

Review paper

Current advancements and future directions in contrast-enhanced ultrasound (CEUS) in liver oncology: from quantification to theranostics

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Abstract

Contrast-enhanced ultrasound has evolved into a precision supportive tool in managing liver oncology, particularly for hepatocellular carcinoma. It has moved beyond simple visual impressions to objective, quantitative data using dynamic contrast-enhanced ultrasound, perfusion curve analysis, and 3D-volumetric assessments. Furthermore, the integration of fusion imaging and artificial intelligence-driven pipelines has made ultrasound indispensable for guiding complex interventional procedures in real-time.

In parallel, emerging technologies like targeted microbubbles and ultrasound-targeted microbubble destruction are pushing the field toward molecular imaging and theranostics, turning ultrasound into an active participant in therapy. This review examines these clinical applications, takes a critical look at persistent hurdles, and identifies current research directions.

Key words: CEUS, fusion imaging, UTMD, contrast-enhanced ultrasound, 3D-CEUS, targeted microbubbles.

Introduction

This article is a narrative review centred on contrast-enhanced ultrasound (CEUS) in liver oncology, with emphasis on (i) focal liver lesion characterisation in chronic liver disease, (ii) assessment of residual viable tumour after locoregional therapies, and (iii) interventional guidance and intraprocedural decision support. The literature was selected to prioritise peer-reviewed clinical studies, consensus/technical reviews, and major translational reports published predominantly within the last 5 years, supplemented by earlier foundational work where it remains directly relevant.

Quantitative analysis in CEUS

A primary development in CEUS is the transition from qualitative pattern recognition [1-3] toward the use of quantitative, reportable parameters. Semi-quantitative or

quantitative descriptors of enhancement – including wash-in slope, time to peak, peak intensity, and area under the curve – are provided through time – intensity curve (TIC) analysis and dynamic CEUS (DCE-CEUS). In liver oncology, these parameters are considered advantageous because the vascular phenotype may be reflected and longitudinal comparisons after therapy can be supported, provided that acquisition and post-processing are sufficiently standardised [4,5].

In the setting of locoregional therapy, such as ablation or embolisation, imaging evaluates whether residual viable tumour warranting early retreatment persists or if complete devascularisation has been achieved. The conspicuity of enhancing viable components within treated areas is improved by CEUS (Figure 1), which can be performed repeatedly at the point of care. Comparative diagnostic performance data regarding focal liver lesion characterisation are summarised in Table 1 [3].

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Authors' contribution:

A Study design · B Data collection · C Statistical analysis · D Data interpretation · E Manuscript preparation · F Literature search · G Funds collection

