries and is expected to continue to increase over the following ten years [3]. HCC has an insidious onset and most patients are unaware of when to begin treatment because of the lack of specific findings in the early HCC stages.

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Corresponding author: Fang Nie
Ultrasound Medical Center,
Lanzhou University Second Hospital,
Lanzhou, 730000, China
E-mail: ery_nief@lzu.edu.cn

The previous clinical gold standard for the diagnosis of HCC is based on tissue biopsy because molecular identification can also be performed while identifying the pathological subtype. However, this invasive examination carries possible risks for patients because of its inevitable flaws. Thus, diagnosis based on noninvasive criteria is now widely available worldwide [4,5].

Contrast-enhanced ultrasound (CEUS) is recommended as a promising method for the noninvasive diagnosis of HCC because it is well-established for unique microperfusion imaging and is repeatable in focal liver lesions. It also offers major advantages in screening surveillance and diagnosis of HCC. The use of CEUS in the diagnosis of liver diseases has expanded recently and is no longer restricted to a single imaging modality. The CEUS LI-RADS classification is a useful supplement to the monitoring of high-risk HCC patients. Previous studies have confirmed that some new algorithms and criteria based on CEUS LI-RADS improve the specificity and accuracy of detecting HCC [6,7]. In addition, imaging of novel ultrasound contrast agents in the Kupffer phase (KP) also greatly improves the sensitivity of detecting tumors [8]. However, inevitable deficiencies in CEUS are also important factors affecting the diagnosis, and the future goal lies in improvement rather than complete elimination.

In this review, we will discuss the imaging features of CEUS in the diagnosis of HCC, including the use of combined relevant biomarkers, novel contrast agents, and the advantages and potential CEUS exerts in radiomics, as well as new challenges faced in the future.

Diagnosis and differential diagnosis of CEUS in HCC

Background

HCC development goes through a long process. There are two sets of blood supply systems in the liver, respectively the hepatic artery and portal vein blood supply. When accompanied by cirrhosis or hepatitis, HCC undergoes a multistage morphological evolution from regenerative nodule through dysplastic nodule to HCC. However, during this multi-stage evolution, the blood supply of nodules changes, resulting in a decrease in normal portal blood flow and an increase in the arterial blood flow of newly formed tumor arteries [9,10]. The mechanisms involved in this alteration are complex and are generally thought to manifest as alterations in inflammation-related pathways and modulation of immune cells [11-13].

After conventional ultrasound (CUS) and Doppler ultrasound (DUS), contrast-enhanced ultrasound (CEUS) is the third ultrasound revolution technology that is currently widely used in clinical practice. Based on the CUS, ultrasound microbubble contrast agents are applied to enhance tissue and organ visualization by displaying blood perfusion in real time [14,15]. At present, the most used contrast agents are second-generation acoustic contrast agents, including Sonazoid (SZ; perflubutane) and SonoVue (SV; sulfur hexafluoride), which can be injected intravenously as a bolus to achieve specific imaging through pulmonary circulation vessels and organs. Compared to contrast-enhanced CT and contrast-enhanced MRI, CEUS has the advantages of real-time dynamic imaging, repeatable procedures, and allowing preference in patients with poor renal function.

CEUS performance in HCC

CEUS technology is widely used in focal liver lesions, which makes clear imaging of atypical focal liver lesions that are difficult to detect by CUS (but also nodules that are not clear by CT and MRI), and higher stages of characterization and detection of malignant tumors [16]. Surprisingly, CEUS has also made great achievements in pathological correlation analysis as well as a local assessment after tumor percutaneous ablation and transarterial chemoembolization [17-19]. The European Federation of Societies for Ultrasound in Medicine and Biology (EFSUMB) guidelines recommend that the CEUS process be divided into three phases, including the arterial phase, the portal venous phase, and the delayed phase. These phases are defined as beginning 20 seconds after peripheral intravenous injection and lasting until 30-45 seconds for the arterial phase, 31-120 seconds for the portal venous phase, and 121-360 seconds for the delayed phase, respectively [20] (fig 1). In the majority of cases, benign liver lesions detected by CEUS usually show no enhancement or iso-enhancement in all phases. On the contrary, if hypo-enhancement occurs, it is necessary to consider whether there is a risk of carcinogenesis or the possibility of a tumor. The typical CEUS enhancement pattern of HCC is arterial phase enhancement earlier than liver parenchyma, and portal venous or delayed phase washout faster than liver parenchyma (fig 2).

