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Contrast-Enhanced Ultrasound (CEUS) for the Diagnosis and Management of Hepatocellular Carcinoma: Current Status and Future Trends

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Abstract
Purpose of Review This review discusses the use of contrast-enhanced ultrasound (CEUS) for liver lesion detection and characterization focusing on hepatocellular carcinoma (HCC). It reviews the uses of CEUS in managing patients with HCC, such as in image-guided intervention, assessment of tumor viability after locoregional treatment, and differentiation of bland from tumor thrombus. Finally, it highlights potential future uses of microbubbles in targeted HCC detection and drug delivery.
Recent Findings The high temporal resolution of ultrasound relative to CT and MRI and its sensitivity to microbubbles enables precise analysis of enhancement profiles at miniscule contrast doses, enabling confident diagnosis of HCC and differentiation of HCC from other benign and malignant hepatic lesions.
Summary CEUS has gained widespread acceptance worldwide for the diagnosis and management of patients with HCC. CEUS is poised to make a large clinical impact in the USA given the recent Food and Drug Administration approval of a CEUS agent for use in liver imaging.

Keywords Hepatocellular carcinoma · HCC · Contrast-enhanced ultrasound · CEUS · Ultrasound · Microbubbles · Tumor thrombus · Contrast agents

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Introduction

Contrast-enhanced ultrasound (CEUS) was initially proposed in the early 1980s [1, 2]. Contemporary ultrasound agents are microbubble-based—these agents were first approved for cardiac imaging worldwide in the mid 1990s. Their use for non-cardiac imaging did not occur until the introduction of contrast-specific instrumentation in the late 1990s. Modern ultrasound devices are capable of producing microbubble-only images; when microbubbles are imaged with these instruments, a single microbubble can be detected in vivo. This revolution led to wide approval and use throughout Europe, Canada, and Asia over the next two decades. Unfortunately, CEUS of the liver has made little inroads into clinical practice in the USA during that same period, primarily because there was no Food and Drug Administration (FDA)-approved agent for liver imaging, and CEUS was not reimbursed until recently. During this same period, CT and MR grew to become the dominant imaging tools despite their relatively limited access, higher cost, and greater risk of contrast reactions. However, the recent approval of a microbubble agent for liver imaging, in combination with the increasing number of patients at risk for hepatocellular carcinoma (HCC) in the USA requiring evaluation and management, the emphasis on cost containment, and changes in practice patterns and reimbursement, all work in favor of increasing utilization of CEUS [3].

CEUS for liver imaging, particularly for lesion detection, characterization, and management, has emerged as one of the dominant indications worldwide. Along with providing a general introduction to the use of CEUS, this article will highlight recent advances in its use for the diagnosis of hepatocellular masses and the differentiation of lesion subtypes, the differentiation of HCC from other liver malignancies such as metastases and cholangiocarcinoma, its use in the evaluation of response to locoregional treatment, and its role in image-

guided intervention. This review will also highlight some of the advances being made in microbubble-based molecular imaging, functional biomarkers, and targeted drug delivery.

Ultrasound Contrast Agents

FDA-approved microbubble-based ultrasound contrast agents are 1–5 μm in diameter and consist of a phospholipid shell filled with a gas core, either perfluorocarbon (Definity, Lantheus Medical Imaging, N. Billerica, MA) or sulfur hexafluoride (SF6) (Lumason, also known as SonoVue, sulfur hexafluoride lipid-type A microspheres, Bracco Diagnostics Inc., Milan). The gas within their core diffuses into the alveolar space as the microbubbles pass through the pulmonary capillaries and is subsequently exhaled. In the case of Lumason, the blood half-life in human subjects is 6 min, and 80 % of the injected dose is recovered from the exhaled air in 11 min [4]. Sonazoid (perflubutane, Daiichi-Sankyo Company Ltd., Tokyo) is only approved in Japan and Korea—this agent is also phagocytosed by Kuppfer cells, extending microbubble survival and enabling imaging in the late parenchymal phase (>5 min) when tumors appear as filling defects within the liver [5]. There is no renal clearance for microbubbles; therefore, microbubbles are safe in patients with compromised renal function, particularly those that cannot tolerate CT or MR contrast [6••].

