

Contrast-enhanced Ultrasound Imaging: State of the Art

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The introduction of a variety of ultrasound contrast agents has a significant impact on the utilization of diagnostic ultrasound in many clinical settings. This article is intended to provide an overview of the principles behind ultrasound contrast agents along with contrast-specific imaging techniques and a review of their current and potential clinical applications. Ultrasound contrast agents have dramatically improved the sensitivity and specificity of current ultrasound diagnoses and have the potential of expanding the already broad range of ultrasound applications. Future improvements in imaging techniques combined with new developments of contrast agents will make ultrasound an even more powerful diagnostic modality.

KEY WORDS — harmonic imaging, microbubble, tumor diagnosis, ultrasound contrast agents, ultrasound imaging technology

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Introduction

Contrast enhancement has become a routine part of clinical radiography, computed tomography (CT), and magnetic resonance imaging (MRI), increasing their diagnostic capabilities. During the last two decades, contrast-enhanced ultrasound imaging has been investigated and has gradually emerged in clinical settings. Concurrent with technological improvements in ultrasound scanning equipment, contrast agents have been developed to meet the demands of this rapidly expanding field of imaging.

The rapid development of contrast agents for ultrasound is precipitated by the performance limits of ultrasound imaging and Doppler techniques. As ultrasound is used to study smaller and deeper structures in the body, the spatial resolution of grayscale imaging and Doppler sensitivity becomes impaired to the degree that it impacts the clinical utility of ultrasound. Contrast agents promise to improve the sensitivity and specificity of current ultrasound diagnoses and have the potential to expand the already broad range of ultrasound applications. This article is intended to provide an overview of the principles behind ultrasound

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contrast agents and a review of the clinical applications in which they are useful.

Types of Contrast Agents Available

During the last decade, many new ultrasound contrast agents have been developed, which are characterized by both smaller microbubble mean size ($< 10 \mu\text{m}$) and prolonged persistence within the cardiovascular circulation (increased from less than 1 minute to as much as 10 minutes). Various techniques are used to combine materials that control the microbubble surface (the encapsulating shell) with gases that inhibit diffusion and microbubble dissolution (air vs. heavy gases such as perfluorocarbons). At present, several contrast agents have been approved for clinical use while others are in various stages of development (Table).

Basic Principles

Vascular ultrasound contrast agents consist of gas-filled microbubbles stabilized by a thin shell (Fig. 1). They are typically $< 8 \mu\text{m}$ in diameter, which allows them to pass through the pulmonary circulation

and systemic capillary beds [1]. When administered intravenously, ultrasound contrast agents improve the detection of blood flow and depiction of the vasculature in a variety of structures compared to conventional (i.e. non-contrast) ultrasound due to increased signal-to-noise ratio (SNR). These agents significantly enhance the acoustic backscatter from blood in both Doppler and grayscale modes, due to the large impedance difference between the gas and the surrounding blood. Previous techniques have used the enhancement of Doppler flow signals (either color Doppler or power Doppler) to visualize the presence of a contrast agent in the vessels or

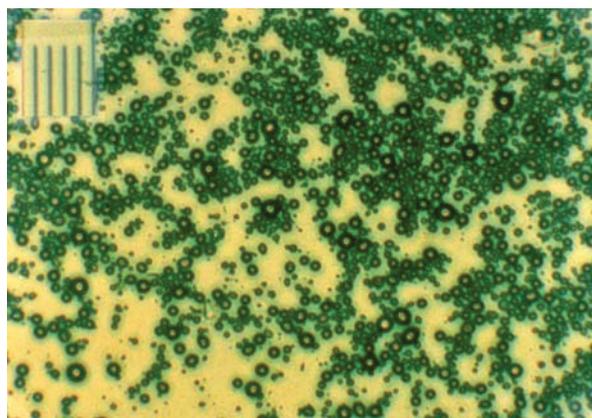


Fig. 1. Microscopic view of microbubbles 2–8 μm in size.

Table. List of ultrasound contrast agents

Name	Shell composition	Gas	Manufacturer
AI-700	Polymer	Perfluorocarbon	Acusphere
biSphere	Gelatin/polymer	Air	Point Biomedical
BR14	Phospholipid	Perfluorobutane	Bracco Diagnostics
BY 963	Lipid	Air	Byk-Gulden
Levovist	Galactose/palmitic acid	Air	Schering
Definity	Lipid	Perfluoropropane	Bristol-Myers Squibb
Imagent	Surfactant	Perfluorocarbon	Imcor Pharmaceuticals
Optison	Albumin	Perfluoropropane	GE Healthcare
Sonazoid	Lipid	Perfluorobutane	GE Healthcare
SonoVue	Surfactant	SF ₆	Bracco Diagnostics
MRX-408	Lipid/ligand oligopeptide	Perfluoropropane	ImaRx
Quantison	Albumin	Air	Andaris Ltd
QFX	Albumin	Perfluorocarbon	Guangzhou Nanfang Hospital

organs of interest. Due to artifacts associated with color flow imaging (e.g. color blooming and poor spatial resolution), this may not be an ideal method of imaging with ultrasound contrast agents. In certain contrast-specific imaging modes, the SNR can be further improved by suppression of tissue signals. The improved SNR can also be exploited in non-vascular structures like the urinary bladder, fallopian tubes and lymphatic channels.

In order to better separate signals from tissue and the contrast agent, tissue signal suppression has to be carried out. This can be achieved by phase/pulse cancellation, coded pulses or amplitude modulation. Although there are different methods commercially available with differing levels of sophistication and technology, grayscale phase/pulse inversion is the basis of most of the modes used for grayscale imaging with ultrasound contrast agents [2,3]. This technique cancels first harmonic (linearly scattered) signals by transmitting a pulse sequence where each pulse is an inverted copy of the previous pulse, and then summing the echoes from subsequent pulses (resulting in zero under linear scattering conditions). Hence, echoes from stationary tissue will be suppressed. However, nonlinear echoes arising from contrast microbubbles will not cancel out and, thus, can be preferentially detected and displayed.

Contrast-specific Imaging Technology

Conventional ultrasound systems have technical limitations when used with ultrasound contrast agents. These limitations can ultimately reduce the usefulness of the contrast agent effects. Thus, ultrasound equipment has been and continues to be modified to optimize their use with a variety of ultrasound contrast agents.