CEUS is recommended as one of the imaging surveillance tools for the diagnosis of HCC in the European Association for the Study of the Liver (EASL) guidelines [5]. According to earlier research, 96% of lesions in moderately differentiated HCC exhibited typical arterial phase hyperenhancement and portal venous phase washout, whereas well- and poorly-differentiated HCC exhibited atypical enhancement patterns [21]. It is crucial to pay attention to the washout time during the portal venous phase and delayed phase extension. Therefore, the high vascular expression of HCC in the arterial phase makes it present a characteristic feature of rapid enhancement in the arterial phase of CEUS, which has important clinical significance for the differential diagnosis of HCC. However, this global rapid enhancement feature is also manifested in hypervascular metastatic liver cancer, but whether hypervascular metastasis or hypovascular metastasis, such lesions usually typically exhibit a "black hole sign" on the liver background and even washout earlier in the arterial and portal venous phases [16,22, 23].

Previous studies have confirmed that the washout time of HCC in the portal venous phase and delayed phase is closely related to the degree of cancer cell differentiation, and the worse the tumor differentiation, the earlier

phase	start (s)	end (s)
arterial	10-20	30-45
portal venous (PV)	30-45	120
late	> 120	bubble disappearance (approx. 4 – 6 min)
Kupffer phase*	10min	-

Fig 1. Vascular phases in CEUS of the liver (*Kupffer phase is a unique post vascular phase for Sonaziod CEUS) [20, adapted].

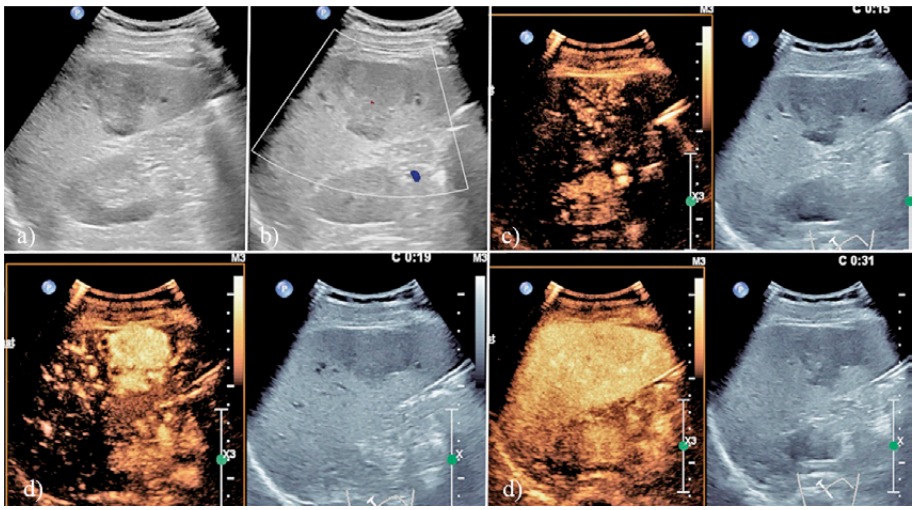

Fig 2. Hepatocellular carcinoma was pathologically confirmed in a 46-year-old man; a) the transverse sonogram showed a hypoechoic mass with irregular margins; b) color Doppler sonogram evidenced a small amount of blood flow around and inside the lesion; c) after ultrasound contrast agent injection, at 15s the lesion began to enhance; d) at 19s the lesion enhancement peaked; e) and after 31s the lesion began to wash.

the washout time in the portal venous phase and delayed phase. However, the early contrast agent washout time does not rule out the possibility of non-HCC [24,25]. Because of the overlapping nature of enhancement patterns and the nonspecific nature of clinical laboratory parameters, there are some difficulties in differentiating HCC from other non-HCCs, such as intrahepatic cholangiocarcinoma (ICC), which always enhances later than HCC on CEUS, flushes faster, or presents other enhancement patterns and features (peripheral ring enhancement, homogeneous/heterogeneous hyperenhancement) [26,27]. In conclusion, CEUS reflects the malignant degree and biological behavior of HCC to a certain extent in different phases, providing important information for guiding treatment and judging prognosis.