CEUS agents are administered intravenously, typically through a 20-gauge or larger angiocatheter. A dose of 5×10^8 microbubbles is typically administered in a volume of only 0.2 ml (Definity) or 2 ml (Lumason). This, in combination with the relatively short half-life, allows for multiple repeat injections during the same examination. A typical liver diagnostic study requires 2 to 4 injections—the total volume required is available in a single vial. Because of the miniscule dose required, and because the microbubbles are rapidly exhaled, approved agents are incredibly safe, and allergic reactions are rarely encountered [7].

Microbubbles have three unique properties that make them ideal for liver imaging. First, CEUS offers true blood pool imaging. The contrast agents used in CT and MRI leak through the endothelium and rapidly equilibrate with the interstitial space [8]. Although smaller than red blood cells, the relatively large size of microbubbles restricts their diffusion into the extravascular space. The entire vascular space becomes highly echogenic on real-time imaging, enabling the distinct visualization of arteries and veins within solid organs, which is not possible with CT and MR beyond their respective contrast agent's first pass through the vasculature bed. Small vessels, such as capillaries, are below the resolution of most clinical imaging systems. However, when filled with microbubbles, they are easily distinguished.

Second, there is interplay between the microbubbles and the ultrasound device used to image them. When exposed to ultrasound, microbubbles not only reflect sound because of their gas core but also oscillate due to their highly elastic lipid shell. This oscillation converts microbubbles into local ultrasound transmitters. With sophisticated multi-pulse transmission and signal processing, echoes from background tissue can be subtracted from echoes received from bubbles in real-time. Using a dual display mode, standard gray scale (B-mode) images can be viewed alongside contrast-only images [9].

Finally, the dynamic oscillation, particularly at high pressures, destabilizes the lipid shell, which then fragments. Once destroyed in this way, the released gas core is exhaled. Microbubble destruction is in fact a powerful clinical tool. Adjusting instrument settings allows for the manipulation of image contrast to favor the visualization of regions with high flow rates such as vessels (rapid microbubble refill) or regions with high fractional blood volume (perfused tissue) [10, 11]. In fact, similar to photobleaching in fluorescence microscopy, relative blood flow and fractional blood volume can be calculated in this way [12, 13].

Imaging HCC by CEUS

HCC Imaging Characteristics and Subtype Differentiation

The incidence of hepatocellular carcinoma (HCC) has significantly increased in the USA over the past 3 decades, with a substantial proportion of cases attributable to hepatitis C virus infection [14]. Given the increasing prevalence of obesity and other factors contributing to liver disease, it is conceivable that HCC will become an even greater healthcare burden in the USA. Burgeoning healthcare costs and increasing emphasis on efficiency and cost-effectiveness of diagnostic tests gives ultrasound an economic advantage over CT or MR [15, 16]. In addition, the recent focus on the carcinogenic effects of ionizing radiation, and fears over the use of iodinated and gadolinium-based contrast agents, may prioritize CEUS in the HCC imaging armamentarium.

Currently, the most accepted indications for CEUS evaluation in the liver include the expedited characterization of lesions initially found on screening ultrasound, the interrogation of small lesions inadequately characterized on contrast-enhanced-MR (CEMR) or contrast-enhanced-CT (CECT), and when there is a contraindication to the use of iodinated or gadolinium contrast agents [6••]. The superior temporal resolution not only makes CEUS a key problem solving modality that may be performed in conjunction with CECT or CEMR but also allows CEUS to be utilized as a primary modality in the characterization, detection, and monitoring of focal liver lesions [17–19].

CEUS enhancement characteristics of HCC reflect the pathophysiological changes underlying the progression of a regenerative to dysplastic cirrhotic nodule, followed by well-differentiated and ultimately poorly differentiated HCC. A corresponding decrease in normal portal vascular tracts with an increase in arterial neovascularization accompanies the progression of a liver lesion through this spectrum [20, 21]. Homogenous or heterogeneous arterial phase enhancement (within 30 s after administration) is a hallmark of HCC in CT, MR, and catheter angiography, as it becomes preferentially supplied by the hepatic arterial circulation. Hypoenhancement relative to surrounding liver (i.e., "washout") in the portal venous or delayed phase is an additional diagnostic feature [22, 23]. This pattern of vascular enhancement and washout on CEUS, including the presence of an enhancing pseudocapsule on the arterial phase, is concordant with the observations seen in CECT and CEMRI (Fig. 1) [24]. Discordance between CEUS and CECT or CEMR is typically due to the higher temporal resolution of CEUS and/or the fact that microbubbles act as a true blood pool agent without the extravascular, interstitial enhancement seen with iodinated and gadolinium agents [25].