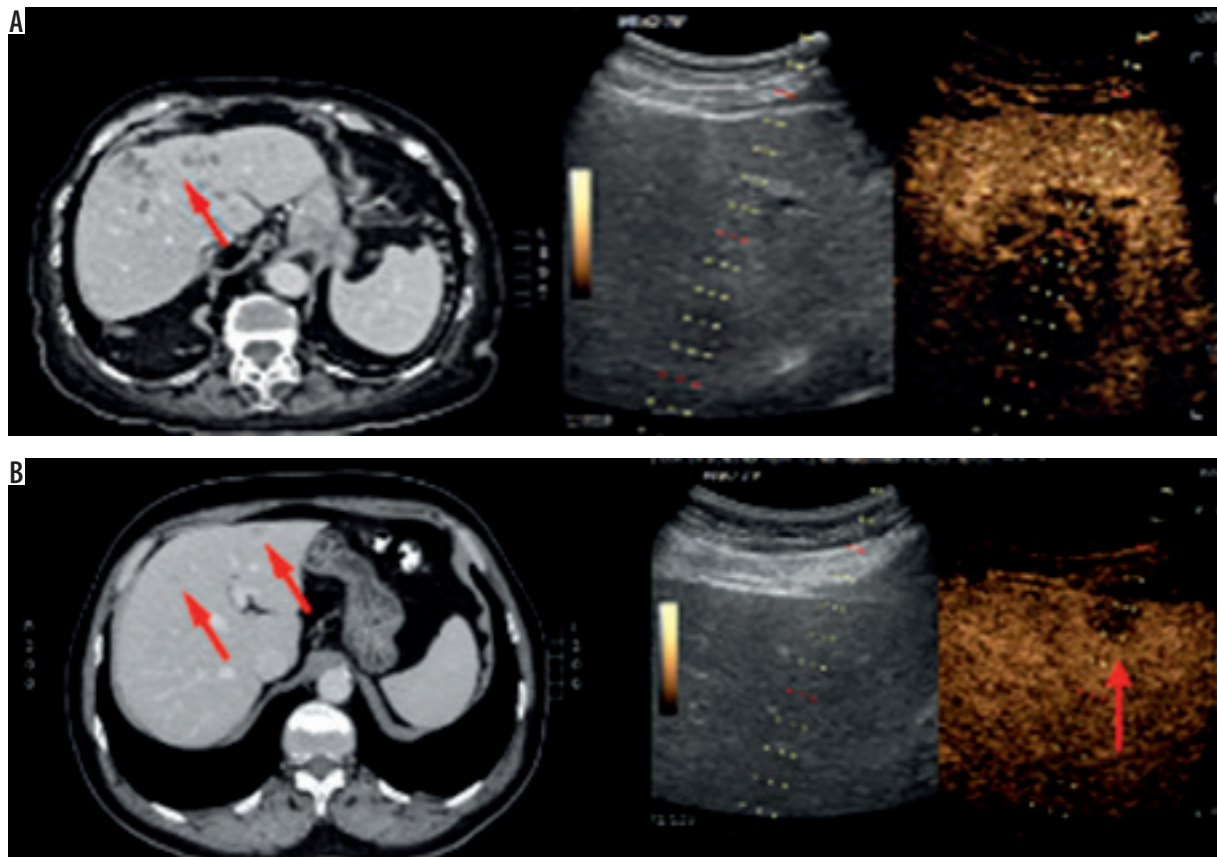


Figure 1. A-B) Increased visibility of the tumour facilitates biopsy of a focal liver lesion

Table 1. Data synthesis: The comparative analysis across both liver and renal studies demonstrates the superior diagnostic profile of CEUS. In liver imaging, CEUS significantly outperformed CT/MRI in terms of sensitivity and accuracy ($p < 0.0001$). Similarly, in the evaluation of unclear renal masses, CEUS maintained an exceptional sensitivity (99.1%) and a higher specificity (80.5%) compared to CT (47.4%). The high negative predictive value (NPV) observed in renal assessments (94.3%) indicates CEUS as a key tool for the non-invasive exclusion of malignancy, especially where cross-sectional imaging results remain indeterminate

Organ/Study type	Sample size	Diagnostic metric	CEUS (SonoVue®)	CT/MRI (combined)	p -value/Significance
Liver lesions/ Trillaud <i>et al.</i> 2009	$N = 134$	Sensitivity	98.2%	68.5%	< 0.0001
		Specificity	88.2%	74.6%	< 0.029
		Accuracy	92.7%	71.9%	< 0.0001
Renal lesions/ Marschner <i>et al.</i> 2020	$N = 255$	Sensitivity	99.1%	97.1% (CT)/96.4% (MRI)	High (all $> 96\%$)
		Specificity	80.5%	47.4% (CT)/75.0% (MRI)	CEUS $>$ CT/MRI
		NPV	94.3%	81.8% (CT)/85.7% (MRI)	Reliable exclusion

Within the scope of treatment monitoring, two approaches have been proposed: size-based and viability-based response assessments. While RECIST 1.1 remains a widely utilised standard across solid tumours, size reduction is often observed later than the biologic response following locoregional therapies in liver oncology [6]. Therefore, enhancement-based concepts focusing on viable tumour are better aligned with the clinical requirement for early detection of residual activity. In this context, CEUS is viewed as conceptually consistent with enhancement-focused response frameworks (e.g. mRECIST/EASL) because persisting arterial hyperen-

hancement within treated lesions or ablation zones can be depicted, thereby supporting timely retreatment decisions [4,5].

Despite these advantages, quantitative CEUS remains sensitive to acquisition settings, vendor-specific processing, motion artifacts, ROI placement, and heterogeneous background liver parenchyma, particularly in cases of cirrhosis. Unless harmonised protocols and transparent reporting of technical parameters are established, quantitative results remain difficult to generalise across different centres and devices, which limits their application as trial-grade endpoints.

3D-CEUS and volumetric assessment

Heterogeneity or extent may be underestimated by two-dimensional CEUS when lesions are irregular, multifocal, or partially visualised, particularly within post-treatment beds. Sampling bias is addressed by 3D-CEUS and volumetric approaches, which enable the evaluation of enhancing volume instead of single-plane enhancement (Figure 2) [1].

In the context of post-ablation monitoring, this approach is particularly relevant for ablative margin (AM) assessment and the early prediction of local tumour progression. The evidence comparing 2D and 3D CEUS-derived approaches after RFA is summarised in Table 2 [7].

Routine adoption remains limited by practical barriers, including availability, acquisition time, breath-hold constraints, motion artifacts, and variability in post-processing. The clinical impact of 3D-CEUS is expected to depend upon integration with automated segmentation, longitudinal registration, and standardised reporting, rather than on volumetrics alone [1,7-9].

Fusion imaging and procedure guidance

Real-time ultrasound (including CEUS) is integrated with pre-procedural computed tomography (CT) or magnetic

resonance imaging (MRI) through fusion imaging [10]. In the field of liver oncology, lesion localisation is improved, the targeting of lesions with poor B-mode visibility is enabled, and more confident biopsy or ablation is supported [11,12]. Functional information is added by CEUS through the highlighting of viable vascularity and the delineation of residual enhancement within treated zones.

