REVIEW ARTICLE

Decoding Hepatocellular Carcinoma Heterogeneity with Multimodal Ultrasound and Acoustic Radiomics

Jiaying Cao[a], Wenying Li[a], Yijie Qiu[a], Li Wei[a], Ying Wang[a], Yunlin Huang[a], Yi Dong[a,], Christoph F Dietrich[a,b]*

[a] *Department of Ultrasound, Xinhua Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai, China;*
[b] *University Hospital Frankfurt, Frankfurt Main, Germany*

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Abstract: Hepatocellular carcinoma (HCC) is characterized by intratumoral heterogeneity (ITH), in which spatially and temporally evolving tumor subclones differ in angiogenesis, hypoxia, immune composition, proliferation, metabolism, and differentiation. This diversity determines tumor progression, recurrence, therapeutic resistance, and clinical outcomes among patients with similar disease stage. Molecular profiling has established ITH as a hallmark of HCC biology, while radiomics, radiogenomics, and habitat imaging based on computed tomography (CT) and magnetic resonance imaging (MRI) have demonstrated that clinically relevant heterogeneity can be assessed noninvasively. Ultrasound offers unique advantages for longitudinal tumor characterization because it is real-time, radiation-free, widely available, portable, and repeatable. Beyond conventional B-mode imaging, modern ultrasound interrogates complementary biological dimensions through contrast-enhanced ultrasound (CEUS), shear-wave elastography (SWE), quantitative ultrasound and radiofrequency (QUS/RF) analysis, and advanced microvascular imaging. Together, these techniques characterize tumor perfusion, biomechanical properties, tissue microstructure, and vascular architecture, providing a multidimensional assessment of intratumoral heterogeneity beyond conventional morphology. This narrative review summarizes the biological foundations of intratumoral heterogeneity in HCC and the benchmark established by CT- and MRI-based radiomics before examining how multimodal ultrasound may provide a biologically informed characterization of tumor heterogeneity. We discuss the emerging role of acoustic radiomics and artificial intelligence in integrating acoustic biomarkers to predict microvascular invasion, histological grade, recurrence risk, treatment response, and longitudinal changes in tumor biology. We propose a testable framework-the acoustic heterogeneity signatures-rather than an established clinical tool. The challenge is no longer to demonstrate that ultrasound contains biologically relevant heterogeneity, but to establish standardized acquisition protocols, biologically validated imaging biomarkers, and multicenter external validation. If these challenges can be overcome, multimodal ultrasound may evolve from a diagnostic imaging modality into a dynamic and interpretable biomarker platform for precision management of HCC, enabling individualized risk stratification, treatment selection, response assessment, and longitudinal disease monitoring.

Key words: Hepatocellular carcinoma; Multimodal ultrasound; Contrast-enhanced ultrasound; Acoustic radiomics; Artificial intelligence; Microvascular invasion

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Hepatocellular carcinoma (HCC) remains one of the leading causes of cancer-related mortality worldwide despite major advances in surveillance, surgical techniques, locoregional interventions, and immunotherapy-based systemic treatment [1,2]. A major reason for the marked variability in clinical out-

* *Corresponding author: Department of Ultrasound, Xinhua Hospital Affiliated to Shanghai Jiaotong University School of Medicine 1665 Kongjiang Road, Shanghai, 200092, China. (Yi Dong)*
e-mail: drdaisydong@hotmail.com (Y D)

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come among patients with apparently similar tumor stage is intratumoral heterogeneity (ITH). Rather than representing an uniform neoplasm, HCC comprises spatially and temporally evolving subclonal populations that differ in angiogenesis, hypoxia, proliferation, immune microenvironment, metabolism, and cellular differentiation [3-5]. This biological diversity drives tumor progression, metastatic potential, recurrence, and resistance to targeted and immune-based therapies, making ITH a fundamental determinant of prognosis rather than merely a descriptive pathological feature [3,6].

Current assessment of tumor heterogeneity remains limited. Percutaneous biopsy samples only a small fraction of the lesion and is inherently susceptible to sampling bias, while important prognostic features such as microvascular invasion (MVI), histological grade, and satellite nodules are usually established only after surgical resection. Likewise, established staging systems, including the Barcelona Clinic Liver Cancer (BCLC) classification and the tumor-node-metastasis (TNM) system, provide valuable prognostic stratification but do not fully capture the underlying biological diversity that governs tumor behavior and treatment response [4,7]. Consequently, there is a growing need for noninvasive imaging biomarkers capable of characterizing tumor heterogeneity across the entire lesion and monitoring its evolution longitudinally throughout the disease course.

Cross-sectional imaging has demonstrated that this goal is achievable. Radiomics, radiogenomics, and habitat imaging based on computed tomography (CT) and magnetic resonance imaging (MRI) have shown that imaging can capture biologically meaningful heterogeneity associated with MVI, histological grade, molecular subtype, recurrence risk, and treatment response [8-12]. These approaches have established the conceptual benchmark for noninvasive phenotyping of HCC.

Ultrasound offers unique advantages for extending this paradigm. It is the most widely available imaging modality worldwide, provides real-time assessment without ionizing radiation, is inexpensive, repeatable, and ideally suited for longitudinal monitoring. Beyond conventional B-mode imaging, modern ultrasound interrogates complementary biological dimensions through contrast-enhanced ultrasound (CEUS), shear-wave elastography (SWE), quantitative ultrasound and radiofrequency (RF) analysis, and advanced microvascular imaging. Nevertheless, much of the rich acoustic information contained within the raw ultrasound signal remains underutilized because conventional clinical imaging relies primarily on processed, envelope-detected, and log-compressed images, while quantitative acoustic features are rarely exploited in routine practice.