In general, during insonation, ultrasound contrast agents produce a linear response at low acoustic pressures (<50 kPa), which means the microbubbles will undergo rhythmic oscillations at a resonant frequency (f_0). A nonlinear contrast agent response occurs at intermediate pressures (from

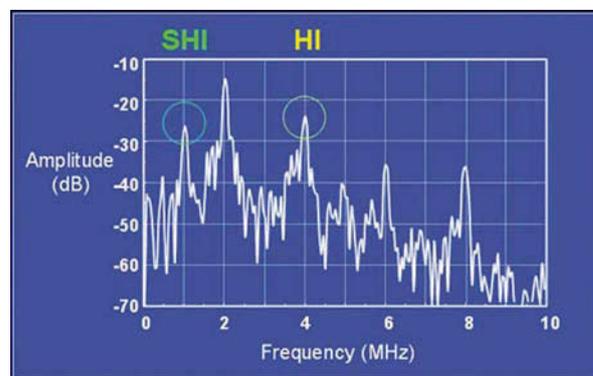


Fig. 2. Spectrum of a microbubble agent showing the fundamental ($f=2$ MHz), second harmonic (HI at 4 MHz), and subharmonic (SHI at 1 MHz) components.

approximately 50 to 500 kPa). This nonlinear response consists of an asymmetric change in microbubble size due to greater resistance to compression than expansion, which produces harmonic and subharmonic signal components (i.e. frequency components at $2f_0$, $3f_0$, $4f_0$, etc., and at $1/2f_0$, $1/3f_0$, etc.) (Fig. 2) [4]. Eventually, at higher acoustic pressures (> 500 kPa; although these pressure levels vary significantly from agent to agent), the contrast microbubbles will be disrupted and destroyed.

Harmonic Imaging

Harmonic imaging (HI) is one such modification which was originally developed for contrast-enhanced ultrasound imaging. The phenomenon of harmonic generation is not confined to microbubble-based contrast agents but can also be induced in native tissue, and this forms the basis of native tissue harmonic imaging (THI). HI uses the same broadband transducers used for conventional imaging, but the ultrasound system is configured to primarily receive echoes at the second harmonic frequency (i.e. twice the transmit frequency). HI provides a way to better differentiate areas with and without contrast microbubbles. Therefore, contrast-enhanced HI has the potential to demonstrate, in real-time, grayscale blood flow imaging (i.e. perfusion imaging) (Fig. 3). The frequencies and parameters of wideband HI that are used depend on

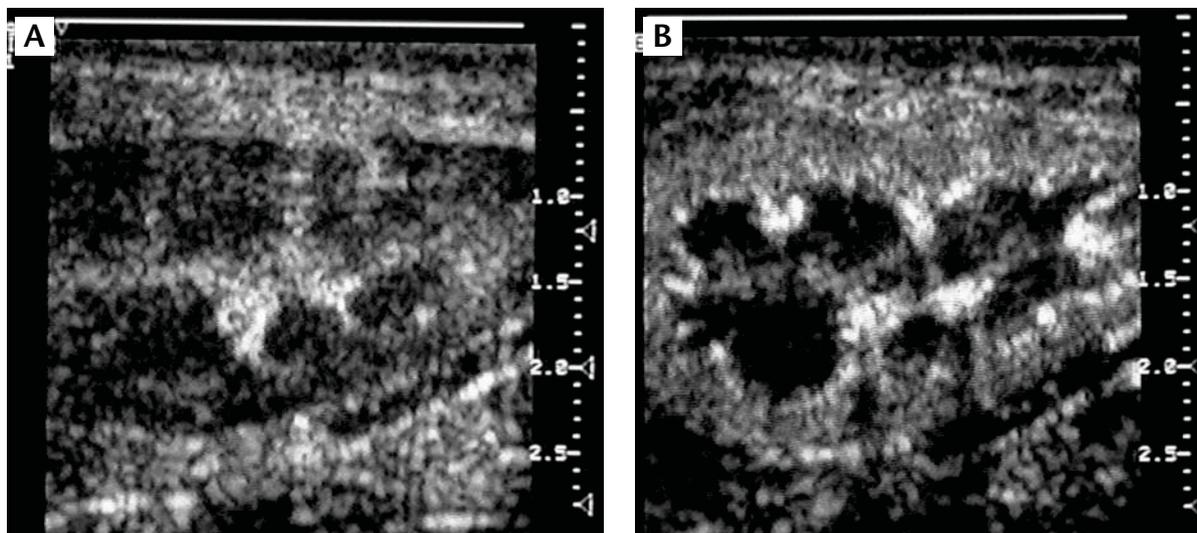


Fig. 3. Compared with fundamental imaging (A), second harmonic imaging (B) of a kidney after contrast injections shows better enhancement and vascular definition.

the specific characteristics (i.e. microbubble size and shell composition) of the ultrasound contrast agent being utilized. Selection of the appropriate agent and imaging mode allows optimization of the utility of contrast-enhanced ultrasound imaging [5].

Initially, HI relied on simple filtering techniques to extract the harmonic microbubble signals, but more sophisticated processing schemes have emerged over the recent years. Currently, the mode with the best SNR and suppression of unwanted tissue signal is wideband HI. Further improvements are possible with the use of pulse inversion HI [6–8]. Additional details on the equipment and software available for ultrasound contrast imaging may be found in the recent EFSUMB guideline paper [9].

Intermittent Imaging

In order to enhance detection of tumor neovascularity with contrast agents, these agents must traverse into these smaller vessels. Conventional ultrasound systems, however, often deliver power levels that are sufficient to destroy contrast microbubbles, especially when using a high mechanical index (MI). If the microbubbles are destroyed before they reach the neovasculature, the desired

enhancement of flow in tumor vessels will not be observed. A potential solution to this problem is the use of intermittent ultrasound imaging, which has been shown to increase ultrasound contrast enhancement [10–12]. The degree of enhancement with intermittent imaging is dependent upon flow rate, acoustic power output and the frequency of insonation [13]. With continuous grayscale ultrasound imaging, contrast microbubbles within the imaging plane may be destroyed during the acquisition of each frame of the image. Since a typical grayscale ultrasound image is refreshed at 30 frames per second, the available contrast agent for each new image frame is that amount which enters the imaging plane in $1/30^{\text{th}}$ of a second. In this short time between frames, contrast may enter larger vessels, but will not generally reach the micro-circulation. With intermittent imaging, the ultrasound beam is turned off for longer periods between each image frame. More contrast microbubbles then enter the imaging field during this interscan period, resulting in increased echo-enhancement. Furthermore, the contrast material will have time to traverse further into the capillary bed.

Flash echo imaging is a particular combination of regular and intermittent grayscale imaging techniques, consisting of low power real-time monitor pulses transmitted continuously, while microbubble

destruction is achieved with intermittent higher power flash pulses [14]. These two modes are displayed simultaneously in a dual screen format. Each sequence of multiple-frame (1–15 frames) flash pulses can be triggered manually or electronically (e.g. every 1–8 seconds). The principle of microbubble destruction in intermittent mode has been used by investigators to measure mean myocardial microbubble velocity and to assess the microvascular cross-sectional area during constant infusion of contrast [15]. The product of these two estimates is the mean myocardial blood flow (i.e. a measure of myocardial perfusion). To date, flash echo imaging is the only quantitative method for ultrasound perfusion estimation (in mL/min/g) that has been developed.

Continuous Imaging

Although intermittent imaging can obtain high contrast within a single frame, this imaging mode is not real-time, which may impair the visualization of some structures and the utility of the modality. In the last few years, a number of other methods have been introduced (some commercially), such as pulse inversion HI, superharmonic imaging, power harmonics, coded harmonic angio and agent detection imaging [16–21], which employ low acoustic power settings and contrast-specific processing to produce a real-time display of the region of interest. The visualization of the enhancement pattern of the normal and abnormal tissues can significantly improve the diagnostic capability of ultrasound imaging.