Mapping is conducted using cross-sectional imaging; subsequently, targeting is facilitated by fusion-assisted navigation, and immediate assessment of residual viability is provided via CEUS to determine if additional treatment is required. A synthesis of clinical utility and technical performance metrics for fusion workflows is summarised in Table 3 [10].

However, fusion benefits are found to be heterogeneous and case-dependent. Outcomes can be affected by registration errors, liver deformation due to respiration and probe pressure, and operator dependence, all of which should be explicitly acknowledged. Endpoints reflecting clinical utility – including procedural success, duration, complication rates, and the frequency with which intraprocedural CEUS evokes a change in therapy (e.g. additional passes, alternative approaches, or escalation to another modality) – should be prioritised in future comparative studies [10-12].

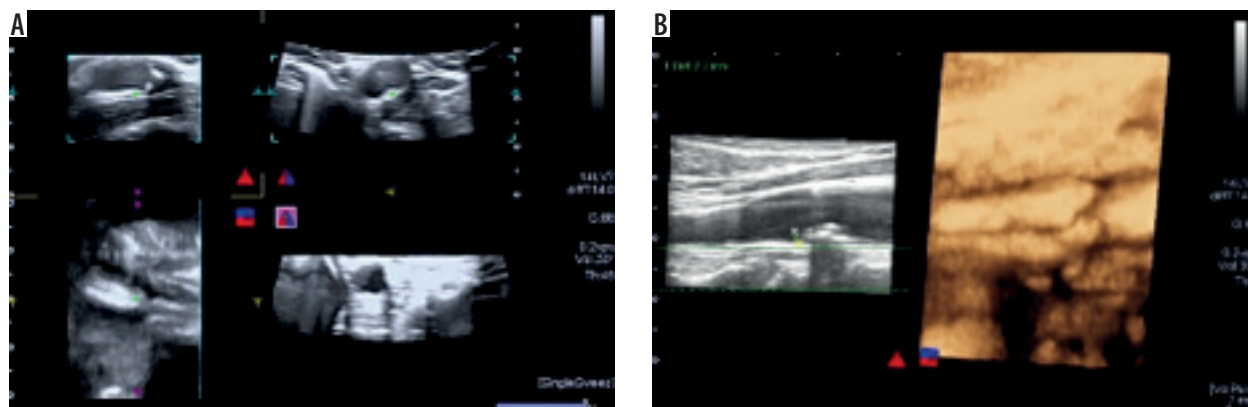


Figure 2. Volumetric image – contrast-enhanced ultrasound. Carotid arteries. A) Orthogonal imaging. B) 4D rendering

Table 2. 2D vs. 3D assessment in post-ablation monitoring 3D-contrast-enhanced ultrasound (CEUS)/fusion imaging significantly outperformed 2D-CEUS by reducing registration time (5.0 vs. 9.3 min) and eliminating the sampling errors of single-plane scans. While 2D-CEUS misclassified 26% of insufficient margins as “safe,” 3D volumetric assessment achieved 100% sensitivity in detecting residual tumors, whereas 2D imaging reported only 55.6% ($p = 0.019$)

Study focus	Sample size	Parameter	2D-CEUS (Standard)	3D-CEUS/Fusion	Significance (p-value)
Ablative margin: Long et al. 2024	N = 98	Registration time	9.3 min (manual)	5.0 min (mutomatic)	< 0.0001
		Ablative margin assessment	High risk of overestimation	Precise (volumetric)	26% of cases reclassified
		Local tumour prediction risk (3-year)	27.2% (if AM < 5 mm)	20.7% (if AM < 5 mm)	Both effective ($p < 0.004$)
Therapeutic response: Wang et al. 2025	N = 140	Sensitivity	55.6%	100.0%	0.019
		Specificity	98.4%	96.3%	0.915
		AUC (overall)	0.77	0.98	0.020

Table 3. Clinical synthesis: contrast-enhanced ultrasound (CEUS)-computed tomography (CT)/magnetic resonance imaging (MRI) fusion proved to be a promising alternative in a diagnostic process. While standard CT/MRI was insufficient in 12% of cases, adding CEUS fusion identified additional lesions and caused immediate therapeutic strategy modifications. Furthermore, real-time microvascular imaging enabled a definitive diagnosis in 15% of patients with atypical CT/MRI enhancement patterns. Despite the higher registration success of ultrasound (US)-US fusion (93.8%), its low applicability rate (61.1%) due to poor B-mode visibility makes multimodal fusion a superior solution, offering 99.7% applicability and 99.3% technical efficacy