This narrative review examines how multimodal ultrasound and acoustic radiomics may enable noninvasive decoding of ITH in HCC, and proposes a testable hypothesis generation framework: acoustic heterogeneity signatures. This framework has not yet been established in HCC and should not be regarded as a clinical recommendation. We first summarize the biological foundations of ITH and the benchmark established by CT- and MRI-based radiomics and habitat imaging. We then review the complementary contributions of CEUS, elastography, quantitative ultrasound, radiofrequency analysis, and advanced microvascular imaging, and discuss how artificial intelligence may integrate these modalities into biologically meaningful acoustic heterogeneity signatures. Finally, we highlight the methodological challenges that must be addressed before multimodal ultrasound can evolve from a diagnostic imaging modality into a standardized, interpretable, and clinically validated biomarker platform for precision management of HCC (Fig. 1).

Intratumoral Heterogeneity as A Determinant of Prognosis in HCC

The molecular classification of HCC provides the biological framework for understanding ITH. Transcriptomic profiling has identified reproducible molecular subclasses, including the Hoshida S1, S2, and S3 subtypes, characterized respectively by activation of WNT/transforming growth factor-β signaling, proliferative programs with MYC/AKT activation and progenitor-cell features, and a more differentiated phenotype associated with favorable prognosis [13]. These molecular subclasses were subsequently integrated with genomic analyses from The Cancer Genome Atlas, leading to the widely adopted distinction between proliferative and non-proliferative HCC [4,6,14]. The proliferative class, accounting for approximately half of HCCs, is characterized by TP53 alterations, chromosomal instability, elevated alpha-fetoprotein levels, progenitor-cell characteristics, increased angiogenesis, and aggressive clinical behavior. In contrast, the non-proliferative class is frequently associated with CTNNB1/WNT pathway activation, greater cellular differentiation, and an immune-excluded tumor microenvironment that has been linked to reduced responsiveness to immune checkpoint inhibition [6,14,15].

However, molecular diversity extends well beyond differences between individual tumors. Multiregion sequencing and single-cell analyses have demonstrated that a single HCC lesion is composed of spatially distinct and dynamically evolving subclonal populations that differ in genetic alterations, vascular architecture, hypoxia, immune composition, metabolic activity, and

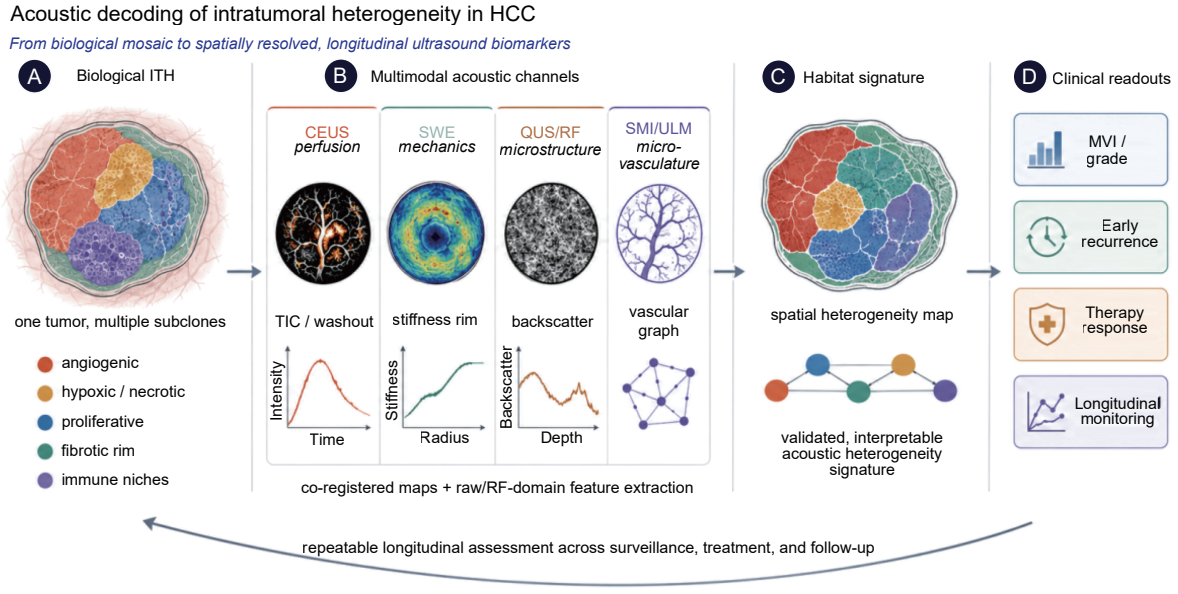


Figure 1 Conceptual framework for decoding intratumoral heterogeneity of hepatocellular carcinoma (HCC) using multimodal ultrasound. HCC is depicted as a spatially heterogeneous biological mosaic composed of distinct tumor habitats. Four complementary acoustic dimensions are interrogated by multimodal ultrasound: tumor perfusion using contrast-enhanced ultrasound (CEUS), biomechanical properties using shear-wave elastography (SWE), tissue microstructure using quantitative ultrasound and radiofrequency (QUS/RF) analysis, and vascular architecture using superb microvascular imaging (SMI) or ultrasound localization microscopy (ULM). Integration of these complementary imaging biomarkers through acoustic radiomics and artificial intelligence enables the generation of a multimodal acoustic heterogeneity signature, with potential applications in noninvasive prediction of microvascular invasion (MVI), histological grade, recurrence risk, treatment response, and longitudinal monitoring for precision management of HCC.

proliferative potential [3,5]. This spatial and temporal heterogeneity evolves during tumor progression and under therapeutic pressure, resulting in continuous clonal selection and adaptation. Consequently, biological characteristics obtained from a single biopsy often fail to represent the complexity of the entire tumor.

Integrative multiregion profiling has further demonstrated that gene-expression signatures derived from multiple tumor regions predict patient survival more accurately than those obtained from a single biopsy, providing direct evidence that conventional tissue sampling systematically underestimates clinically relevant heterogeneity [3,5]. Likewise, distinct spatial vascular phenotypes, such as the vessels that encapsulate tumor clusters (VETC) pattern, illustrate how regional differences in tumor architecture translate into specific mechanisms of dissemination, metastatic behavior, prognosis, and differential response to antiangiogenic therapy [16].