Substantial efforts have been made to correlate enhancement patterns to a lesion's underlying histological differentiation [26, 27]. Although arterial phase enhancement followed by washout is a classic pattern, it is usually only a characteristic of moderately differentiated HCC [28]. A large percentage of well-differentiated HCCs may be hypoechoic or isoechoic to the background liver in the arterial phase [29]. Lesion behavior in the portal venous or delayed phase has therefore been suggested to be more critical in distinguishing benign from malignant lesions in CEUS. Wilson et al. found that washout in the portal venous phase is present in 93 % of malignant lesions analyzed, while diffuse arterial phase enhancement is present in up to 95 % of benign focal nodular hyperplasia lesions (Fig. 2) [28, 30].

CEUS offers a distinct advantage over CECT and CEMR in its ability to assess real-time contrast kinetics. Unlike CT or MRI which acquires images at specific time points tens of seconds or minutes apart, CEUS offers near-continuous imaging, allowing for the calculation of more accurate time enhancement curve (TIC) profiles that can be subsequently analyzed to provide quantitative measures of vascularity [8, 31]. For instance, washout time relative to liver has been proposed to predict HCC differentiation, with a longer time to washout correlating more with well-differentiated HCC vs moderate or poorly differentiated lesions. Lesions that become hypoechoic relative to liver 120 s or longer after injection were shown to have a 98 % sensitivity, 78 % specificity, and 0.96 accuracy in distinguishing well-differentiated HCC from other subtypes [32, 33]. On the other hand, poorly differentiated HCCs are more likely to washout within 1 min post injection [34]. Xu et al. showed that compared to moderately or poorly differentiated HCC, well-differentiated HCCs exhibit longer time to peak enhancement (25.73 ± 4.04 vs 16.78 ± 7.57 s) and contrast-enhanced times (17.10 ± 4.94 vs 11.43 ± 2.09 s),