Study focus/Source	Sample size	Category/Metric	Modality/Comparison	Results (n/%)	p-value/Significance
I. Lesion Conspicuity/ Bo <i>et al.</i> 2016	N = 70	Visibility rate	Conventional US (B-mode)	25/70 (35.7%)	< 0.001
			US + CECT/CEMRI fusion	49/70 (70.0%)	< 0.001
			CEUS + CECT/CEMRI Fusion	67/70 (95.7%)	Ref.
II. Clinical utility/ Rennert <i>et al.</i> 2025	N = 100	Additional lesions	Preliminary CT/MRI vs. fusion	12/100 (12%)	< 0.05
		Definite diagnosis	Preliminary CT/MRI vs. fusion	15/100 (15%)	Clinical gain
III. Technical comparison/ Xu <i>et al.</i> 2018	N = 157	Applicability rate	CT/MRI-CEUS fusion	99.7%	< 0.05
		Registration success	US-CEUS fusion	93.8%	< 0.05
			CT/MRI-CEUS fusion	81.3%	Technical challenge
		Technical efficacy	CT/MRI-CEUS fusion	99.3%	High success

Artificial intelligence in CEUS: segmentation, radiomics, and prediction

Artificial intelligence (AI) has been integrated as a central component of contemporary imaging pipelines. In the context of CEUS, inter-observer variability in ROI selection – identified as a major source of noise in TIC-based quantification – may be reduced through automated segmentation [13].

Beyond segmentation, high-dimensional features can be extracted from CEUS videos and parametric maps via radiomics [14] and machine learning [15] to support lesion classification, risk stratification, and the prediction of recurrence or treatment response [16,17]. Representative deep learning studies and validation setups are summarised in Table 4 [13,17,18].

Concurrently, AI is observed to be particularly vulnerable to domain shift in CEUS, resulting from heterogeneity in vendor processing, contrast dosing, presets, and acquisition protocols. Many published models remain restricted to single-centre cohorts and retrospective vali-

ation, whereby apparent performance is often inflated and transportability is limited. Clinical adoption will necessitate harmonised acquisition, robust feature stability assessment, external validation across multiple devices and centres, and prospective evaluation demonstrating management-changing benefits rather than retrospective discrimination alone [17-19].

Molecular CEUS and targeted microbubbles

Molecular CEUS goes beyond standard perfusion imaging by using microbubbles that bind to specific biomarkers, such as those involved in angiogenesis [19,20]. In liver oncology, this allows us to identify a tumour's biological features and monitor its response at a molecular level [21,22].

Although the technology is promising, it has not reached routine clinical use yet. This is mainly due to strict regulatory hurdles, a lack of standardised agents, and the ongoing need to prove that it offers a real advantage over conventional CEUS, CT, or MRI [19-22].

Table 4. Summary of deep learning models for liver lesion assessment. Because the objectives of these studies vary – ranging from lesion classification to recurrence prediction – different metrics were used to evaluate the performance of each method. The distinction between internal and external validation is also emphasized to show how the models perform both in their original clinical settings and across different hospitals. For segmentation tasks, the Intersection over Union (IoU) was used to measure the overlap between the artificial intelligence (AI)-generated outlines and the manual masks created by an expert. Finally, the "Reference" column indicates the established gold standard used to verify the accuracy of each AI model

Study	Objective	Sample Size (N)	Performance	Reference
Xu <i>et al.</i> 2025	Prediction of HCC early recurrence post-surgery	242 patients (test cohort: 47)	AUC: 0.90 (95% CI: 0.82-0.99)	Clinical features – recurrence
Liu <i>et al.</i> 2022	Diagnosis of HCC (malignancy detection)	303 patients (IV: 50, EV: 50)	AUC (EV): 0.957 (95% CI: 0.90-1.00) AUC (IV): 0.969 (95% CI: 0.89-1.00)	Histopathological confirmation
Mamuleanu <i>et al.</i> 2022	Liver lesion segmentation	49 patients (100 frames in test set)	IoU: 0.839	Expert manual segmentation

Moreover, recent studies show that using novel ultrasound contrast agents – nanospheres – can widely enhance CEUS capabilities. Their size (under 400 nm) is considerably smaller than conventional microbubbles (> 1 µm), thus allowing them to move outside the blood vessel and into the tumour interstitially [23,24].

However, nanospheres tend to present poor selectivity *in vivo* after intravenous administration. This problem is overcome by the use of tumour-specific antibodies conjugated with nanospheres, which is a complex and treacherous process [23].

This approach can be particularly significant for pancreatic ductal adenocarcinoma (PDAC). Unlike many other tumours, PDAC is typically hypovascular and surrounded by a dense fibrotic stroma, which often renders conventional microbubbles ineffective. Targeted nanospheres can move past these vascular limitations, flowing directly into the pancreatic tumour matrix [23]. Such a method could allow for staging of small, otherwise inconspicuous pancreatic lesions.

Another study explored the implementation of nanospheres for facilitating small cell lung cancer imaging, proving that on the mouse model such an approach can translate into significantly higher AUC and peak intensity values [24].