Other Imaging Methods

When imaging small vessels or if the concentration of contrast is low, the echogenicity of contrast microbubbles can be limited. However, collecting images over an extended period of time (e.g. 3–10 seconds) using alternative post-processing techniques (e.g. maximum intensity projection) can achieve a

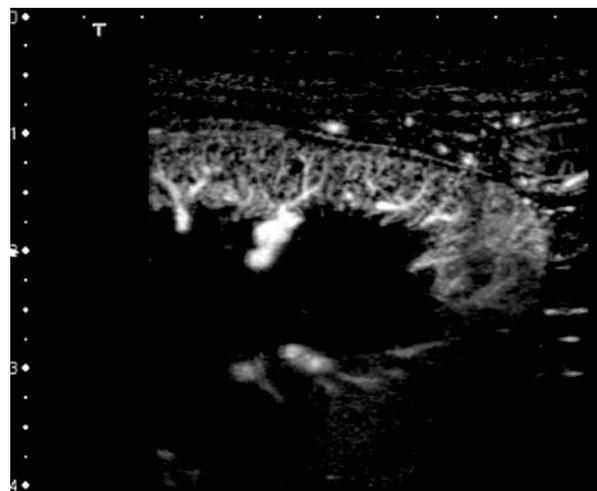


Fig. 4. Contrast-enhanced imaging using micro-flow imaging mode shows a high resolution depiction of normally perfused kidney parenchyma in an animal model.

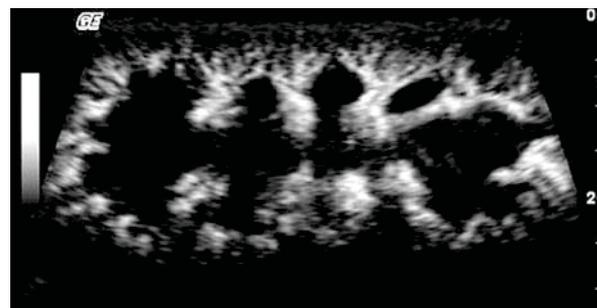


Fig. 5. Subharmonic image of a canine kidney shows the perfusion pattern of vasculature of the kidney with suppression of background tissue.

temporally summed (or compounded) enhanced image. This is the basis of a commercially available technique [3], which has currently been implemented by three manufacturers (Fig. 4).

Under specific conditions, gas microbubbles generate subharmonics, which occur mainly at half the transmitted frequency. Compared to superharmonic imaging, an advantage of subharmonic imaging is that tissue signal is minimal, which results in a high agent-to-tissue ratio (i.e. high SNR). Reports have described the feasibility and implementation of subharmonic ultrasound imaging [22,23] (Fig. 5). Currently, subharmonic ultrasound imaging is still in its early development.

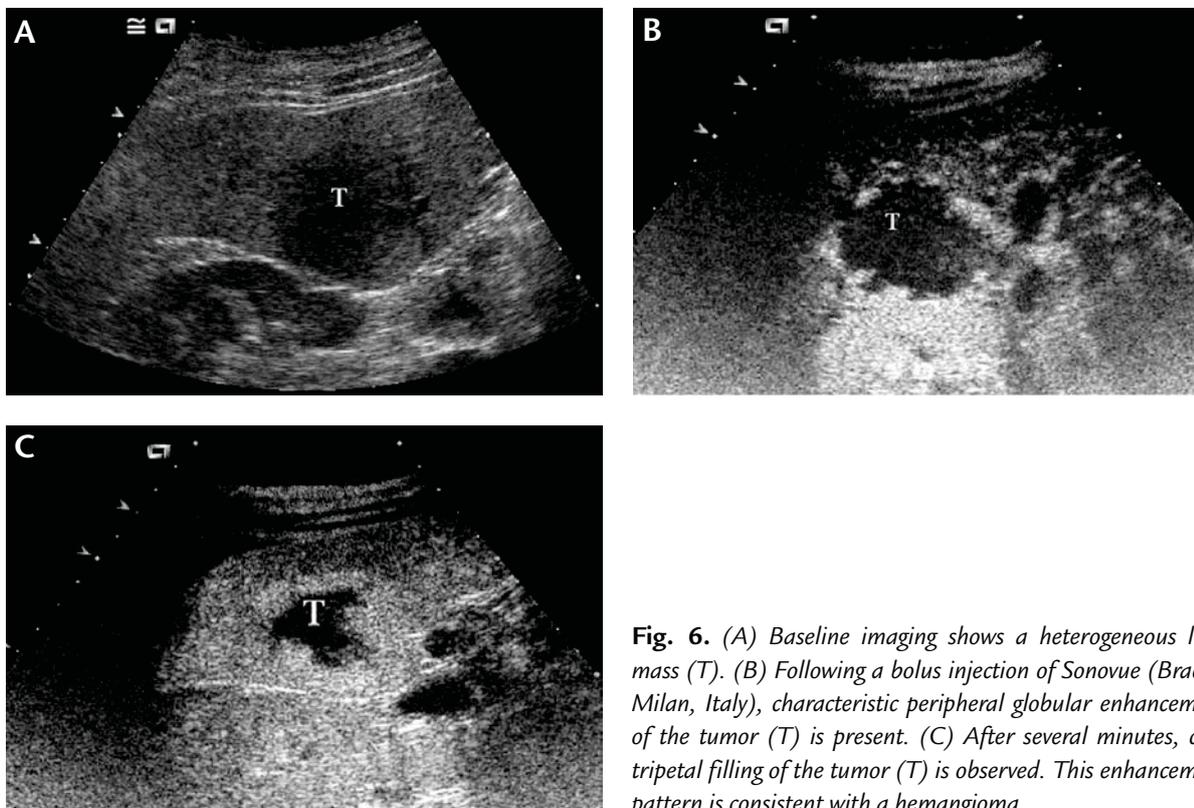


Fig. 6. (A) Baseline imaging shows a heterogeneous liver mass (T). (B) Following a bolus injection of Sonovue (Bracco, Milan, Italy), characteristic peripheral globular enhancement of the tumor (T) is present. (C) After several minutes, centripetal filling of the tumor (T) is observed. This enhancement pattern is consistent with a hemangioma.

Clinical Applications

Clinical applications for ultrasound contrast agents can potentially be found in any structure that is evaluated with conventional ultrasound, with the only exception being the fetus. The major applications of ultrasound contrast agents are in cardiac and hepatic imaging. Other applications are being explored, although there are currently fewer reports on their clinical use. The assessment of vascularity by demonstration of microvessels or increased parenchymal signal intensity provides a new parameter in diagnostic evaluation, just like intravenous contrast has enhanced CT and MRI as well as nuclear imaging.

Hepatic uses

The use of ultrasound contrast agents for evaluation of the liver has become widespread and can be considered routine in parts of Europe and Asia [4]. Current microbubble-based agents are essentially blood pool agents. The type of enhancement demonstrated in the liver is similar to that shown

with contrast-enhanced CT and MRI. Therefore, interpretation of the enhancement patterns in contrast-enhanced ultrasound is similar to those performed with contrast-enhanced CT or MRI. In addition, continuous scanning at low acoustic pressures may reveal dynamic contrast enhancement that can be quite helpful for characterization of a variety of focal liver lesions (Fig. 6).