Collectively, these findings establish intratumoral heterogeneity as a measurable biological property that fundamentally determines tumor aggressiveness, recurrence, therapeutic response, and patient survival. The major challenge is therefore no longer to demonstrate that heterogeneity exists, but to develop robust, noninvasive imaging biomarkers capable of capturing its spatial distribution and temporal evolution throughout the entire tumor. Such biomarkers would enable longitudinal assessment of tumor biology beyond the inherent limitations of focal biopsy and conventional staging systems.

Imaging of Tumor Heterogeneity: The Benchmark Established by CT and MRI

Cross-sectional imaging has fundamentally transformed the concept of tumor characterization from purely anatomical assessment to noninvasive phenotyping of tumor biology. Radiomics has demonstrated that routinely acquired CT and MRI images contain quantitative information reflecting intratumoral heterogeneity, enabling prediction of clinically relevant outcomes such as MVI, histological grade, early recurrence, and overall survival. Meta-analyses of CT- and MRI-based radiomics report pooled areas under the receiver operating characteristic curve (AUROCs) approaching 0.90 for preoperative prediction of MVI, highlighting the considerable potential of imaging-derived biomarkers [8]. However, these encouraging results should be interpreted with caution because methodological heterogeneity between studies is substantial ($I^2 \approx 94\%$) [8], radiomics quality scores remain modest, and external validation is still limited.

Beyond conventional radiomics, radiogenomics has established direct links between imaging phenotypes and underlying molecular pathways. A CT-derived radiogenomic signature has been shown to predict microvascular invasion with high accuracy (the diagnostic accuracy, sensitivity, and specificity was 89%, 76% and 94%, respectively) [9], while characteristic features on gadoxetic acid-enhanced MRI, including rim arterial-

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phase hyperenhancement and hepatobiliary-phase hypointensity, have been associated with proliferative tumor biology and β-catenin pathway activation, respectively [10,11]. These observations demonstrate that imaging features can serve as surrogate biomarkers of molecular tumor characteristics rather than merely depicting anatomical morphology.

More recently, habitat imaging has extended this concept by moving beyond whole-lesion feature averaging toward spatially resolved analysis of intratumoral heterogeneity. Instead of treating the tumor as a single homogeneous region of interest, multiparametric voxel clustering identifies biologically distinct subregions-or habitats-that may represent viable proliferative tissue, hypoxic regions, necrosis, or transitional zones (Fig. 2). By preserving spatial information, habitat imaging more faithfully reflects the biological complexity of HCC and has shown improved performance over conventional whole-tumor radiomics for predicting MVI, recurrence, survival, and treatment response [12].

Importantly, these advances have been achieved almost exclusively with CT, MRI, and, to a lesser extent, positron emission tomography. In contrast, an analogous framework for multimodal ultrasound, integrating perfusion, tissue mechanics, acoustic microstructure, and vascular architecture into spatially resolved acoustic habitats, has yet to be established. This represents both a significant methodological challenge and a major opportunity. Ultrasound possesses unique advantages, including real-time imaging, repeatability, portability, and the ability to combine complementary acoustic modalities within a single examination. However, as with CT and MRI radiomics, robust clinical translation will require standardized acquisition protocols, harmonized feature extraction, biological validation, and rigorous multicenter external validation. Ultimately, the experience gained from CT and MRI provides not only the benchmark but also the roadmap for developing biologically interpretable acoustic biomarkers of intratumoral heterogeneity.

Multimodal Ultrasound for Characterizing Intratumoral Heterogeneity

Unlike conventional cross-sectional imaging, ultrasound interrogates tissue through multiple complementary acoustic mechanisms that reflect different aspects of tumor biology. Rather than relying solely on grayscale morphology, modern ultrasound provides quantitative information on perfusion, tissue mechanics, acoustic microstructure, and vascular architecture, thereby offering a multidimensional characterization of intratumoral heterogeneity. These complementary acoustic dimensions can be acquired repeatedly during routine clinical examinations, making ultrasound uniquely suited for longitudinal assessment of the evolving tumor phenotype.

The biological richness of ultrasound originates from the underlying acoustic signal. Conventional B-mode images represent processed, envelope-detected, and log-