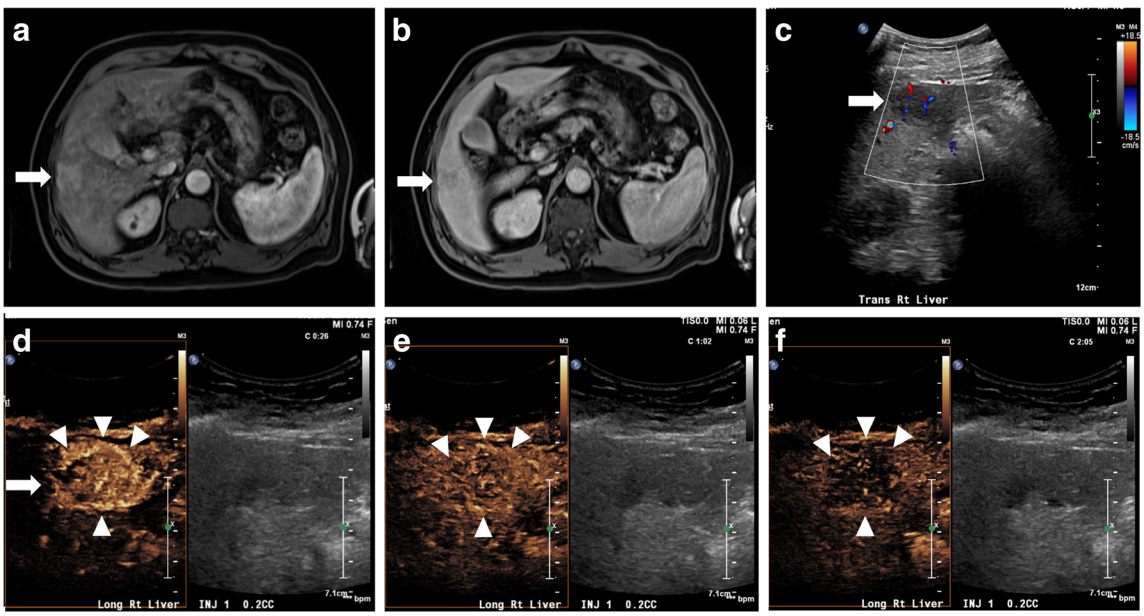

Fig. 1 **a**, **b** Patient with unknown history of liver disease presented with an indeterminate liver lesion on MR (*arrow*). **c** Color Doppler US of the same lesion in **a** and **b**, showing a hypervascular and hypoechoic lesion (*arrow*). **d** Dual display of microbubble-only image (*left hand panel*) and B-mode image (*right hand panel*) shows rapid arterial enhancement 26 s after injection (*arrow*), along with an enhancing pseudocapsule (*arrowheads*). Slight washout on the portal venous phase at 1 min (**e**) followed by marked washout at 2 min (**f**) post injection (*arrowheads*), consistent with a low- to intermediate-grade HCC, as confirmed on biopsy

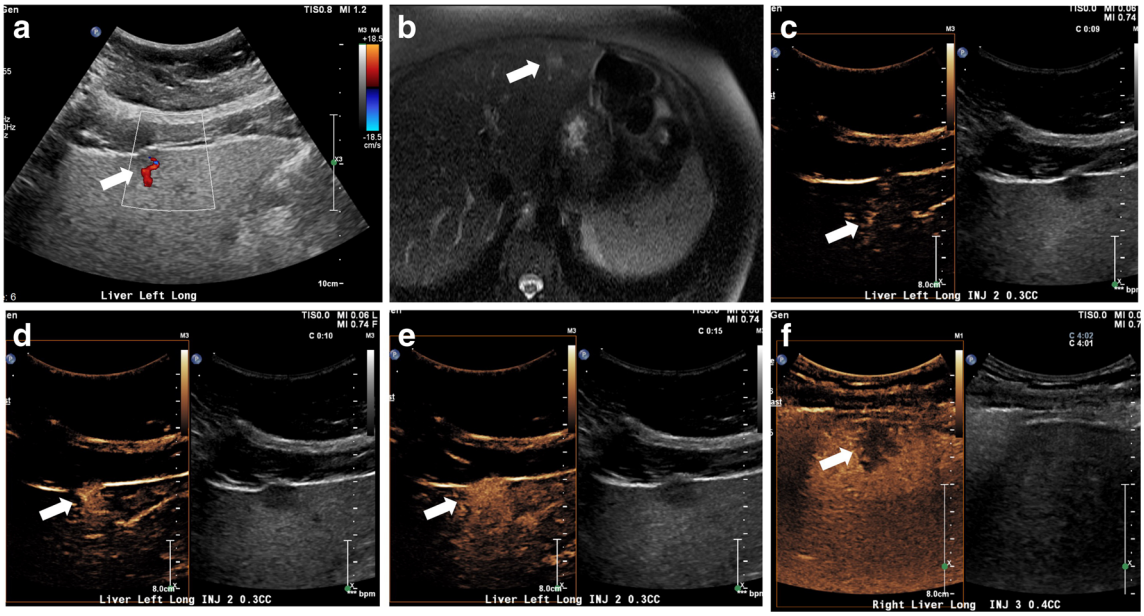

Fig. 2 **a** A fifty-three-year-old female with remote hysterectomy for a stage 1 endometrioid adenocarcinoma and a more recent renal wedge resection for leiomyosarcoma. On a recent ultrasound to evaluate for abnormal liver function tests, a small solid hypoechoic liver lesion with a feeding vessel was identified (*arrow*). **b** MRI showed this same lesion to be hyperintense on T2-weight images (*arrow*), with mild enhancement on the arterial phase (not shown). However, because the lesion was so small, the MRI was felt to be inconclusive. **c** Shortly after microbubble administration, CEUS showed a small feeding vessel, followed by the appearance of an arterial spoke-wheel pattern (**d**), and centrifugal enhancement (*arrow*)in the portal venous phase (**e**) without wash out by 3 min (*not shown*). Biopsy showed normal hepatic structures without evidence of malignancy, suggestive of focal nodular hyperplasia (FNH). **f** In a different patient with history of colorectal cancer, marked washout is seen in a subcapsular liver lesion (*arrow*). This lesion proved to be a colorectal metastasis on subsequent biopsy. Metastatic lesions nearly always show marked washout by the portal venous or delayed phase on CEUS

lesser peak enhancement (1.73 ± 0.54 vs 2.70 ± 1.14) and flatter clearance slope (1.27 ± 0.81 vs 1.87 ± 0.41) [35].