Hepatic pathology can be considered in two main groups: focal lesions and diffuse disease. The characteristic patterns of enhancement in benign and malignant liver lesions have been described [24]. There is good concordance with the enhancement characteristics of focal liver lesions using contrast-enhanced ultrasound with those that have been described for CT and MRI studies [25,26]. This is true for benign and malignant hepatic tumors [27]. There is better characterization of certain focal lesions like hepatocellular carcinoma (HCC) and metastatic lesions (Fig. 7) compared to an unenhanced ultrasound scan [28]. This is especially the case for small (<2 cm) focal liver lesions. Contrast agents have

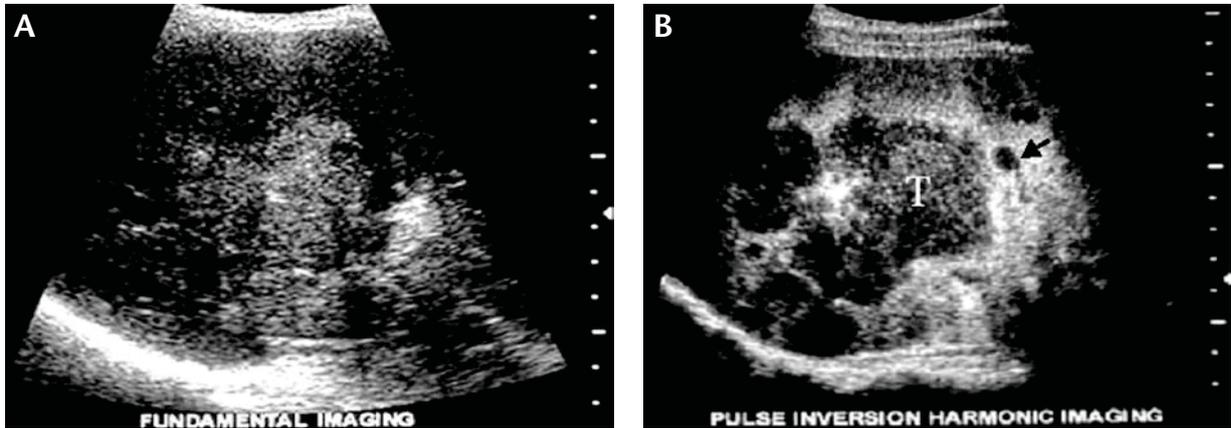


Fig. 7. (A) Conventional grayscale imaging shows suspicious masses within a heterogeneous liver in a patient with colon cancer. (B) Contrast-enhanced pulse inversion harmonic imaging improved the delineation of multilobulated tumors (T) within the enhanced liver parenchyma.

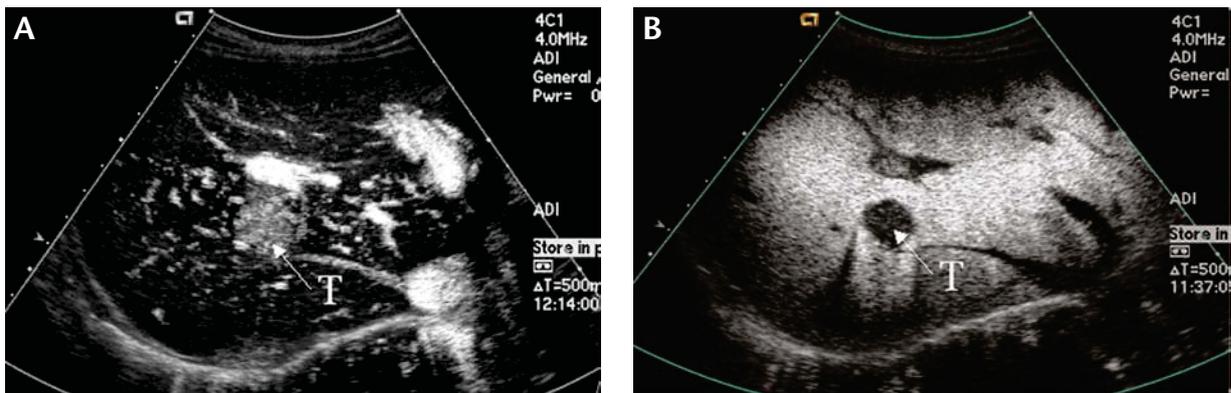


Fig. 8. (A) Harmonic imaging of a hepatocellular carcinoma demonstrates tumor (T) enhancement in its arterial phase using Agent Detection Imaging mode (ADI; Siemens, Issaquah, WA, USA). (B) In its delayed phase, the tumor (T) appears as a hypoechoic lesion within the contrast-enhanced parenchyma.

been shown to be useful in improving the detection of HCC (Fig. 8), in differentiating HCC from regenerating nodules and to detect recurrence in treated lesions. Complementary information may be obtained when compared to contrast-enhanced CT scans [29]. This improvement shows good inter-observer concordance [30]. Focal nodular hyperplasia has a central star-like pattern of enhancement that can be observed with continuous contrast-enhanced ultrasound (Fig. 9) [31]. Due to the transient nature of enhancement, the star-like pattern may not be seen in all examinations, especially if an intermittent mode is used.

In some cases, there is overlap of the benign and malignant features in focal liver lesions on

contrast-enhanced ultrasound, but the use of additional parameters may provide advantages over CT and MRI. For example, hepatic transit time has been found to be useful in monitoring post radiofrequency (RF) ablation procedures [32] and contrast-enhanced power Doppler imaging for radiotherapy [33]. For diffuse liver disease like cirrhosis, global parameters such as hepatic transit time show promise in being able to diagnose cirrhosis without biopsy. A prospective study that assessed the diagnostic accuracy of transhepatic circulatory time with an ultrasound contrast agent demonstrated that the hepatic artery to hepatic vein and portal vein to hepatic vein interval times were significantly shorter in the cirrhosis group

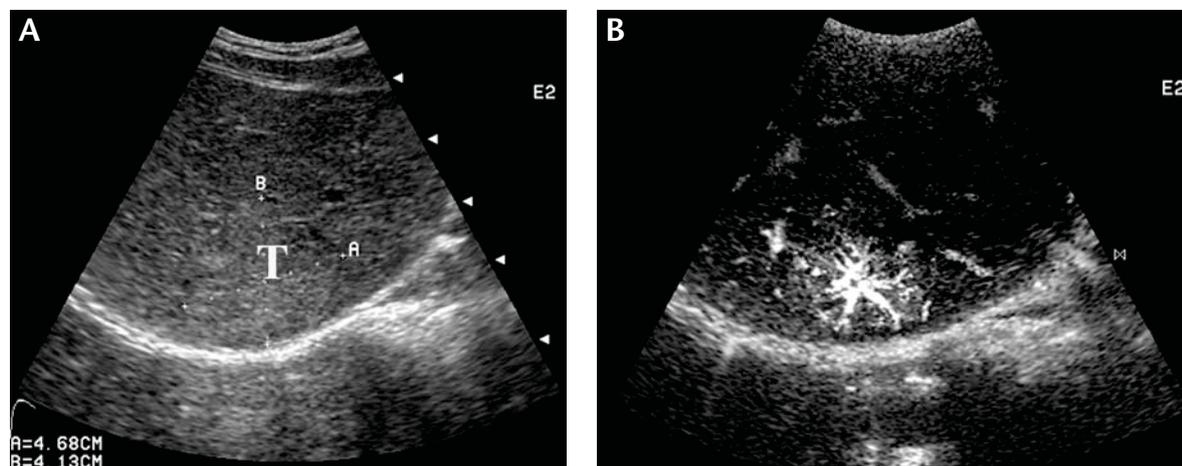


Fig. 9. (A) Conventional grayscale imaging shows an isoechoic lesion (T) in the right lobe of the liver. (B) After a bolus injection of contrast agent, the tumor demonstrates a central star-like pattern of enhancement, consistent with a diagnosis of focal nodular hyperplasia.