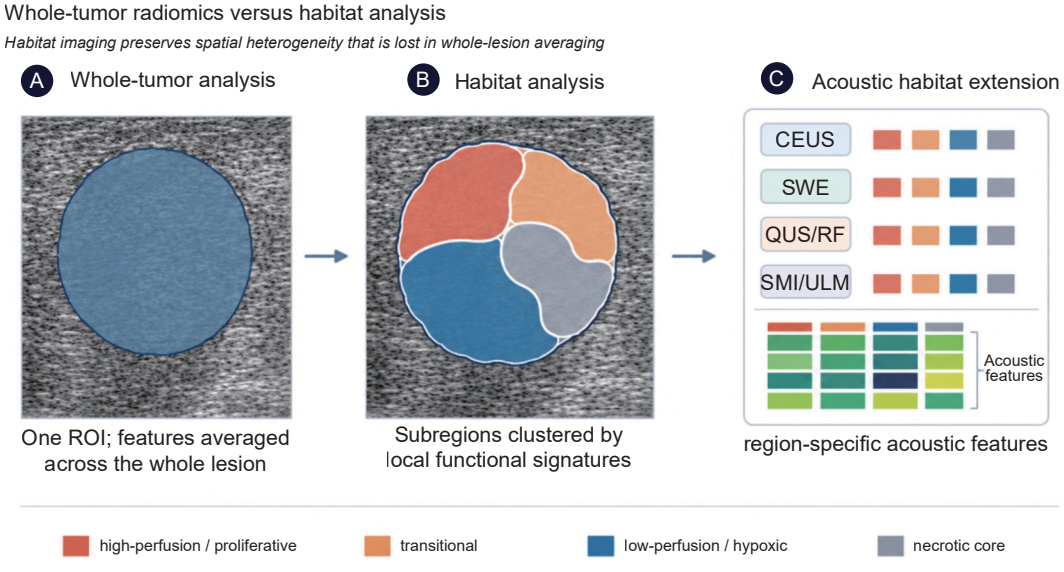


Figure 2 Whole-tumor versus habitat analysis for imaging intratumoral heterogeneity. (A) Conventional whole-tumor radiomics treats the lesion as a single region of interest, averaging quantitative imaging features across the entire tumor and thereby potentially obscuring spatial biological heterogeneity; (B) Habitat analysis preserves spatial information by clustering multiparametric imaging data into distinct imaging habitats that may correspond to biologically different tumor regions, including hyperperfused, transitional, hypoperfused, and necrotic compartments; (C) Conceptual extension to multimodal ultrasound, in which contrast-enhanced ultrasound (CEUS), shear-wave elastography (SWE), quantitative ultrasound/radiofrequency (QUS/RF) analysis, and superb microvascular imaging or ultrasound localization microscopy (SMI/ULM) are spatially integrated to generate region-specific acoustic habitats and a comprehensive multimodal acoustic heterogeneity signature for biological phenotyping of hepatocellular carcinoma. Schematic illustration.

arithmically compressed displays of backscattered echoes, optimized for visual interpretation but containing only part of the available acoustic information. QUS and RF-domain analysis preserve additional signal characteristics that are largely inaccessible on standard B-mode images. Together with CEUS, SWE, and advanced microvascular imaging, these techniques interrogate complementary biological properties rather than redundant information. Their integration forms the conceptual foundation of acoustic radiomics, in which multiple acoustic biomarkers are combined to characterize the spatial and temporal heterogeneity of HCC.

Contrast-enhanced ultrasound: perfusion heterogeneity

Contrast-enhanced ultrasound (CEUS) provides a real-time assessment of tumor perfusion using strictly intravascular microbubble contrast agents. Because the microbubbles remain confined to the vascular compartment, enhancement patterns directly reflect tissue perfusion and vascular architecture rather than extracellular diffusion. Arterial-phase enhancement, washout kinetics, and quantitative time–intensity curve (TIC) analysis provide complementary information on tumor angiogenesis, vascular density, and perfusion heterogeneity (Fig. 3). The CEUS Liver Imaging Reporting and Data System (CEUS LI-RADS) has standardized the diagnostic interpretation of focal liver lesions [17], while perfluorobutane microbubbles (Sonazoid) enable additional late-phase imaging related to uptake by Kupffer cells, allowing defect-reperfusion assessment and improved lesion characterization [18].

More recently, CEUS has evolved beyond lesion detection toward quantitative characterization of tumor biology. Multiparametric perfusion analysis and radiomic feature extraction have demonstrated the ability to predict clinically relevant pathological characteristics, including MVI, histological grade, and proliferative activity. In a prospective multicenter study, a Sonazoid-enhanced CEUS model predicted MVI with an externally validated AUROC of 0.81 [19], while Kupffer-phase radiomics has shown promising results for predicting histological differentiation and Ki-67 expression [20]. These findings suggest that the greatest diagnostic value of CEUS lies not in isolated perfusion parameters but in the integrated analysis of spatially heterogeneous enhancement patterns. Future developments will likely combine dynamic perfusion analysis with regional radiomic features and habitat-based assessment, allowing CEUS to characterize biologically distinct vascular

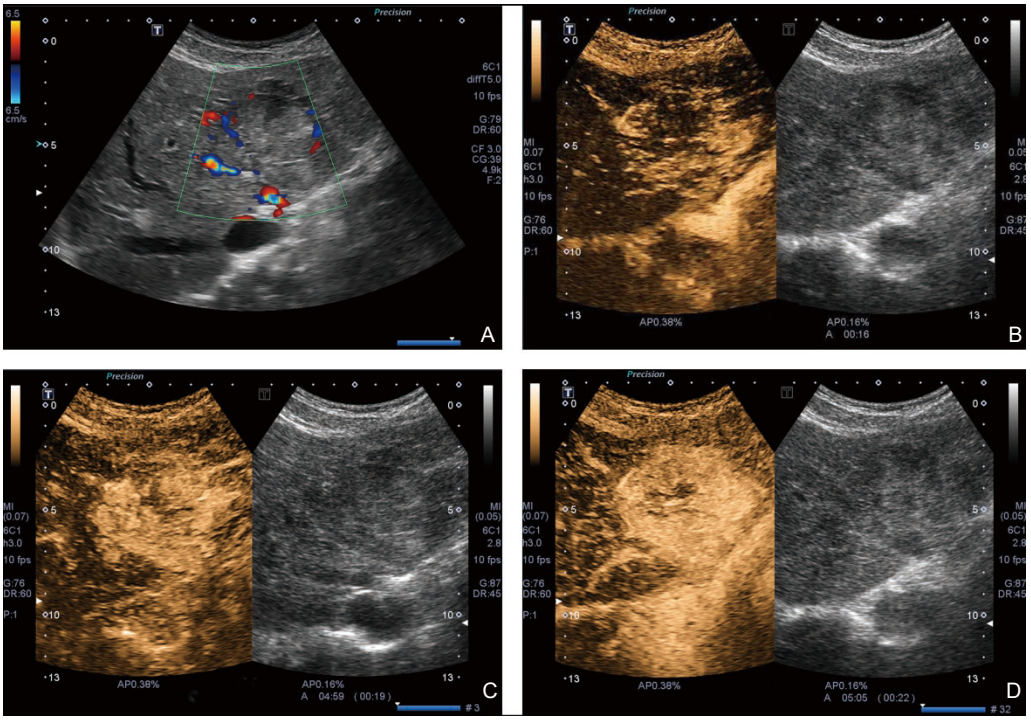


Figure 3 Representative contrast-enhanced ultrasound (CEUS) findings in hepatocellular carcinoma (HCC). A 72-year-old man with HCC confirmed by surgical histopathology (Edmondson–Steiner Grade II). (A) Grayscale ultrasound with color Doppler imaging demonstrates a heterogeneous hyperechoic liver lesion with peripheral vascular signals; (B, C) Following intravenous administration of 2.0 mL SonoVue®, the lesion exhibits heterogeneous arterial-phase hyperenhancement with a characteristic mosaic enhancement pattern; (D) Time-intensity curve analysis demonstrates peak enhancement at 23 s. Washout begins at approximately 45 s, followed by persistent hypoenhancement during the portal venous and late phases, consistent with the typical CEUS enhancement pattern of HCC. This case illustrates the marked intratumoral perfusion heterogeneity that can be visualized by CEUS and forms the basis for quantitative perfusion analysis and acoustic radiomics.