Although currently only available in Japan and Korea, Sonozoid provides additional valuable insight into HCC diagnosis and differentiation given that this agent is trapped in Kupffer cells. Lack of contrast agent uptake on the late phase (>3–5 min) denotes lack of Kupffer cells, a characteristic of malignant lesions, much like the experience with Feridex (iron oxide nanoparticles) in MRI. Unlike the hepatobiliary MR agent gadoxetic acid (Eovist, Bayer Schering Pharma, Berlin) that accumulates in hepatocytes, the delayed Kupffer cell phase with Sonozoid is a better predictor of dysplastic nodules and differentiator of well, moderately, and poorly differentiated HCC than the late phase of Eovist [36]. The advantage of CEUS with Sonozoid is the ability to also assess lesion behavior during the arterial and portal phases, which are less optimally imaged with Eovist [37, 38].

CEUS features of benign lesions are often concordant with enhancement patterns seen on CECT and CEMR, allowing for differentiation from HCC or other malignancies [30, 39]. For example, focal nodular hyperplasia (FNH) exhibits rapid centrifugal enhancement followed by an isoechoic to hyperechoic appearance during the delayed phase (Fig. 2c–e) [40]. A complete discussion of benign lesion characterization is beyond the scope of this review.

Diagnostic Efficacy Compared to CEMR and CECT

A 2011 meta-analysis of 21 studies by Guang et al. showed that CEUS using SonoVue (Lumason in the USA) is at least equal in diagnostic efficacy for HCC compared to CECT and CEMR [41]. However, for lesions smaller than 3.0 cm, CEUS may outperform CEMR and CECT in some cases. Sugimoto et al. compared CEUS to CEMR following Gd-EOB-DTPA (Primovist® in Europe, Eovist in the USA, Bayer Schering Pharma, Berlin) and found CEUS to be superior in assessing arterial hypervascularity of HCC [42]. Takahashi et al. also showed improved arterial phase characterization by CEUS compared to CEMR [43]. This highlights one of the benefits of real-time imaging with CEUS, as it is better able to interrogate the relatively brief though critical arterial phase that may be suboptimally imaged by CECT and CEMR [44].

While CEUS can compete with cross-sectional imaging as a primary modality in diagnosing HCC in some settings, it should also be viewed as an effective problem solving tool in conjunction with CECT or CEMR. CEUS may be useful in confirming the diagnosis of recurrent HCC when CECT demonstrates atypical enhancement patterns (i.e., arterial enhancement without delayed phase washout or vice versa) [45]. Delayed phase imaging using both CEUS and CEMR was

shown to be superior to either modality alone in characterizing liver lesions as benign or malignant with an accuracy approaching 98 % [46•].

A key disadvantage of CEUS is its inability to visualize the entire liver in some patients and is therefore unable to accurately stage disease. However, since a large percentage of HCC recurrence is in the same liver segment, CEUS may be effectively incorporated into post treatment algorithms along with CECT or CEMR, potentially reducing the number of cross-sectional follow-up examinations [47].

The Liver Imaging and Reporting Data System (LI-RADS), supported by the American College of Radiology, is a diagnostic algorithm with standardized terminology, description, and reporting of HCC lesions for CT and MR. This system incorporates expert opinion across other societal guidelines such as the AASLD and input from the OPTN [48]. This reporting system has gained widespread acceptance over the past several years [48] and was recently expanded to incorporate CEUS, which will enable further comparisons with CECT and CEMR, and its impact on the diagnostic algorithm moving forward [49].