UTMD and theranostics

Ultrasound-targeted microbubble destruction (UTMD) utilises acoustic energy to disrupt microbubbles [25]. This process creates a temporary increase in permeability, which facilitates the enhanced local delivery of therapeutics. Within the field of oncology, imaging is used to confirm perfusion and target characteristics, while controlled insonation drives localised delivery or works in synergy with ablative strategies. Successful clinical translation, however, requires meticulous control over acoustic parameters, dosing, and safety monitoring to minimise off-target effects and ensure reproducibility.

Beyond UTMD, additional therapeutic ultrasound strategies – such as combining focused ultrasound with immunotherapy – are currently under clinical investigation. A summary of ongoing trials for these applications is provided in Table 5.

UTMD evolves from the sonoporation concept, which originated from foundational studies on the mechanical index (MI) and its application in CEUS. The MI serves as a standardised estimate of the maximum peak negative acoustic pressure within the tissue, scaled by the square root of the centre frequency; fundamentally, it quantifies the likelihood of microbubble cavitation [26]. From a clinical standpoint, this creates a vital distinction in ultrasound application: while a low MI is essential for stable, non-destructive real-time visualisation, escalating the acoustic intensity facilitates microbubble rupture, i.e. the very mechanism that enables targeted therapeutic delivery.

Empirical research, particularly studies involving the Sonazoid contrast agent, demonstrates that microbubble dissolution begins at moderate pressures, typically within an MI range of 0.4 to 1.0 [27]. However, true inertial cavitation – characterised by violent bubble collapse – is only triggered when the MI exceeds 1.0. This threshold is critical because the resulting disintegration of microbubbles provides the mechanical basis for sonoporation, defined as the use of ultrasound in the presence of cavitation nuclei to induce plasma membrane perforation [28].

In this context, the injected microbubbles serve as the essential cavitation nuclei. Because biological tissues and fluids naturally lack stabilised gas bodies within, they are inherently resistant to cavitation at diagnostic energy levels. By introducing synthetic microbubbles into the circulation, we provide “seeds” or nuclei that concentrate acoustic energy. These gas-filled cores oscillate and eventually collapse under ultrasound exposure, lowering the energy threshold required to generate the mechanical forces necessary for membrane permeabilisation.

In practical terms, while an MI of approximately 0.4 may be viewed as a “cellular massage” that gently disturbs

Table 5. Overview of ongoing clinical trials investigating the therapeutic applications of ultrasound

Name (number) of a trial/Sponsor	Aim of the study	Estimated primary completion date	Estimated study completion date	Results submitted to ClinicalTrials.gov
iFOCUS (NL85300.041.23)/UMC Utrecht	To evaluate the safety, tolerability, and feasibility of combination treatment of HIFU-HT and ICI in adult patients with metastatic or unresectable cancer who have progressive disease after regular treatment.	2030-08-01	2030-08-01	–
Breast-48 (19900)/Patrick Dillon, MD, University of Virginia	To evaluate the use of HIFU combined with pembrolizumab in patients with metastatic breast cancer.	2022-06-01	2022-06-17	2025-11-19 (1 st submission cycle)
Mel 70 (HSR230477)/University of Virginia	To evaluate the safety of FUSA in combination with intratumoral poly(I:CLC) injections for the treatment of melanoma.	2026-08	2026-08	–

the membrane, an MI exceeding 1.0 should be considered a “cellular puncture”. This localised perforation is a primary goal because it transiently disrupts membrane integrity to allow the uptake of exogenous drugs or genetic material. However, the success of this approach relies on a narrow safety window. Live microscopy has shown that sonoporation sites are dynamic; small, site-specific pores can be successfully repaired by the cell’s innate defence mechanisms – such as calcium-dependent resealing – often within 60 seconds. Conversely, excessive acoustic energy may lead to pores larger than 100 μm^2 or severe disruption of the actin cytoskeleton, resulting in irreversible cell lysis or apoptosis. Thus, precisely calibrating the MI is essential to maximise delivery efficiency while maintaining cellular viability.

The clinical viability of this mechanical approach is best exemplified by recent breakthroughs in treating glioblastoma (GBM). While the blood-brain barrier (BBB) remains a formidable obstacle for most chemotherapeutic agents, magnetic resonance-guided focused ultrasound (MRgFUS) has successfully leveraged sonoporation to transiently disrupt this barrier. Clinical data have demonstrated that this technique can achieve a 7.7-fold increase in temozolomide (TMZ) concentration within sonicated areas compared to untreated tissue, notably without inducing long-term neurological complications.

It is important to emphasise, however, that these landmark results were achieved under MRI guidance, which provides excellent anatomical precision but lacks the real-time temporal resolution to monitor microbubble cavitation dynamics directly. The current challenge lies in translating these therapeutic successes from the MRI suite to ultrasound-guided platforms, such as CEUS [29].

UTMD and sonobiopsy

While sonoporation is predominantly utilised for the “inward” delivery of therapeutics, its potential for the “outward” release of tumour-specific biomarkers into the bloodstream is increasingly recognised [30]. In the field of neuro-oncology, this transition allows for a move from purely therapeutic interventions toward a comprehensive diagnostic and monitoring paradigm. The safety profile of this approach has been demonstrated in clinical trials involving patients with Alzheimer’s disease, where it was shown that biological barriers can be opened across multiple brain regions without causing permanent injury [31].