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subregions within individual HCC lesions rather than relying on single global descriptors.

Shear-wave elastography: mechanical heterogeneity

SWE provides a quantitative assessment of the biomechanical properties of HCC, offering insights into tumor heterogeneity beyond conventional morphology. By measuring shear-wave propagation, SWE estimates tissue elasticity, while emerging shear-wave dispersion and viscoelastic techniques may additionally characterize viscosity-related properties. These biomechanical parameters reflect the complex interplay of extracellular matrix remodeling, stromal organization, fibrosis, tumor cellularity, necrosis, and the peritumoral desmoplastic response, thereby providing complementary information on the tumor microenvironment.

Growing evidence suggests that the peritumoral mechanical phenotype may be more informative than intratumoral stiffness alone. In patients with hepatitis B virus-related HCC, a two-dimensional SWE-based four-region nomogram predicted recurrence-free survival with a concordance index of 0.77, with increased peritumoral stiffness emerging as an independent prognostic factor [21]. Likewise, the "stiff rim" sign and increased stiffness within the peritumoral shell have been proposed as imaging correlates of capsular invasion, stromal remodeling, and tumor-host interactions, all of which are closely linked to aggressive biological behavior.

From the perspective of intratumoral heterogeneity, SWE contributes a distinct mechanical dimension that complements the vascular information obtained from CEUS. Rather than depicting perfusion, elastography characterizes the spatial distribution of tissue mechanical properties, potentially identifying regions of fibrosis, viable tumor, necrosis, or stromal reaction within and around the lesion. Such regional biomechanical mapping may ultimately become an important component of multimodal acoustic habitat analysis.

Despite its considerable promise, several technical challenges currently limit the reproducibility and clinical translation of SWE-derived biomarkers. Elasticity measurements remain influenced by lesion depth, acoustic attenuation, respiratory and cardiac motion, probe compression, acquisition protocol, and inter-vendor variability. Furthermore, standardized acquisition procedures, harmonized quantitative parameters, and universally accepted cutoff values have not yet been established. Consequently, future studies should focus on multicenter standardization, biological validation, and external validation before SWE-derived heterogeneity biomarkers can be incorporated into routine clinical decision-making.

Quantitative ultrasound and radiofrequency analysis: microstructural heterogeneity

QUS and RF-domain analysis provide access to the intrinsic acoustic information contained within the backscattered ultrasound signal, enabling characterization of tissue microstructure beyond the spatial resolution of conventional B-mode imaging. Whereas standard B-mode images represent processed, envelope-detected, and logarithmically compressed signals optimized for visual interpretation, QUS analyzes the original acoustic data to derive quantitative descriptors of tissue architecture. Parameters derived from envelope statistics, including the Nakagami shape parameter, together with spectral features such as the backscatter coefficient and RF power spectrum, reflect the density, size, distribution, and organization of sub-resolution acoustic scatterers, thereby providing indirect information on tissue composition and microstructural heterogeneity [22,23].

Unlike CEUS and elastography, which primarily interrogate vascular and biomechanical properties, QUS and RF analysis capture a distinct microstructural dimension of tumor biology. Changes in cellular architecture, extracellular matrix organization, fibrosis, necrosis, and tumor cellularity all influence acoustic scattering behavior and may therefore generate measurable imaging biomarkers. By quantifying these subtle alterations, RF-domain analysis has the potential to reveal biological heterogeneity that remains invisible on conventional grayscale imaging.

Although experience in HCC is still limited, the initial results are highly encouraging. A radiomics model based on raw RF signals predicted microvascular invasion (MVI) with an AUROC of 0.95 in a preliminary single-center study, outperforming conventional grayscale radiomic features [24]. Nevertheless, the cohort comprised only 42 patients, and the absence of external validation precludes conclusions regarding generalizability. These findings should therefore be regarded as proof of concept rather than established clinical evidence.

To date, the most mature clinical applications of QUS have focused on diffuse liver diseases, including hepatic steatosis, fibrosis, and treatment monitoring after thermal ablation, where quantitative acoustic parameters have demonstrated good reproducibility and biological relevance. In contrast, the application of QUS and RF-domain analysis to focal liver tumors remains in its infancy. Wider access to raw RF data from clinical ultrasound systems, standardized acquisition protocols, harmonized feature extraction, and prospective multicenter validation will be essential for clinical translation.

From the perspective of multimodal imaging, QUS contributes the microstructural component of an integrated acoustic heterogeneity signature. Combined with CEUS-derived perfusion, SWE-derived biomechanical information, and advanced microvascular imaging, RF-domain biomarkers have the potential to provide a comprehensive, biologically interpretable characterization of intratumoral heterogeneity in HCC. Such integration represents one of the most promising future directions for ultrasound-based radiomics and precision imaging.

Microvascular and super-resolution imaging: vascular architectural heterogeneity

Advanced microvascular imaging contributes a fourth and complementary acoustic dimension by characterizing the architectural organization of the tumor microvasculature. Unlike CEUS, which primarily assesses tissue perfusion and vascular dynamics, these techniques visualize the morphology and spatial distribution of small blood vessels, providing structural information that reflects tumor angiogenesis, vascular remodeling, and intratumoral vascular heterogeneity.