Distinguishing HCC from Cholangiocarcinoma and Metastases

Reliably distinguishing HCC from intrahepatic cholangiocarcinoma in the cirrhotic liver is a difficult task on imaging, though remains critical in guiding clinical management. The American Association for the Study of Liver Diseases (AASLD) notably removed CEUS from its updated 2011 recommendations for nodule workup in cirrhotic patients for this very reason, as a small percentage of both malignancies share some common features on CEUS [50]. Since those guidelines were released, efforts have been made to more reliably distinguishing HCC and cholangiocarcinoma, such as showing that cholangiocarcinoma more frequently exhibits rim-like enhancement on the arterial phase [51]. Another key differentiating observation is that cholangiocarcinomas tend to washout much faster than HCCs, likely due to the increased fibrotic components seen in this malignancy [51, 52•]. A study by Han et al. suggested that using a lesion to liver background ratio of below 0.4 at 3 min post injection provides greater than 90 % sensitivity, specificity, and accuracy for diagnosing cholangiocarcinoma, even when compared to poorly differentiated HCCs that exhibit a faster washout than well-differentiated HCCs [53]. However, pitfalls remain in those tumors that contain both HCC and cholangiocarcinoma histology. These combined biphenotypic tumors may show CEUS features resembling both tumor types, as has been reported with CT and MRI, and suspicion for this type should be raised when there is a concurrent rise in both AFP and CA-19-9 tumor markers [54].

Metastatic disease from an extrahepatic malignancy is often listed in the differential diagnosis when a new liver lesion is encountered; however, metastases are relatively uncommon in the setting of cirrhosis given that a diseased liver is considered an inhospitable tissue. While the presence of liver metastases can be reasonably postulated for patients having a known extrahepatic primary malignancy, metastases universally demonstrate rapid washout, or marked hypoechogenicity, compared to background liver in the portal venous or delayed phases, and like cholangiocarcinomas, tend to washout faster than HCCs (Fig. 2f) [55]. When arterial enhancement is present, it is usually in a rim-like or "chaotic" pattern [6••, 39].

Differentiating Malignant vs Benign Venous Thrombus

Portal vein thrombosis is not uncommon in cirrhosis, either as a sequela of decreased portal venous flow or from HCC invasion. Distinguishing between the two is critical as malignant vascular invasion has significant negative prognostic implications and excludes therapies such as surgical resection and transplantation in most cases [56–58]. According to Dodd et al., the detection of an arterial waveform on Doppler US has a specificity of 95 %, but only a modest sensitivity of 62 % for diagnosis of malignant thrombus [59]. Malignant thrombus demonstrates similar features to HCC tumors on CEUS, with arterial phase enhancement followed by washout [60•]. Tarantino et al. showed that enhancement on CEUS was 88 % sensitive while detection of flow on Doppler US was only 20 % sensitive for diagnosis of malignant thrombus [61]. Rossi et al. corroborated this increased sensitivity of CEUS vs Doppler US in detecting malignant thrombus [62]. CEUS was also shown to be 100 % sensitive and 98 % specific in detecting and characterizing biopsy-proven malignant portal vein thrombus in patients with HCC, as compared to 67.6 and 60 %, respectively for CECT [63].

Improving Imaging Guidance and Assessing Treatment Response to Locoregional Therapies

CEUS can provide improved intraprocedural guidance by increasing the conspicuity of lesions not readily apparent on conventional ultrasound or CT [64•]. Since multiple microbubble injections can be given in the same session, CEUS is ideal for guiding needle placement, unlike in CECT or CEMR where only one contrast dose can be administered. Several studies have shown improved lesion conspicuity over standard ultrasound, resulting in greater yield at biopsy and more successful radiofrequency ablation [65, 66].

CEUS is excellent in detecting residual tumor blood flow after treatment with transarterial chemoembolization (TACE),

indicating residual or recurrent viable tumor (Fig. 3). Lipiodol, which may be used as an embolic agent in TACE, is highly attenuating and can therefore mask lesion enhancement on post-procedural CECT studies; by at least one report, CEUS was more accurate and sensitive in detecting residual tumor after TACE when lipiodol was used [67]. Because of the high sensitivity to microbubbles on real-time imaging, CEUS is able to detect miniscule residual tumor blood flow just days to weeks after treatment [68, 69]. This capability is significant as standard practice calls for CECT or CEMR 1–3 months after TACE to assess tumor response [69]. CEUS just 1 day after TACE more accurately detected residual viable tumor compared to CECT at 1 month, enabling more aggressive and expedited follow-up [70•]. Even when imaged at 1 month after TACE, Cho et al. reported that CEUS detected residual tumor missed by CECT and CEMR in nearly 50 % of patients [71].