Potential for CEUS LI-RADS classification

One specificity of HCC diagnosis is that it can be diagnosed based on relevant imaging findings without a pathological diagnosis, so imaging is very important for accurate diagnosis and staging of HCC. To classify and assess the risk of intrahepatic lesions in patients at high risk for HCC, the CEUS-based liver imaging reporting and data system (CEUS LI-RADS) classification was proposed in 2016 and updated in 2017. CEUS LI-RADS aims to emphasize the PPV and specificity of diagnosing HCC rather than the sensitivity of diagnosis [28]. In the liver observations, it was determined that benign was classified as LR-1, possibly benign as LR-2, HCC moderately suspicious as LR-3, HCC severely suspicious as LR-4, and confirmed HCC was classified as LR-5. Liver lesions were classified in LR-5 if they showed APHE (not rim or peripheral discontinuous), late (≥60s), and mild washout [29] (fig 3). If the liver lesions are thought to be categorized in the LR-5 class, these patients can consider beginning HCC standardized clinical treatment without the requirement for an invasive biopsy because the LR-5 class has a very high specificity for HCC [30,31].

Arterial phase hyperenhancement(APHE)	No APHE		APHE (not rim[b],not peripheral discontinuous globular[c])	
Nodule size(mm)	< 20	≥20	< 10	≥10
No washout of any type	CEUS LR-3	CEUS LR-3	CEUS LR-3	CEUS LR-4
Late and mild washout	CEUS LR-3	CEUS LR-4	CEUS LR-4	CEUS LR-5

Fig 3. ACR CEUS LI-RADS v2017 Core diagnostic table [20, adapted].

If early washout time was adjusted to <45s, the specificity of diagnosing HCC significantly increased to 95.61%, and the incidence of HCC misdiagnosed as ICC decreased from 12.3% to 4.4% [6]. However, another study did not show similar results; that is, the modified LR-5 had increased sensitivity and decreased specificity for the diagnosis of HCC [32]. The reason for analyzing this difference was that the non-HCC tumors included in the LR-M category were different in the two studies due to the highly similar CEUS findings of HCC and ICC. In a validation on the diagnostic performance of CEUS LI-RADS in HBV-related large-scale populations, the specificity and PPV of LR-M were significantly increased in non-HCC malignancies and the accuracy of LR-5 in diagnosing HCC increased from 81% to 87% after reclassification of the LR-M nodular subgroup into LR-5 [7], indicating that we should not be limited to LR-5 alone when exploring the predictive potential of CEUS LI-RADS for HCC, while LR-M is also very important for improving the accuracy and specificity of HCC, which has also been demonstrated in other studies [33-35].

Of course, not only do LR-5 and LR-M nodules require great attention in imaging diagnosis and clinical treatment, but physicians and patients usually ignore LR-3 and LR-4 nodules because such nodules are not very malignant and aggressive. In other words, different patterns of CEUS liver lesions (LR-3 and LR-4) did not show superior clinical results and long-overall survival compared with LR-5 lesions [36]. Other arterial phase characteristics, such as ill-defined borders, disorganized vessels in the lesion, and circumferential arteries around the lesion, have also been reported in HCC in addition to APHE, and these additional features have significantly improved the specificity of CEUS LI-RADS for HCC diagnosis.

There are distinct differences in the biological behavior between HCC and non-HCC malignancies, leading to different optimal treatments and clinical prognoses. CEUS LI-RADS is the widest reference for HCC diagnosis, however, it does not entirely exclude non-HCC malignancies. By combining tumor markers and prior cancer history, it may be possible to reduce misdiagnosis to some extent and increase HCC specificity.