This emerging diagnostic method, termed sonobiopsy, utilises focused ultrasound and microbubbles to facilitate “two-way trafficking” between brain tissue and the peripheral vasculature. By briefly opening the blood-brain barrier, biomarkers such as circulating tumour DNA and mRNA are released into the plasma for collection. It must be noted that the first human study on this technique, published in 2023, was conducted on a limited cohort of only five participants; however, the results confirmed the

feasibility of enriching biomarkers in the bloodstream [32]. This technique addresses the primary limitations of conventional liquid biopsies, where brain-derived markers are often too diluted or restricted by the blood-brain barrier to be detected. By enhancing the sensitivity of blood tests in a non-invasive and repeatable manner, sonobiopsy allows for the tracking of tumour changes without the risks associated with invasive brain surgery. However, this technique remains insufficiently investigated and requires further research.

Limitations and standardisation roadmap

Standardisation is deemed critical for the successful translation of CEUS innovations into routine liver oncology practice. Key barriers, such as vendor-dependent quantification, inconsistent acquisition protocols, variable operator expertise, and limited multi-centre prospective evidence, are frequently identified as obstacles to widespread implementation.

For quantitative CEUS, the inclusion of minimum reporting items is required; these should encompass system configurations (including mechanical index), contrast agent specifications and dosage, timing and breath-hold strategies, ROI/segmentation methodologies, motion management, reference standards, and reproducibility metrics. Regarding AI-based applications, the transparent reporting of preprocessing, calibration, external validation, and robustness across multiple vendors is considered essential.

A pragmatic roadmap is proposed, incorporating harmonised protocols, shared benchmark datasets, and prospective studies anchored in management-changing endpoints, such as retreatment decisions based on the early assessment of residual viability [1,2,4,5,15,16,19].

Discussion

The clinical value of CEUS in liver oncology is increasingly defined not by the replication of CT or MRI, but by advantages that are found difficult to achieve with other modalities [1-3]. These include real-time assessment of vascularity, repeatability without ionising radiation, and feasibility at the point of care. These strengths are identified as most critical in focal liver lesion characterisation in the context of chronic liver disease, early detection of residual viable tumour following locoregional therapies, and interventional guidance when rapid, iterative imaging is required.

Current advances in quantification, volumetrics, and AI are interpreted as systematic efforts to convert these pragmatic advantages into standardised outputs stable enough to influence clinical decisions and trial endpoints [1,2,4].

Objective enhancement descriptors are offered by quantitative CEUS, but the translation into clinically actionable parameters remains incomplete. Reproducibility is frequently compromised by cirrhosis-related heterogeneity,

motion artifacts, ROI dependence, and vendor-specific variability. Consequently, parameters that are not only measurable but decision-relevant are prioritised, particularly those enabling the early identification of residual viability that triggers retreatment [5,6]. In this context, enhancement-focused response concepts are considered more aligned with the clinical question than size-only assessments, while RECIST 1.1 is maintained as an important cross-tumour reference standard for general oncologic trials [6].

Sampling bias may be reduced and post-ablation margin evaluation improved through 3D-CEUS and volumetric assessment, although their impact is expected to depend on workflow integration and automation [1,7]. Similarly, procedural reliability is enhanced by fusion imaging, which provides immediate post-treatment verification; however, the benefit is noted to be case-dependent and limited by registration errors and operator dependence [10-12]. Meaningful evidence is therefore expected to emphasise procedural outcomes and management changes rather than surrogate technical metrics alone [7,10].

AI is recognised as a highly visible direction, but the risk of overestimating its clinical readiness is acknowledged. Domain shift is identified as a core problem in CEUS due to vendor and protocol variability, and a lack of robust external validation is observed in many current studies. Future work must prioritise harmonised acquisition, multi-centre validation, and the prospective demonstration that AI-assisted CEUS improves clinical decisions and patient outcomes [14-19].

Finally, a compelling theranostic narrative is offered by molecular CEUS and UTMD, although these technologies require further research. Their translation is seen to depend on rigorous safety characterisation, standardisation of targeted agents, regulatory progress, and trials

demonstrating incremental benefit beyond established systemic and locoregional strategies [19-22].

Conclusions

CEUS is considered to have turned into a clinically valuable modality in liver oncology by enabling real-time, repeatable assessment of tumour vascularity and immediate post-procedural evaluation of residual viability. Over the next decade, progress is expected to be judged primarily by management-changing benefit – particularly the earlier and more reliable detection of residual viable tumour after locoregional therapy – rather than by incremental improvements in standalone diagnostic performance [1,2,4,5].