Contrast-free microvascular imaging techniques, such as superb microvascular imaging (SMI), employ advanced clutter-suppression algorithms to detect slow-flow signals within small vessels without the need for contrast agents. In focal liver lesions, SMI-derived vascular indices have demonstrated significant correlations with histological microvessel density and improve visualization of intratumoral vascular architecture compared with conventional Doppler imaging [25]. These observations suggest that microvascular imaging can provide noninvasive surrogate markers of tumor angiogenesis and vascular complexity.

An even higher level of spatial resolution is achieved with ultrasound localization microscopy (ULM). By localizing and tracking individual microbubbles over thousands of sequential frames, ULM reconstructs vascular networks beyond the conventional acoustic diffraction limit, enabling visualization of vessels approaching the capillary scale [26]. Recent advances have demonstrated the feasibility of ULM in the human liver using high-frame-rate clinical ultrasound systems, achieving several-fold improvements in vascular resolution during short breath-hold acquisitions [27]. Building upon these developments, the emerging field of ultrasound microvasculomics applies high-throughput quantitative feature extraction to super-resolution vascular maps, generating imaging biomarkers that describe vessel density, branching topology, tortuosity, connectivity, and network organization [28].

From the perspective of intratumoral heterogeneity, microvascular imaging complements perfusion-based CEUS by depicting the structural organization rather than the functional consequences of tumor angiogenesis. Together, these approaches offer the potential to characterize regional vascular phenotypes associated with aggressive tumor biology, hypoxia, microvascular invasion, and therapeutic response, thereby providing an essential component of multimodal acoustic habitat analysis.

Despite their considerable promise, several challenges currently limit clinical implementation. Image quality remains sensitive to respiratory and tissue motion, prolonged acquisition times, and two-dimensional sampling of inherently three-dimensional vascular networks. Furthermore, standardized acquisition protocols, harmonized quantitative vascular biomarkers, and robust histopathological validation against human microvascular architecture are still lacking. Prospective multicenter studies incorporating three-dimensional imaging, motion correction, and biological validation will be required before microvascular imaging and super-resolution ultrasound can become routine biomarkers of vascular heterogeneity in HCC.

Artificial Intelligence and Acoustic Radiomics

Artificial intelligence (AI) provides the computational framework for integrating multimodal ultrasound into biologically meaningful imaging biomarkers of intratumoral heterogeneity. While individual ultrasound modalities interrogate complementary aspects of tumor biology-including perfusion, biomechanics, microstructure, and vascular architecture-AI enables these diverse acoustic signals to be combined into quantitative models that capture the complex spatial and temporal organization of HCC.

The foundation of this approach is acoustic radiomics, which transforms medical images into high-dimensional quantitative data by extracting large numbers of imaging features beyond visual perception. These include first-order intensity descriptors, texture features, wavelet transformations, envelope statistics, spectral parameters, shape descriptors, and regional or habitat-based features that collectively quantify tumor heterogeneity [22,23,29]. Machine-learning algorithms subsequently integrate these quantitative imaging biomarkers with clinical, laboratory, and pathological variables to generate predictive models, whereas deep-learning approaches directly learn hierarchical feature representations from imaging data without requiring predefined feature engineering. To avoid repeating generic radiomics limitations, we focus here on HCC-specific barriers, including cirrhosis and steatosis confounding QUS, respiratory motion and depth effects on RF signals, variability in CEUS phases and contrast agent

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dose, SWE confounding by portal hypertension, vendor-dependent raw RF access.

Recent studies demonstrate the potential of AI-assisted ultrasound for precision oncology. A spatiotemporal deep-learning model based on CEUS achieved a test-set AUROC of 0.865 for preoperative prediction of MVI and independently stratified both recurrence-free and overall survival [30]. Likewise, a multicenter study comparing handcrafted radiomics with deep-learning approaches demonstrated improved robustness of deep-learning models across institutions, although performance declined during independent cross-vendor external validation, highlighting the persistent challenge of generalizability [31].

Beyond prediction alone, AI offers the opportunity to integrate the complementary information provided by CEUS, SWE, QUS, radiofrequency analysis, and advanced microvascular imaging into a unified acoustic heterogeneity signature. Such multimodal models may ultimately characterize distinct biological habitats within individual tumors, providing a noninvasive representation of spatial heterogeneity that more closely reflects the underlying tumor microenvironment than any single imaging modality.

However, successful clinical translation requires considerably more than high discriminatory performance. Future AI models must demonstrate robust calibration, prospective multicenter validation, cross-vendor reproducibility, and resilience to variations in acquisition protocols and patient populations. Equally important is biological interpretability. Explainable AI techniques, including gradient-weighted class activation mapping (Grad-CAM) [32] and Shapley additive explanations (SHAP), can provide insight into model decision-making, but their biological fidelity and clinical reliability require rigorous validation rather than assumption. Ultimately, the value of acoustic radiomics will depend not only on predictive accuracy but also on transparency, reproducibility, and its ability to improve clinical decision-making beyond established imaging and staging systems. In this review, we use "acoustic radiomics" specifically for AI analysis of raw RF or QUS parametric maps, as distinct from conventional B-mode radiomics, which uses post-processed B-mode images as input.

Clinical Applications

The clinical relevance of noninvasive characterization of intratumoral heterogeneity extends throughout the entire management pathway of HCC, from diagnosis and treatment planning to response assessment and longitudinal surveillance. By providing a comprehensive representation of tumor biology, multimodal ultrasound has the potential to complement conventional staging systems and support more individualized clinical decision-making.

In the preoperative setting, imaging-derived biomarkers of tumor heterogeneity may improve risk stratification by predicting biologically aggressive features such as MVI, histological grade, and early recurrence. Such information could influence surgical planning, including the extent of resection, postoperative surveillance strategies, and selection of patients who may benefit from neoadjuvant or adjuvant treatment approaches. Similarly, more accurate assessment of tumor biology may contribute to refining patient selection for liver transplantation when integrated with established clinical criteria rather than replacing them.