(7.4 ± 1.7 s and 1.9 ± 1.5 s, respectively) compared with those in the noncirrhosis group (normal: 15.6 ± 2.1 s and 11.1 ± 1.7 s, $p < 0.001$ and $p < 0.001$; and hepatitis: 12.8 ± 4.1 s and 7.8 ± 4.4 s, $p < 0.001$ and $p < 0.002$, respectively) [34]. Hepatic transit time can also distinguish between cirrhosis and severe hepatitis from mild hepatitis, although there is overlap between severe hepatitis and cirrhosis.

Interventional and intraoperative uses

The ablation of lesions with RF is a technique that is increasing in popularity, especially in the treatment of unresectable liver lesions. The ideal method of monitoring ablation during the procedure itself and in post-ablation follow-up has not been established. Contrast-enhanced ultrasound has been used in diagnosis of lesions before ablation as well as monitoring the outcome of ablation procedures in the liver (Fig. 10) [35].

Contrast-enhanced ultrasound has the potential to be very useful as it allows real-time assessment of lesion vascularity [36] and is similar to dynamic CT in its sensitivity and specificity [37,38]. Grayscale pulse inversion HI is superior to power Doppler contrast-enhanced ultrasound [39], and both are superior to conventional ultrasound [40] in demonstrating residual tumor after thermal ablation. Similar findings have been described in a preliminary report on ablation of renal lesions [41]. The use of



Fig. 10. Pulse inversion harmonic imaging of a liver tumor post radiofrequency ablation demonstrates a small residual viable nodule (arrows) at the periphery of the lesion.

contrast-enhanced ultrasound for intraoperative evaluation of focal liver lesions should promise to improve detection of sub-centimeter nodules, show nodular vascularity with greater detail, and potentially improve clinical outcomes [42].

Contrast-enhanced ultrasound imaging has been used to delineate thermal lesions from viable tissue during RF ablation of the prostate in an animal model. The initial results demonstrated that contrast-enhanced imaging is able to guide and monitor the RF ablation of the entire prostate (Fig. 11). This technique holds a great potential for future use in humans as an innovative treatment of prostate cancer [43].

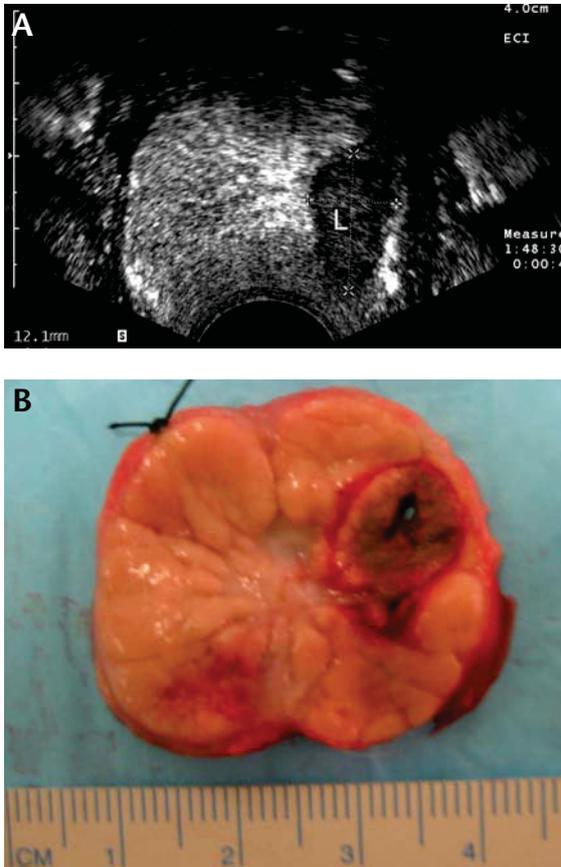


Fig. 11. (A) Contrast-enhanced pulse inversion harmonic imaging of a canine prostate after radiofrequency ablation identifies a demarcated thermal lesion (L) within contrast-perfused normal parenchyma. This information is useful for monitoring radiofrequency ablation of the entire prostate. (B) Pathologic specimen showing the coagulated lesion corresponding to the ultrasound findings.

Echocardiographic uses

In the United States and Europe, the Food and Drug Administration (FDA) and the European Union have approved several contrast agents for use during echocardiography examinations with the specific indication of providing ventricular opacification and enhancement of endocardial border definition in patients with technically suboptimal echocardiograms (Fig. 12). Contrast echocardiography includes applications for the right ventricle such as demonstration of shunts, abnormalities in the position or presence of the great vessels, and for the left ventricle such as cardiac structure, valvular function and wall motion. Additional applications include perfusion quantification and reperfusion assessment of the myocardium [44,45].

The administration of contrast agents has been shown to enable more accurate measurement of left ventricular volume, ejection fraction, diagnosis and grading of valvular disease, intracardiac thrombus detection, aortic dissection, detection of complications of myocardial infarction (such as ventricular rupture and aneurysm formation), and improved assessment of systolic function compared to conventional ultrasound imaging. In stress echocardiography, contrast agents increase the number of interpretable segments, which allows accurate assessment of left ventricular function [46]. At the myocardial level, contrast agents can be used to diagnose