Correlation between CEUS and HCC tumor markers

It is undeniable that AFP has been considered the most important and widely used serum biomarker for the diagnosis of HCC. Previous studies have shown that when the AFP cut-off value is 20 ng/mL, the sensitivity for the diagnosis of HCC (147 studies) is 60% and the specificity is 84%, and the sensitivity of combined AFP and US for the diagnosis of HCC rises to 96%, while its specificity for the diagnosis alone is similar [37]. In using CEUS, which is more diagnostic for HCC, CEUS combined with AFP has higher diagnostic performance for HCC and facilitates differentiation from other PLCs [38,39]. AFP-L3 is a subtype of AFP that is mainly produced by liver tumor cells, and in the detection of HCC, the cut-off value most commonly used for AFP-L3 is currently 10%. High AFP-L3 serum values before HCC treatment may predict poor patient prognosis [40], and in a statistical model used to assess HCC, called the GALAD model, the investigators found that AFP-L3 did not act as an independent predictor [41], but the enhancement pattern of CEUS in HCC and high AFP-L3 may be negatively correlated with overall survival in HCC patients [42]. It is also a predictor of HCC recurrence. Also found to be highly expressed in the serum of HCC patients is des-gamma-carboxy prothrombin (DCP), also known as prothrombin induced by vitamin K deficiency or antagonist-II (PIVKA-II). Several studies have reported that DCP has better sensitivity and specificity than AFP, but the combination of the two can improve diagnostic efficacy [43,44].

A small number of studies have investigated the correlation between ultrasound or CUES and the expression of other tumor markers such as glypican-3, cytokeratin 19 (CK19), and epidermal growth factor receptor (EGFR), although current studies have affirmed that these parameters are associated with tumor progression and poor prognosis in HCC [45-47]. EGFR may be negatively correlated with microvessel density, peak time, and washout time exhibited by CEUS [48]. Quantitative parameters of CEUS reflect tumor differentiation to some extent, and during HCC dedifferentiation, reduced normal blood supply to the liver and tumor neovascularization leads to tumor proliferation, thereby decreasing the expression of tumor suppressors [49]. We conjecture that this association can also be reflected in the correlation between the expression of other tumor-related factors and CEUS, while future research directions continue to explore the potential of tumor microvessel density and angiogenesis, and the advantage of CEUS microperfusion can just be a strong adjunct.

A special ultrasound contrast agent

Sonazoid CEUS (SZ-CEUS), in contrast to Sonovue CEUS (SV-CEUS), offers a particular phase of imaging known as the post vascular phase. In hepatic CEUS imaging, KP imaging can be performed in the late vascular phase (10 min after contrast agent injection). In the nor-

mal liver, Sonazoid microbubbles can be phagocytosed and absorbed by Kupffer cells and show a significant enhancement effect, but hepatic malignancies have few Kupffer cells, resulting in significant contrast agent loss in the KP and the formation of KP defects. KP defects are defined as non-enhancing or significantly hypo-enhancing lesions relative to the liver [50,51], which further improves the diagnostic performance of SZ-CEUS for HCC.

In addition, in patients with rough liver parenchyma and unclear appearance on two-dimensional ultrasound, SZ-CEUS with KP can be used to detect small HCCs in surveillance, which lowers the rate of patients being incorrectly referred for CT/MRI [8,52]. Several researchers added SZ-CEUS to the LI-RADS classification and found that LR-5 utilizing modified criteria significantly increased the sensitivity of diagnosing HCC without reducing specificity [53,54]. During HCC evolution, Kupffer cell counts decrease with poor HCC differentiation, demonstrating that the KP are of great interest for differentiating solid tumors.

In a diagnosis of high-risk HCC patients using both SZ-CEUS and SV-CEUS, the frequency of mild and late washout (79%>53%) and sensitivity for the diagnosis of HCC (79%>54%) were higher in SZ-CEUS, while there was no significant difference in specificity (both 100%). This suggests that hypo-enhancement of liver nodules in the KP phase has a higher detection value for malignant tumors [55]. CUS may be difficult to differentiate a dysplastic nodule from early HCC, but hypo-enhancing vascularized nodules in the late vascular phase (KP) using SZ-CEUS may be useful in early HCC [56]. Compared to CUS, further combining HCC lesions in the KP with radiomics, showed better diagnostic performance and facilitated the identification of preoperative microvascular invasion in high-risk HCC patients [57].