Current evidence demonstrates encouraging, although still evolving, diagnostic performance. Meta-analyses report pooled AUROCs of approximately 0.81 for ultrasound-based radiomics in predicting MVI, while deep-learning models have shown promising internal performance that generally decreases during independent external and cross-vendor validation [31,33] (Table 1). These observations emphasize both the potential of acoustic biomarkers and the continuing need for standardized acquisition, multicenter validation, and robust model generalization.

Beyond preoperative risk assessment, multimodal

Table 1 Representative reported performance of imaging biomarkers for preoperative prediction of microvascular invasion in hepatocellular carcinoma (non-head-to-head comparison)

Modality/model	Study design	AUROC (internal/external)	Principal limitation
CT/MRI radiomics	Meta-analysis	0.90 (pooled)	$I^2 \approx 94\%$; low Radiomics Quality Score [8]
CEUS deep learning (spatiotemporal)	Single center	0.865 (test)	Small independent test set [30]
Sonazoid-CEUS	Prospective, multicenter	0.81 (external)	Single nodule ≤ 5 cm [19]
Ultrasound radiomics	Meta-analysis	0.81 (pooled)	Small, partly overlapping samples [33]
Ultrasound deep learning	Cross-institutional	0.80-0.82/0.67-0.69	Generalization gap on external data [31]
Original-RF radiomics (current authors)	Single center	0.95 (internal)	$n = 42$; no external validation [24]

AUROC, area under the receiver operating characteristic curve; CEUS, contrast-enhanced ultrasound; CT, computed tomography; MRI, magnetic resonance imaging; RF, radiofrequency. Bracketed numbers denote references. Values are reported as published; direct comparison across studies is limited by differences in cohort, reference standard, and validation design

ultrasound may provide valuable prognostic information. Quantitative biomarkers derived from CEUS and shear-wave elastography have demonstrated associations with recurrence-free and overall survival, suggesting that imaging can capture biological characteristics linked to long-term outcome [21,30]. Furthermore, imaging is increasingly being explored as a noninvasive tool for predicting and monitoring response to locoregional therapies, including transarterial interventions, as well as systemic treatments such as targeted therapy and immunotherapy. By repeatedly characterizing changes in perfusion, tissue mechanics, vascular architecture, and acoustic microstructure, ultrasound may enable dynamic monitoring of treatment-induced alterations in tumor biology. Integration of imaging phenotypes with molecular and immunological characteristics also offers the prospect of identifying imaging biomarkers associated with the tumor immune microenvironment and therapeutic responsiveness [12].

Ultimately, the clinical value of multimodal ultrasound will not be determined solely by predictive accuracy or improvements in AUROC. Its true impact will depend on whether imaging-derived heterogeneity biomarkers provide incremental prognostic information beyond established staging systems, improve risk reclassification, influence therapeutic decision-making, and ultimately translate into better patient outcomes. Prospective studies demonstrating clinical utility and cost-effectiveness will therefore be essential before acoustic heterogeneity signatures can be incorporated into routine precision management of HCC (Fig. 4).

Challenges and Future Directions

Despite rapid technological progress, several important challenges must be overcome before multimodal ultrasound can become a clinically established biomarker of intratumoral heterogeneity. The principal limitation is no longer the ability to generate increasingly sophisticated imaging biomarkers, but rather to demonstrate that these biomarkers are robust, reproducible, biologically meaningful, and clinically actionable.

The first challenge is generalizability. Many radiomic and deep-learning models demonstrate excellent performance in single-center cohorts but show substantially reduced accuracy when evaluated across different institutions, ultrasound systems, and acquisition protocols [29,31]. Unlike CT and MRI, ultrasound remains highly operator dependent and lacks a standardized biomarker framework comparable to the Image Biomarker Standardization Initiative (IBSI). This limitation is particularly relevant for quantitative RF-domain analysis, where differences in beamforming, signal processing, and proprietary reconstruction algorithms currently hinder direct comparison between vendors.