Near-term clinical impact is predicted to arise from pragmatic integration: standardised quantitative protocols, workflow-optimised fusion imaging for selected interventional cases, and AI tools that reduce operator dependence while remaining robust across vendors and centres. In contrast, molecular CEUS and UTMD are represented as promising longer-horizon directions that will require rigorous safety evaluation and clinical trials demonstrating incremental benefit beyond established liver oncology strategies. Overall, harmonisation, external validation, and the use of prospective, clinically actionable endpoints are considered essential to enable wider adoption and reproducible patient benefit [7,9-11,14-22].

Disclosures

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4. Conflicts of interest: None.

References

1. Dietrich CF, Correas JM, Cui XW, Dong Y, Havre RF, Jenssen C, et al. EFSUMB technical review – update 2023: dynamic contrast-enhanced ultrasound (DCE-CEUS) for the quantification of tumor perfusion. *Ultraschall Med* 2024; 45: 36-46.
2. Dietrich CF, Nolsøe CP, Barr RG, Berzigotti A, Burns PN, Cantisani V, et al. Guidelines and good clinical practice recommendations for contrast enhanced ultrasound (CEUS) in the liver – update 2020 – WFUMB in cooperation with EFSUMB, AFSUMB, AIUM, and FLAUS. *Ultraschall Med* 2020; 41: 562-585.
3. Trillaud H, Bruel JM, Valette PJ, Vilgrain V, Schmutz G, Oyen R, et al. Characterization of focal liver lesions with SonoVue-enhanced sonography: international multicenter-study in comparison to CT and MRI. *World J Gastroenterol* 2009; 15: 3748-3756.
4. Cerrito L, Ainora ME, Cuccia G, Galasso L, Mignini I, Esposto G, et al. Dynamic contrast-enhanced ultrasound in the prediction of advanced hepatocellular carcinoma response to systemic and locoregional therapies. *Cancers (Basel)* 2024; 16: 551. DOI: 10.3390/cancers16030551.
5. Nam K, Stanczak M, Lyshchik A, Machado P, Kono Y, Forsberg F, et al. Evaluation of hepatocellular carcinoma transarterial chemoembolization using quantitative analysis of 2D and 3D real-time contrast enhanced ultrasound. *Biomed Phys Eng Express* 2018; 4: 035039. DOI: 10.1088/2057-1976/aabb14.
6. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). *Eur J Cancer* 2009; 45: 228-247.
7. Long H, Zhou X, Zhang X, Ye J, Huang T, Cong L, et al. 3D fusion is superior to 2D point-to-point contrast-enhanced US to evaluate the ablative margin after RFA for hepatocellular carcinoma. *Eur Radiol* 2024; 34: 1247-1257.
8. Ying X, Dong S, Zhao Y, Chen Z, Jiang J, Shi H. Research progress on contrast-enhanced ultrasound (CEUS) assisted diagnosis