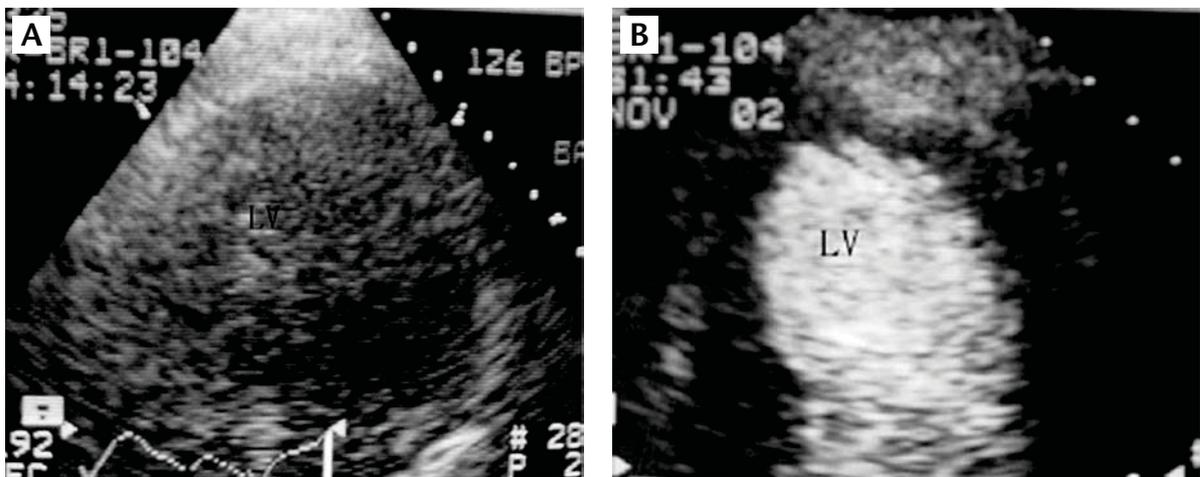


Fig. 12. (A) Suboptimal echocardiography of the left ventricle (LV) shows inadequate endocardial border definition. (B) After intravenous contrast injection, complete opacification of the left ventricle (LV) is obtained and the endocardial borders are clearly seen.

infarction and assess tissue viability. Coronary artery stenoses can be localized and their severity quantified using contrast-enhanced intermittent HI. Coronary perfusion rates may be calculated using microbubble destruction and reperfusion techniques [47].

Cerebrovascular uses

Significant limitations exist in current transcranial Doppler ultrasound examinations. These include low reproducibility, inter-investigator variability, and inadequate access through the skull. Thus, transcranial Doppler examinations can be improved with the use of ultrasound contrast agents [48]. Ultrasound contrast agents provide better delineation of normal blood flow, occlusions, pseudo-occlusions, stenoses, and collaterals in the extracranial and intracranial vascular beds [49]. For examination of the extracranial carotid arteries, contrast administration can increase visualization of the residual lumen, increase diagnostic confidence, and decrease the number of indeterminate examinations. However, the applications of contrast agents to the carotid artery are in their infancy. Clinical trials are necessary to determine optimal techniques for contrast-assisted carotid imaging.

Thyroid and parathyroid uses

Ultrasound contrast agents have been used to obtain time-intensity curves of flow through thyroid nodules. This has the potential to differentiate between benign and malignant lesions and to characterize hypovascularized malignant nodules that could not be observed without contrast [50]. A more rapid time to peak signal intensity has been documented in malignant thyroid nodules [51]. In parathyroid lesions that do not show flow with conventional ultrasound, contrast agents can provide useful information by visualizing typical color Doppler signals of the parathyroid lesions. This can help to distinguish parathyroid nodules from thyroid lesions [52].

Gastrointestinal uses

Contrast-enhanced ultrasound has been used to evaluate patients with portal hypertension by enhancing the Doppler signal and permitting

better visualization of esophageal varices transabdominally [53] and perforating veins in recurrent varices [54]. This technique has great potential for improving early detection of visceral varices and monitoring of therapeutic response.

Bowel pathology may be diagnosed on the basis of altered vascularity. Ultrasound contrast agent can demonstrate ischemia [55] and help differentiate benign from malignant gastrointestinal stromal tumors [56]. Assessment of inflammatory bowel disease can also be improved with the use of contrast agents by demonstrating increased vascularity in affected segments [57]. This is of benefit in determining if active disease is present or only fibrosis even when the bowel wall does not show significant thickening [58]. The same reasoning applies in the evaluation of pathologic hyperemic bowel, e.g. in acute appendicitis [59].

The use of orally administered ultrasound contrast agents for bowel evaluation has also been shown to be of value [60]. Oral contrast agents appear to significantly improve the ability to image both normal and abnormal structures in the gastrointestinal tract (Figs. 13 and 14), and to provide an acoustic window for evaluation of adjacent organs such as the pancreas (Fig. 15). Acoustic artifacts associated with bowel gas often prevent complete ultrasonic evaluation of the pancreas,



Fig. 13. Following the ingestion of oral contrast, ultrasound imaging shows a uniform and homogeneous echogenicity within the stomach (ST). Note that a demarcated defect on the posterior wall of the stomach is clearly seen, which corresponds to an ulceration lesion.

which has led to CT being the primary choice for the evaluation of this organ. Evaluation of oral ultrasound contrast agents has shown significant improvement in visualization of the stomach, pancreas and adjacent structures. These oral agents could be coupled in the future with an intravenous contrast agent, enhancing the ability of ultrasound to detect pancreatic tumors.

Gallbladder and biliary tree uses

There are a few published reports that describe the use of ultrasound contrast agents for the evaluation of abnormalities of the gallbladder and biliary tree.



Fig. 14. Transverse ultrasound imaging of the stomach (ST) after ingestion of oral contrast shows irregular thickening of the walls of the pylorus with a narrowed gastric cavity, consistent with gastric cancer in the pyloric region.

One report described how the detection and staging of malignant hilar obstructions of the biliary tree was improved by the use of Levovist in the post-vascular phase of sonography compared with conventional sonography [61]. Some studies described how ultrasound contrast agents can increase visualization of the vasculature in the gallbladder wall and hyperemia of the liver parenchyma adjacent to the gallbladder in cases of acute cholecystitis [62,63].

Renal uses

Contrast-enhanced ultrasound of the kidney can provide a clear and detailed view of renal vascularity, with early enhancement in the arterial phase followed by an intense and uniform enhancement in the renal cortex (i.e. perfusion imaging). The enhancement then extends to the pyramids until they become isoechoic with the cortex.

The application of ultrasound contrast agents in the characterization of renal tumors has great promise. Contrast-enhanced ultrasound has the potential to perform dynamic time-contrast intensity curves [64], characterize focal renal lesions [65], evaluate for the presence of a pseudocapsule in renal cell carcinoma [66], and evaluate the collecting system for vesicoureteric reflux [67]. Other potential renal applications for contrast imaging include the evaluation of renal perfusion, kidney transplants, and monitoring tumor ablation procedures.

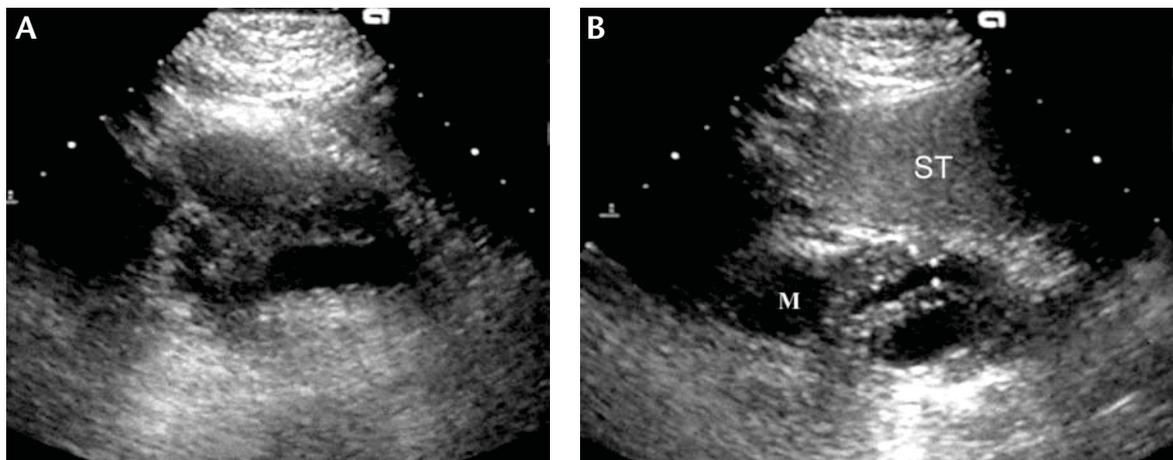


Fig. 15. (A) Pre-contrast transverse imaging of the mid epigastrium reveals partial obscuration of the head of the pancreas. (B) Post-contrast imaging shows a hypoechoic mass (M) at the head of the pancreas through a contrast-filled stomach (ST). Dilatation of the pancreatic duct is also seen.