The second challenge is reproducibility. Quantitative

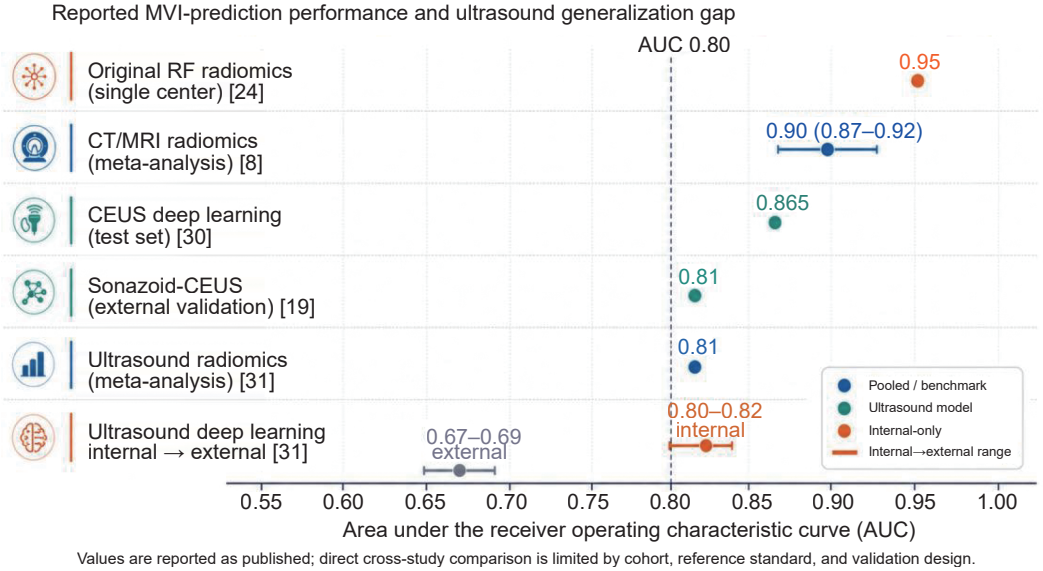


Figure 4 Representative reported diagnostic performance of imaging biomarkers for the preoperative prediction of microvascular invasion (MVI) in hepatocellular carcinoma (HCC) (non-head-to-head comparison). Dots represent reported areas under the receiver operating characteristic curve (AUROCs) or pooled estimates from published studies, whereas horizontal bars indicate 95% confidence intervals or the range between internal and external validation when available. The dashed vertical line denotes an AUROC of 0.80, a commonly accepted threshold for good diagnostic discrimination. Although several imaging approaches-including CT/MRI radiomics, contrast-enhanced ultrasound (CEUS), ultrasound radiomics, deep learning, and radiofrequency (RF)-based analysis-have demonstrated promising diagnostic performance, the decline observed between internal and external validation of ultrasound deep-learning models highlights the persistent challenge of model generalizability. Due to differences in study design, patient populations, reference standards, and validation strategies, the parallel display in the figure is only used to illustrate the research background, and the ranking is based solely on reported values and does not represent the level of evidence.

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imaging features are influenced by numerous technical factors, including acquisition settings, probe orientation, respiratory and cardiac motion, lesion segmentation, and image preprocessing. Higher-order texture features may also be sensitive to annotation variability and uncertainty in pathological reference standards, particularly for endpoints such as microvascular invasion or recurrence [8,29]. Standardized acquisition protocols, phantom-based calibration, harmonized feature definitions, and reproducibility-aware feature selection will therefore be essential prerequisites for multicenter studies.

A third obstacle is access to quantitative acoustic data. Although conventional B-mode images are universally available, the original RF signals that contain much of the microstructural information remain inaccessible on many clinical ultrasound platforms. Vendor-specific data formats, proprietary beamforming algorithms, and differences in gain compensation and signal processing currently limit interoperability and restrict the development of truly vendor-independent acoustic biomarkers. Broader availability of standardized RF data and open acquisition interfaces would substantially accelerate methodological development and external validation.

Finally, successful clinical implementation requires transparent and biologically interpretable artificial intelligence. Predictive performance alone is insufficient for clinical adoption. Future models should provide reliable calibration, uncertainty estimation, and interpretable explanations of their predictions. Explainable AI methods, including Grad-CAM and SHAP, offer promising approaches to understanding model behavior, but their biological validity and clinical reliability require prospective evaluation rather than assumption. Ultimately, imaging biomarkers must demonstrate not only statistical accuracy but also a meaningful relationship to underlying tumor biology and an ability to improve clinical decision-making.

Future research should therefore focus on prospective, multicenter, externally validated studies incorporating standardized multivendor acquisition, access to raw acoustic data, phantom calibration, biologically validated reference standards, rigorous external validation, and comprehensive reporting of discrimination, calibration, clinical utility, and decision-curve analyses. Integration of complementary ultrasound modalities-including contrast-enhanced ultrasound, shear-wave elastography, quantitative ultrasound, radiofrequency analysis, and advanced microvascular imaging-offers the opportunity to develop a unified multimodal acoustic heterogeneity signature that captures perfusion, biomechanical properties, tissue microstructure, and vascular architecture within a single imaging framework.

Ultimately, the future of ultrasound lies not in replacing existing imaging modalities but in providing a repeatable, real-time, and biologically informed assessment of tumor heterogeneity throughout the disease course. If supported by rigorous standardization, biological validation, and prospective clinical evaluation, acoustic decoding has the potential to evolve from an emerging research concept into a practical imaging biomarker platform for precision management of hepatocellular carcinoma, enabling improved risk stratification, treatment selection, response monitoring, and personalized patient care.

Conclusion

Intratumoral heterogeneity is a defining hallmark of hepatocellular carcinoma and a principal determinant of tumor progression, recurrence, therapeutic resistance, and patient outcome. Over the past decade, radiomics, radiogenomics, and habitat imaging based on CT and MRI have demonstrated that clinically relevant aspects of tumor biology can be assessed noninvasively, establishing an important paradigm for precision oncology.

Multimodal ultrasound now offers a complementary and uniquely versatile approach to this challenge. Contrast-enhanced ultrasound characterizes perfusion heterogeneity, shear-wave elastography interrogates biomechanical properties and the peritumoral microenvironment, quantitative ultrasound and radiofrequency analysis provide insights into tissue microstructure beyond the spatial resolution of conventional B-mode imaging, and advanced microvascular imaging depicts the architecture of tumor angiogenesis. Individually, these modalities provide complementary biological information; collectively, they have the potential to generate a comprehensive acoustic heterogeneity signature that reflects the complex spatial and temporal organization of HCC. The proposed multimodal acoustic habitat framework has not yet been established in HCC. Current evidence supports individual modalities, but not their integration into an acoustic heterogeneity signature; this review is therefore hypothesis-generating.

The integration of these complementary acoustic dimensions through radiomics and artificial intelligence represents a promising step toward biologically informed imaging biomarkers capable of supporting preoperative prediction of microvascular invasion and histological grade, individualized risk stratification, treatment selection, response assessment, and longitudinal monitoring. However, successful clinical translation will require standardized acquisition protocols, access to quantitative acoustic data, biologically validated imaging biomarkers, prospective multicenter studies, and rigorous external validation.

Ultimately, the future of ultrasound lies not only in

detecting liver tumors but in decoding their biological behavior. If these methodological challenges can be addressed, multimodal ultrasound may evolve from a predominantly diagnostic imaging modality into a real-time, repeatable, and interpretable biomarker platform that enables precision management of hepatocellular carcinoma and advances truly personalized oncologic care.

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Authors' Contributions

DY and DC conceived of and designed the study; CJY and LWY drafted the manuscript; QYJ, WL, WY and HYL revised the article.

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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

Not applicable.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Consent for publication

Not applicable.

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