- and treatment in liver-related diseases. *Int J Med Sci* 2025; 22: 1092-1108.
9. Liu J, Guo Y, Sun Y, Liu M, Zhang X, Zheng R, et al. Three-dimensional ultrasound fusion imaging in precise needle placement for thermal ablation of hepatocellular carcinoma. *Int J Hyperthermia* 2024; 41: 2316097. DOI: 10.1080/02656736.2024.2316097.
 10. Calandri M, Mauri G, Yevich S, Gazzera C, Basile D, Gatti M, et al. Fusion imaging and virtual navigation to guide percutaneous thermal ablation of hepatocellular carcinoma: a review of the literature. *Cardiovasc Intervent Radiol* 2019; 42: 639-647.
 11. Li K, Su ZZ, Xu EJ, Ju JX, Meng XC, Zheng RQ. Improvement of ablative margins by the intraoperative use of CEUS-CT/MR image fusion in hepatocellular carcinoma. *BMC Cancer* 2016; 16: 277. DOI: 10.1186/s12885-016-2306-1.
 12. Tahmasebi A, Siu Xiao T, Wessner CE, Kuon Yeng Escalante CM, Lyschchik A, Forsberg F, et al. Feasibility of contrast-enhanced ultrasound fusion with pretreatment MR/CT for recurrence detection in renal cell carcinoma patients during post-ablation surveillance. *Ultrasound Med Biol* 2025; 51: 1336-1342.
 13. Mămuleanu M, Urhūţ CM, Săndulescu LD, Kamal C, Pătraşcu AM, Ionescu AG, et al. Deep learning algorithms in the automatic segmentation of liver lesions in ultrasound investigations. *Life (Basel)* 2022; 12: 1877. DOI: 10.3390/life12111877.
 14. Bi WL, Hosny A, Schabath MB, Giger ML, Birkbak NJ, Mehrtash A, et al. Artificial intelligence in cancer imaging: clinical challenges and applications. *CA Cancer J Clin* 2019; 69: 127-157.
 15. Ting Sim JZ, Fong QW, Huang W, Tan CH. Machine learning in medicine: what clinicians should know. *Singapore Med J* 2023; 64: 91-97.
 16. Lin WP, Xing KL, Fu JC, Ling YH, Li SH, Yu WS, et al. Development and validation of a model including distinct vascular patterns to estimate survival in hepatocellular carcinoma. *JAMA Netw Open* 2021; 4: e2125055. DOI: 10.1001/jamanetworkopen.2021.25055.
 17. Xu W, Zhang H, Zhang R, Zhong X, Li X, Zhou W, et al. Deep learning model based on contrast-enhanced ultrasound for predicting vessels encapsulating tumor clusters in hepatocellular carcinoma. *Eur Radiol* 2025; 35: 989-1000.
 18. Liu L, Tang C, Li L, Chen P, Tan Y, Hu X, et al. Deep learning radiomics for focal liver lesions diagnosis on long-range contrast-enhanced ultrasound and clinical factors. *Quant Imaging Med Surg* 2022; 12: 3213-3226.
 19. Rix A, Palmowski M, Kiessling F. Molecular ultrasound imaging: clinical applications. *Radiologe* 2015; 55: 956-963 [Article in German].
 20. Wang X, Bove AM, Simone G, Ma B. Molecular bases of VEGFR-2-mediated physiological function and pathological role. *Front Cell Dev Biol* 2020; 8: 599281. DOI: 10.3389/fcell.2020.599281.
 21. Herr FL, Antons MJ, Blume LV, Hirner-Eppeneder H, Kloiber-Langhorst S, Cimic A, et al. Contrast-enhanced ultrasound with VEGFR2-targeted microbubbles for monitoring combined anti-PD-L1/anti-CTLA-4 immunotherapy effects in a murine melanoma model with immunohistochemical validation. *PLoS One* 2025; 20: e0326675. DOI: 10.1371/journal.pone.0326675.
 22. Qiu YJ, Cao JY, Liao JH, Duan Y, Chen S, Cheng R, et al. CXCR4-targeted ultrasound microbubbles for imaging and enhanced chemotherapy/immunotherapy in liver cancer. *Acta Biomater* 2025; 197: 416-430.
 23. Yang H, Zhao P, Zhou Y, Li Q, Cai W, Zhao Z, et al. Preparation of multifunctional nanobubbles and their application in bimodal imaging and targeted combination therapy of early pancreatic cancer. *Sci Rep* 2021; 11: 6254. DOI: <https://doi.org/10.1038/s41598-021-82602-9>.
 24. Wang JP, Zhou XL, Yan JP, Zheng RQ, Wang W. Nanobubbles as ultrasound contrast agent for facilitating small cell lung cancer imaging. *Oncotarget* 2017; 8: 78153-78162.
 25. Wang Y, Liu C, Lyu R, Zhang Y. Application and prospects of ultrasound combined with immunotherapy in cancer treatment of intensive care. *Front Immunol* 2025; 16: 1670527. DOI: 10.3389/fimmu.2025.1670527.
 26. Dietrich CF, Averkiou M, Nielsen MB, Barr RG, Burns PN, Calliada F, et al. How to perform contrast-enhanced ultrasound (CEUS). *Ultrasound Int Open* 2018; 4: E2-E15. DOI: 10.1055/s-0043-123931.
 27. Shi WT, Forsberg F, Tornes A, Ostensen J, Goldberg BB. Destruction of contrast microbubbles and the association with inertial cavitation. *Ultrasound Med Biol* 2000; 26: 1009-1019.
 28. Tu J, Yu ACH. Ultrasound-mediated drug delivery: sonoporation mechanisms, biophysics, and critical factors. *BME Front* 2022; 2022: 9807347. DOI: 10.34133/2022/9807347.
 29. Park SH, Kim MJ, Jung HH, Chang WS, Choi HS, Rachmilevitch I, et al. One-year outcome of multiple blood-brain barrier disruptions with temozolomide for the treatment of glioblastoma. *Front Oncol* 2020; 10: 1663. DOI: 10.3389/fonc.2020.01663.
 30. Izhar M, Thakur A, Park DJ, Chang SD. Ultrasound mediated blood-brain barrier opening increases brain tumor biomarkers: a review of preclinical and clinical trials. *J Liq Biopsy* 2024; 6: 100277. DOI: 10.1016/j.jlb.2024.100277.
 31. Meng Y, Goubran M, Rabin JS, McSweeney M, Ottoy J, Pople CB, et al. Blood-brain barrier opening of the default mode network in Alzheimer's disease with magnetic resonance-guided focused ultrasound. *Brain* 2023; 146: 865-872. Erratum in: *Brain* 2023; 146: e56. DOI: 10.1093/brain/awad082.
 32. Yuan J, Xu L, Chien CY, Yang Y, Yue Y. First-in-human prospective trial of sonobiopsy in high-grade glioma patients using neuronavigation-guided focused ultrasound. *NPJ Precis Oncol* 2023; 7: 92. DOI: 10.1038/s41698-023-00448-y.