Prostate uses

In order to more accurately detect the presence of prostate cancer, researchers have focused upon the detection of neovascularity in prostate cancer. The vascular supply to malignant prostate tissue differs from the vascularity of normal prostate tissue in density and distribution of microvessels. Studies of the prostate demonstrate a clear association of increased microvessel density with the presence of cancer [68]. Quantitative assessment of microvascular density may actually provide important data to guide therapeutic decisions [69]. Unfortunately, the microvessels that proliferate around and within prostate cancers are below the limits of the resolution of conventional Doppler ultrasound.

One potential solution to this problem may be the use of ultrasound contrast agents to detect flow in microvessels associated with cancer. An early study suggested that contrast-enhanced color flow detection of increased vascularity was associated with the presence of prostate cancer [70]. Several additional studies have demonstrated

Doppler enhancement of vessels in prostate cancer [71,72]. These preliminary studies suggested that contrast agents might be useful in ultrasound evaluation of prostate vascularity and improve the detection of cancer (Fig. 16). Both contrast-enhanced color Doppler and harmonic grayscale imaging have been used successfully to improve imaging of prostate cancer and to guide targeted biopsy for definitive diagnosis of prostate cancer [73–77].

Pancreatic uses

The pancreas may be evaluated with contrast-enhanced ultrasound via a transabdominal approach or endoscopically [78]. Preliminary reports show that contrast-enhanced ultrasound improves the conspicuity of pancreatic carcinoma compared to conventional ultrasound [79,80]. Quantitative analysis of the amount of post-contrast enhancement may be useful in separating benign and malignant pancreatic lesions [81]. Further work is needed to validate these early findings.

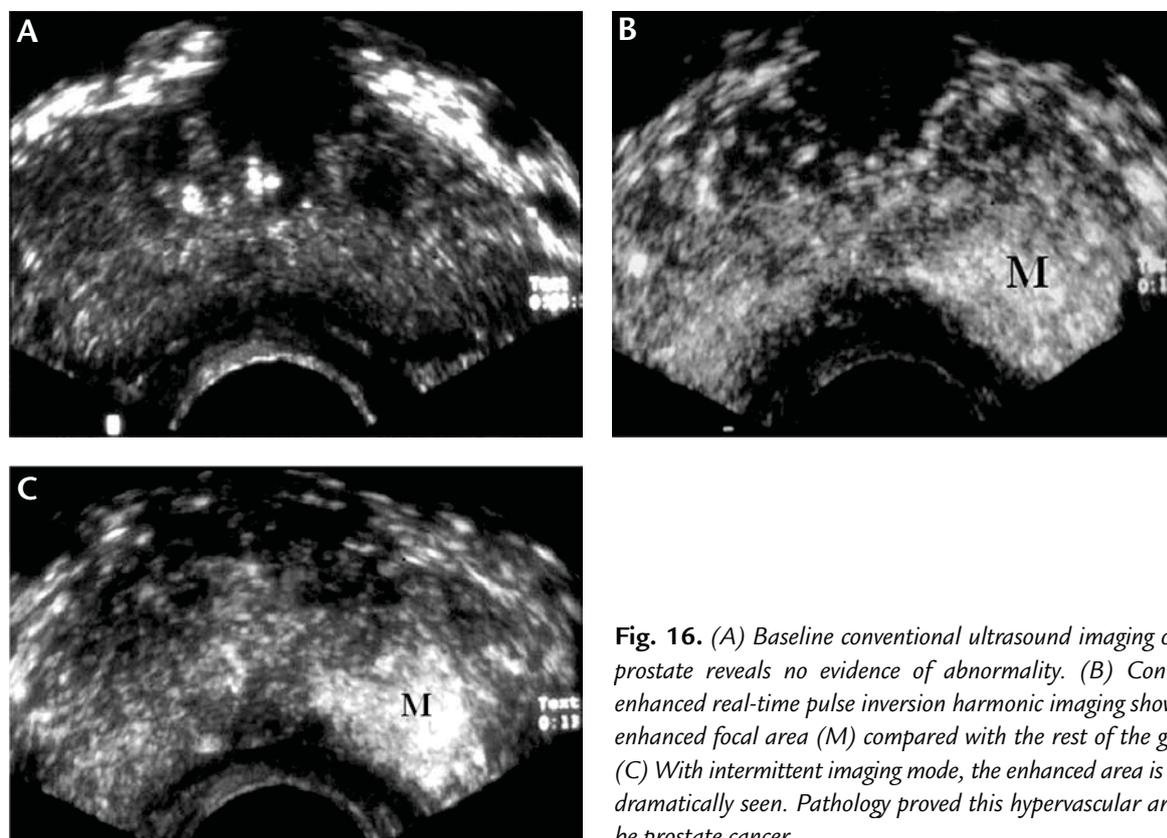


Fig. 16. (A) Baseline conventional ultrasound imaging of the prostate reveals no evidence of abnormality. (B) Contrast-enhanced real-time pulse inversion harmonic imaging shows an enhanced focal area (M) compared with the rest of the gland. (C) With intermittent imaging mode, the enhanced area is more dramatically seen. Pathology proved this hypervascular area to be prostate cancer.

Vascular uses

Preliminary reports have described how contrast-enhanced ultrasound of the renal arteries can improve visualization of the renal arteries and its branches, improve the detection of renal artery stenosis, reduce the duration of an examination and improve the accuracy of these examinations [82,83]. Improved detection of intra- and extrarenal arteries with contrast-enhanced color Doppler imaging provides a superior roadmap of the vessels themselves and allows for more accurate placement of the spectral Doppler sample volume. Several clinical contrast studies in the evaluation of renal artery stenosis (RAS) have produced encouraging results [82,83]. In a multicenter trial, 191 patients referred for renal arteriography were examined with contrast-enhanced ultrasound. The ability to image the renal arteries improved from 75% to 90% after contrast administration ($p < 0.001$). Accuracy in diagnosing RAS $> 50\%$ increased from 65% on non-contrast evaluations to 78% with the use of contrast [84,85].

Trauma uses

Traumatic lesions have common enhancement features on cross-sectional imaging, independent of the organ and tissues involved. The arterial tree is invariably involved in all organs and contrast-enhanced ultrasound easily detects parenchymal lesions, such as lacerations and hematomas, providing detailed information on them [86]. The findings were consistent with those of CT exams, suggesting that contrast-enhanced ultrasound could be a reliable technique for the evaluation of post-traumatic parenchymal damage.