CEUS-based radiomics

The diagnostic outcomes are generally subjective and may result in decreased sensitivity for detecting HCC, regardless of whether CUS or CEUS is being used. Radiomics obtains a large number of quantitative features for classification and prediction by high-throughput extraction and quantification of image materials to non-invasively and comprehensively assess tumor heterogeneity [58,59]. CT and MRI-based radiomics have shown good performance in lesion detection and classification, microvascular invasion, and prognosis prediction in HCC [60-62]. In recent years, with CEUS mode and selection optimization, the CEUS-based radiomics has also been gradually appreciated. Researchers have tried to optimize the detection and differentiation of HCC from hepatocellular cholangiocarcinoma using CEUS-based radiomics characteristics [63].

Traditional CUS-based radiomics models have good predictive value for differentiating benign from malignant liver tumors [64], but they do not well differentiate the liver malignancies. The accuracy of CEUS-based radiomics in the diagnosis of HCC and metastatic liver cancer reached more than 85% in the detection of focal liver lesions when compared with CUS-based radiomics [65,66], confirming that the combination of blood perfusion information extracted from CEUS and tumor biological characteristics is very beneficial for the detection of HCC. An advanced machine learning method based on convolutional neural networks combined in CUS and CEUS images achieved more than 97% accuracy for the automatic diagnosis of HCC. At the same time, noise and artifacts are unavoidable in CEUS image acquisition, and the application of wavelet denoising and multi-plane wave compounding methods can improve the imprecision and inaccuracy caused by the low signal-to-noise ratio [67,68].

To measure the comparability of CUS and CEUS for HCC and ICC presentation, a unique T-AFML technique, producing diagnostic metrics congruent with pathological results has already been published [69]. This method is expected to be applied to the differentiation of different histological types of HCC, but there are still some limitations in expanding clinical application and diagnostic performance. Additionally, the combination of CEUS and deep learning enables preoperative prediction of microvascular invasion and prognosis, as well as noninvasive application to the assessment of progression-free survival in local treatment of HCC patients, and they can promote optimized treatment options for patients with early HCC.

Fusion Imaging

Fusion imaging refers to CEUS combined with CT/MRI co-imaging, which uses special localization to realize the integration and reconstruction of images, realize the accurate position tracking of the ultrasonic transducer, facilitate real-time accurate localization of target lesions, and improve the sensitivity and specificity of diagnosis by displaying the lesion morphology and peripheral vascularization [70-72].

It has been reported that fusion imaging can accurately identify the underlying entity of focal liver lesions (FLLs) and improve the accuracy of their detection, even if the lesions were previously diagnosed as indeterminate FLLs on CT/MRI or were undetectable on the first CEUS

examination performed [71,73]. For some early HCC or atypical HCC, CEUS may present with short arterial phase enhancement time or insignificant portal venous and delayed washout, and CT/MRI multiphase scans are more helpful in the detection of lesions, especially in liver nodules less than 10 mm in diameter, nodules difficult to be detected by CUS. Fusion imaging increases the sensitivity of detecting these minimal lesions from 50.7% to 83.57%, while they have a great risk of developing malignant tumors [74,75]. Real-time imaging fusion of CEUS with dynamic CT/MRI has proved to be a safe and effective method for accurate localization of HCC. CEUS/MRI imaging fusion, which is currently commonly utilized, offers considerable utility in HCC assessment before radiofrequency ablation, achieving successful ablation by shortening time and exact localization.

Conclusion

The application of CEUS as a noninvasive imaging method in the qualitative diagnosis of HCC has shown some advantages. Of course, there are flaws, such as the possibility to be affected by respiration during ultrasound scanning and the low detection rate for deeply situated lesions, which should be modified rather than fully excluded in future studies. Our goal is dedicated to the improvement of CEUS technology and further deepening the study of HCC diagnosis, which is expected to be applied in the early diagnosis of HCC, thus exerting greater clinical value and helping patients to improve their prognosis.

Conflict of interest: none

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