Similar CEUS performance was shown when assessing other locoregional treatments, such as radiofrequency or microwave ablation. As tumor progression after ablation most often occurs locally, directed CEUS is a feasible alternative to CECT or CEMR in follow-up [47]. Several studies have shown that CEUS is at least as accurate as CEMR and CECT in monitoring for residual tumor after ablation [72–74]. Another unique aspect of CEUS is its ability to assess the ablation zone during treatment to provide immediate real-time feedback of treatment efficacy. When used in this way, there was practically zero recurrence by one study [75]. CEUS helped convert 21.8 % inadequately ablated lesions to adequately treated by the end of the ablation session in another report [76].

Finally, the use of CEUS for intraoperative localization of HCCs and the detection of additional lesions was more sensitive for small HCCs than preoperative CECT. Mitsunori et al. reported that intraoperative CEUS found 8 new lesions in 7 of 52 patients [77].

Future Trends

CEUS for Monitoring HCC Response to Chemotherapy

The tyrosine kinase inhibitor sorafenib is a systemic antiangiogenic agent that has shown a 3-month survival benefit in patients with advanced HCC [78]. Relative tumor perfusion can be quantified by CEUS allowing the detection of flow changes induced by antioangiogenic therapies, making it a potentially valuable tool in monitoring of early therapeutic response [79, 80]. A positive response may be characterized by decreased blood flow and increased mean transit time, as was shown in an animal xenograft model of HCC [81]. In general, post therapeutic reduction in HCC enhancement has been shown to predict improved overall survival in patients with intermediate or advanced HCC [82].

Given the substantial cost, side effects, and low tolerance of sorafenib and other antiangiogenic agents, the ability to predict early tumor response is of immense value to appropriately stratify and individualize treatment strategies. Perfusion parameters calculated by CEUS at just 2 and 4 weeks after initiation of sorafenib was predictive of longer progression free survival [83]. One report showed that CEUS perfusion wash-in 7 days after initiation of therapy was strongly associated with progression free and overall survival [84], while another reported that CEUS 1 month after treatment was a better predictor of disease non-progression than CECT acquired at 2 months [85]. Similarly, a decrease in tumor blood volume measured on CEUS just 3 days after initiation of bevucizimab (Avastin) can remarkably predict progression free and overall survival at 2 months [86]. Given its ability to predict therapeutic response so shortly after initiation, CEUS will likely play a key role in determining efficacy of newer antiangiogenic agents proposed for HCC [87].

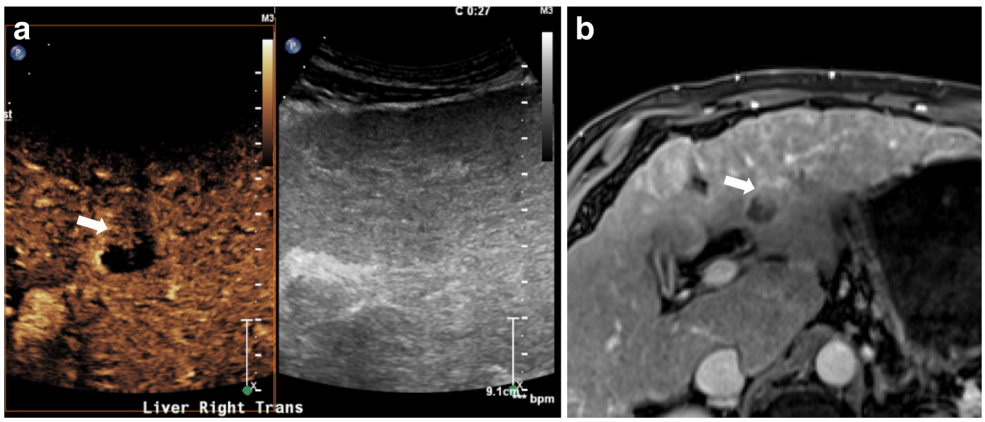

Fig. 3 Cirrhotic patient with known HCC, status post TACE, with clinical suspicion for residual or recurrent disease. **a** CEUS image (*left hand panel*) shows residual enhancing nodularity in the previously treated lesion (*arrow*), which is difficult to see on the corresponding B-mode image (*right hand panel*). **b** CEMR performed concurrently demonstrates this same lesion with residual enhancing nodularity, similar to that seen on CEUS (*arrow*). When there is clinical suspicion of recurrence with a single treated lesion after TACE, CEUS may be more efficient than repeat CEMR