In the evaluation of trauma to the abdomen, contrast-enhanced ultrasound has the advantage of demonstrating viable tissue and improving contrast between traumatic lesions and remaining normal parenchyma. Although superior to conventional ultrasound [87], contrast-enhanced ultrasound is not yet a replacement for contrast-enhanced CT [88]. However, contrast-enhanced ultrasound has several advantages over CT, including the ability to be performed at bed-side within the intensive care

unit and to be used for serial evaluations to determine if active bleeding is present. Thus, additional research in this area is warranted.

Lymphatic uses

Microbubble-based ultrasound contrast agents have been injected subcutaneously to enhance detection of lymphatic channels (LCs) and sentinel lymph nodes (SLNs) [89,90]. Clinically, SLN mapping is important for tumor staging (such as breast, skin, and gastrointestinal tumors) and to determine the use of adjuvant therapies. With traditional methods (e.g. lymphoscintigraphy or injection of blue dyes and surgical dissection), it is impossible to demonstrate the internal architecture of the SLN, which is important for the detection of metastatic spread to SLNs (initial accuracy 86%). Contrast-enhanced lymphosonography is a minimally-invasive technique to localize draining LCs and SLNs (Fig. 17) and has the ability to evaluate SLNs for metastases (Fig. 18). This new technique also has the potential to enhance ultrasound-guided biopsies of SLNs for improved tissue sampling for definitive pathologic assessments.

Gene and Drug Delivery and Targeted Agents

Functional ultrasound imaging of specific tissues using targeted microbubbles represents a new approach that departs from the concept that

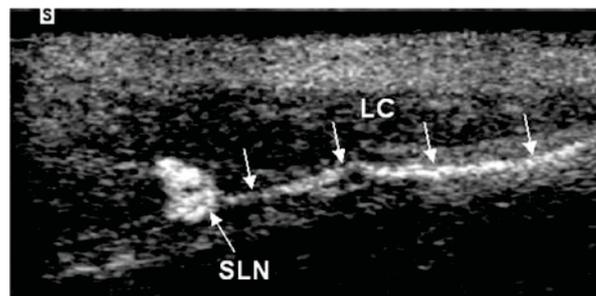


Fig. 17. Following subcutaneous injection of contrast agent in an animal model, pulse inversion harmonic imaging identifies a contrast-filled lymphatic channel (LC; arrows) and its sentinel lymph node (SLN).

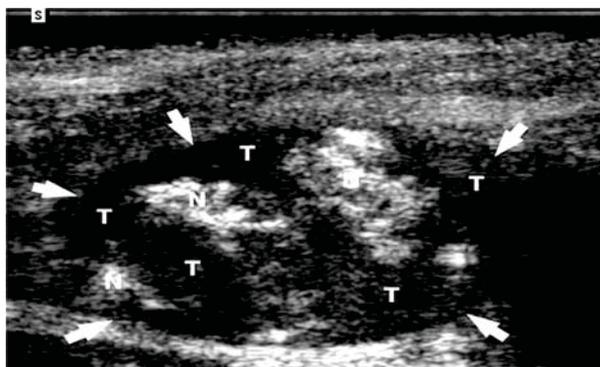


Fig. 18. In a swine melanoma model, contrast-enhanced ultrasound imaging identifies a metastatic sentinel lymph node (N), which appears as partially enhanced normal lymphatic tissue and hypoechoic tumor deposits (T).

microbubbles passively transit the microcirculation like red blood cells. Targeted ultrasound imaging involves the design and synthesis of microbubbles that will adhere to endothelium or other targets under disease-specific conditions (e.g. inflammation). To the extent that the microbubbles are designed to adhere to molecular epitopes on the surface of abnormal endothelium, targeted contrast imaging could provide capabilities for *in vivo* ultrasonic detection of phenotypic features of endothelium that predate clinical disease and/or are otherwise not detectable using currently available technologies [91–93]. Investigators have demonstrated that microbubbles can be phagocytosed intact by activated neutrophils and monocytes and can be detected by ultrasound imaging. These findings indicate that contrast-enhanced ultrasound may provide a useful means for the noninvasive assessment of inflammation and to follow the response to treatment [94].

Targeted imaging using contrast microbubbles has advantages over other molecular imaging methods. Unlike nuclear imaging approaches, ultrasound contrast microbubbles stay within the vascular space and have a short circulation time. This is useful to the extent that the technique is not susceptible to nonspecific signals resulting from extravasation of the imaging agent or retention in non-target organs such as the liver. However, these features are intrinsically limited to the interrogation

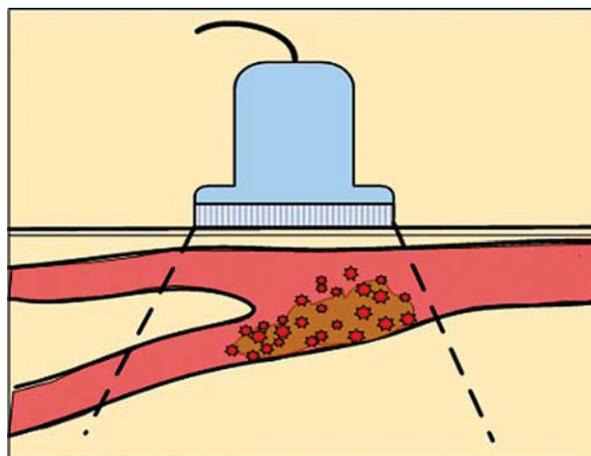


Fig. 19. Diagram showing a targeted contrast agent used for thrombolysis.

of phenomena occurring on the surface of endothelial cells, thus excluding its application to many important physiologic processes that occur intracellularly and outside of the vascular space, for example, gene therapy. Recent reports of acoustically active nanoparticle emulsions (or gas-filled nanobubbles) capable of exiting the vascular space may offer an exciting solution to this challenge [95].

Targeted microbubbles may ultimately have utility beyond their diagnostic attributes. Ultrasonic destruction of microbubbles appears to enhance delivery of genes, drugs, and lysis of clots (Fig. 19) [96,97]. The ability to target therapeutics by designing the delivery agent (microbubble) to be able to reach the site of interest may ultimately prove to be another powerful clinical application of this exciting technology.

Summary

In conclusion, present and future ultrasound contrast agents should provide for increased diagnostic capabilities in a variety of normal and abnormal vascular applications. These agents will enhance tumor vascularity, delineate areas of ischemia, and improve visualization of many abnormalities, including the detection and characterization of tumors in such organs as the liver. Ultrasound contrast agents that are non-toxic, intravenously injectable, and

stable for recirculation are already being used routinely in a variety of clinical applications. Future developments including the continued modification of ultrasound equipment to better exploit the enhancement properties of contrast agents should increase the capability of these agents to improve the sensitivity and specificity of ultrasound imaging. The field of targeted ultrasound imaging is still in its infancy, and as with the field of molecular imaging in general, much remains to be done to develop this area into a clinical reality.

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