Molecular Imaging

Microbubbles are a robust template for US-based molecular imaging, which will likely play a large future role in personalized medicine. For instance, microbubbles can be decorated with various ligands, turning the bubbles into targeted probes. As an example, BR55 (Bracco Suisse SA, Geneva, Switzerland) is a microbubble agent targeted to VEGFR-2 that preferentially accumulates in tumors overexpressing VEGFR-2 [88]. Using this agent, differential microbubble accumulation was shown 14 days after the initiation of sorafenib in mice harboring HCC xenografts, correlating with decreased VEGFR-2 expression, indicating that this agent may be useful in monitoring changes in tumor ligand expression after therapy [89]. Microbubbles can conceivably be developed to target ligands or other biomarkers discovered in HCC to improve disease diagnosis and treatment monitoring. Unfortunately, because of their relatively large size, current microbubbles can only target ligands along the vessel endothelium.

Therapeutic Agent Delivery

One of the most powerful extensions of CEUS is the use of microbubbles as drug delivery vehicles, either alone or in synergy with other treatments such as high-intensity focused ultrasound (HIFU) or TACE. When microbubbles are insonated at high power (above the FDA limits), they undergo cavitation, resulting in microjets that disrupt the membrane of adjacent cells to deliver their load into the cytosol, a technique known as sonoporation [90]. Disruption can also be induced in the endothelial lining, enabling the delivery of therapeutics across an otherwise tight endothelial barrier. A recent preclinical study by Chowdhury et al. demonstrated the ability of microbubble-mediated delivery of microRNAs in doxorubicin-resistant HCC resulted in enhanced apoptosis, decreased tumor volume and resensitization to doxorubicin [91•]. HCC-bearing animals treated with doxorubicin-loaded microbubbles in the presence of a destructive ultrasound pulse were shown to have smaller tumor sizes, higher intratumoral doxorubicin concentrations, and overall increased survival compared to cohorts treated without a destructive US pulse [92]. Microbubbles targeted to integrin $\alpha_v\beta_3$ and carrying the double suicide CD/TK gene were shown to effectively transfect HCC HepG2 cells both in vitro and in vivo when insonated with US, resulting in improved cell cycle arrest and apoptosis compared to controls [93]. As new drugs, gene therapies, and nucleic acids are introduced as potential therapies for HCC, US-mediated microbubble delivery promises to be an efficient delivery vehicle as compared to systemic or untargeted therapies alone.

Conclusion

CEUS for the diagnosis and monitoring of HCC promises to make a substantial impact in the USA, as it has in other countries now that an FDA-approved and reimbursable non-cardiac indication is available. The superior temporal resolution and high sensitivity of ultrasound to microbubbles, even at miniscule doses, allows for the interrogation of variable enhancement patterns that can be missed on cross-sectional CEMR or CECT, enabling lesion detection and characterization and more precise histologic differentiation. While CEUS will likely not replace CEMR or CECT, especially in the setting of staging or multifocal disease, it is a valuable adjunct tool in the care of cirrhotic patients, particularly for those patients with contraindications to iodinated or gadolinium contrast agents. The ability of CEUS to detect residual tumor blood flow or changes in perfusion indices shortly after local or systemic therapy has allowed it to better predict long-term outcome and may enable more individualized patient management. CEUS will likely play an ever-increasing role in image guidance for increased efficacy of locoregional treatments. Finally, the ability to target microbubbles to certain ligands may broaden their use as an imaging biomarker, and microbubbles may provide a potential vehicle delivery system for novel drug and gene therapies.

Compliance with Ethical Standards

Conflict of Interest Dr. Christopher D. Malone and Dr. David T. Fetzer declare that they have no conflicts of interest. Dr. Robert F. Mattrey declares honorarium payment from Bracco Diagnostic Inc., outside of the submitted work.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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- • Of importance
- •• Of major importance

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