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Tissue harmonic and contrast-specific imaging: back to gray scale in ultrasound

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Abstract The development of new US techniques that produce images based on nonlinear acoustic effects of US interaction with matter or microbubble contrast agents has opened new prospects for gray-scale US in native tissue and contrast imaging. Tissue harmonic imaging uses higher frequencies generated on propagation of the US beam through matter to improve image quality and resolve small anatomic structures and details, and is becoming a routine approach in US examination of many abdominal districts. Contrast-specific imaging techniques display enhancement of US agents in gray-scale with optimal contrast and spatial resolution, and offer high sensitivity either to microbubble movement or to microbubble destruction in dependence of the level of the applied acoustic peak pressure. Owing to the ability to exploit the microcirculation, contrast-specific techniques have enabled the evolution of contrast US from vascular imaging to the imaging of perfused tissues. Several studies have shown that these methods can substantially improve US detection and characterization of focal liver lesions, and promising results have been reported in other areas of investigation. This article reviews physical principles, technical issues, and clinical applications of tissue harmonic and contrast-specific imaging. It is foreseen that the new gray-scale US techniques will rapidly become a tool in numerous clinical scenarios.

Keywords Tissue harmonic imaging · Ultrasound contrast agents · Ultrasound technology

Introduction

Over the past decade, the most important advances in US have taken place in the Doppler technology. The combination of color and power Doppler imaging with US contrast agents, in fact, has dramatically improved the ability of the Doppler techniques to explore the vascular system, allowing evaluation of even small and deep vessels.

However, the development of new US techniques that produce images based on nonlinear acoustic effects of US interaction with matter or microbubble contrast agents – including tissue harmonic and contrast-specific imaging – has recently brought attention back to gray-scale US. Tissue harmonic imaging, by using higher frequencies generated on US propagation through matter, substantially improves image quality with respect to conventional (fundamental mode) US. Contrast-specific imaging techniques, by displaying microbubble enhancement in gray-scale, maximize contrast and spatial resolution, and enable the evaluation of the microcirculation, thus prompting the evolution of contrast US from vascular imaging to the imaging of perfused tissues.

This article gives an overview of tissue-harmonic and contrast-specific gray-scale US imaging by discussing physical principles, technical issues, and potential clinical applications.

Tissue harmonic imaging

Physical principles

In tissue harmonic imaging, harmonic frequencies are generated on propagation of the US beam through tissue, not on reflection from an object such as in contrast harmonic US [1, 2, 3]. Harmonics are generated on pulse propagation because water (and tissue) is not fully incompressible. Slight changes in density, in fact, occur when an acoustic pressure is applied. These changes in density affect the velocity of US propagation through water: US is a little faster at the peaks of waves than in the troughs. This leads to a slight change in the shape of the wave as it propagates. At each instant in wave propagation, a very small amount of harmonics is generated [2].

This phenomenon is defined by the following equation [4]:

$$P_2 \approx (B/A+2)\frac{\pi \cdot f}{2\rho \cdot v^3} d \cdot p_{ac}^2 ,$$

where P_2 is the second harmonic, B/A is a nonlinear parameter of the tissue (i.e., 5.2 for water), f is frequency of insonification, ρ is density of tissue medium, v is acoustic velocity, p_{ac} is applied acoustic pressure, and d is distance of propagation.

From this equation, it can be inferred that harmonic generation increases with distance of propagation and that source pressure and second harmonic pressure have a nonlinear relation. These two aspects have a substantial role in understanding why tissue harmonic imaging can improve image quality.

The growth in harmonic frequencies with distance is a consequence of the mechanism of their generation. The original pulse, in fact, is composed only of the fundamental frequencies and, as soon as it propagates through tissue, energy builds at the second harmonic frequency. After a few centimeters, enough energy has been converted from the fundamental frequency to produce a significant harmonic frequency energy wave. Because much of the artifact in a US image is related to reverberations and scattering at or near the body wall, these artifacts contain relatively little harmonic frequency energy; hence, imaging in the harmonic range will eliminate much of the near-field artifacts [2].

The nonlinear relation between fundamental frequency energy and harmonic frequency energy can be understood by considering that harmonics are generated by the change in density of water and the corresponding changes in US velocity through water. These changes occur more dramatically with higher energy waves than with lower energy waves; thus, the generation of harmonics is related nonlinearly (squared relation between P_2 and p_{ac}) to fundamental frequency energy. Weak fundamental frequencies produce almost no harmonic frequency energy, whereas strong fundamental waves generate considerable harmonic frequency energy. This is crucial because much of the artifacts in US result from aberrant propagation that is considerably weaker than the central imaging beam.

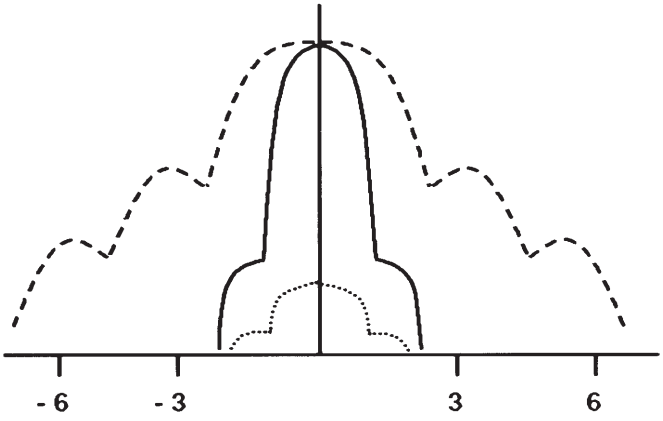


Fig. 1 Beam profiles of fundamental frequency *(dashed line)*, second harmonic frequency *(dotted line)*, and amplified second harmonic frequency *(solid line)*. Second harmonic beam profile, although much weaker than the fundamental one, has substantial reduction of side lobes with respect to central beam. When second harmonic is amplified to bring central beam signal up to the strength of the fundamental frequency, side lobes remain considerably lower in strength than side lobes in fundamental frequency

The beam profile at the fundamental frequency has a strong central lobe and weaker but still significant side lobes, which are responsible for much of the clutter that occurs (Fig. 1). Side-lobe artifacts result when US energy outside the main US beam hits an interface and is reflected back to the transducer, producing artifactual echoes. The beam profile obtained at the second harmonic frequency shows a substantial reduction in side-lobe energy with respect to the central beam. If the second harmonic signal is amplified to bring the central beam signal up to the original fundamental amplitude, the side lobes remain considerably below where they would have been in fundamental frequency energy imaging (Fig. 1). As a result, much of the near-field artifacts can be eliminated [2, 3].

Technical issues

The amount of harmonic frequency energy returning from tissue is much less than that returning at the fundamental frequency. The benefit of harmonic frequency imaging is therefore dependent on elimination of the fundamental frequency. To this aim, instrumentation must meet some important requirements [2]. Firstly,

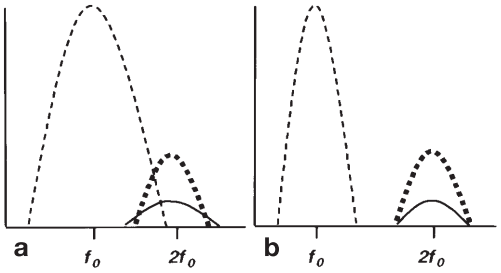


Fig. 2a, b Effects of narrowing of band pulse and sharp filter on frequency spectrum passed to demodulator. **a** When using a wide-band pulse filtered signal keeps substantial fundamental component. **b** In contrast, use of narrow band pulse results in purely harmonic filtered signal

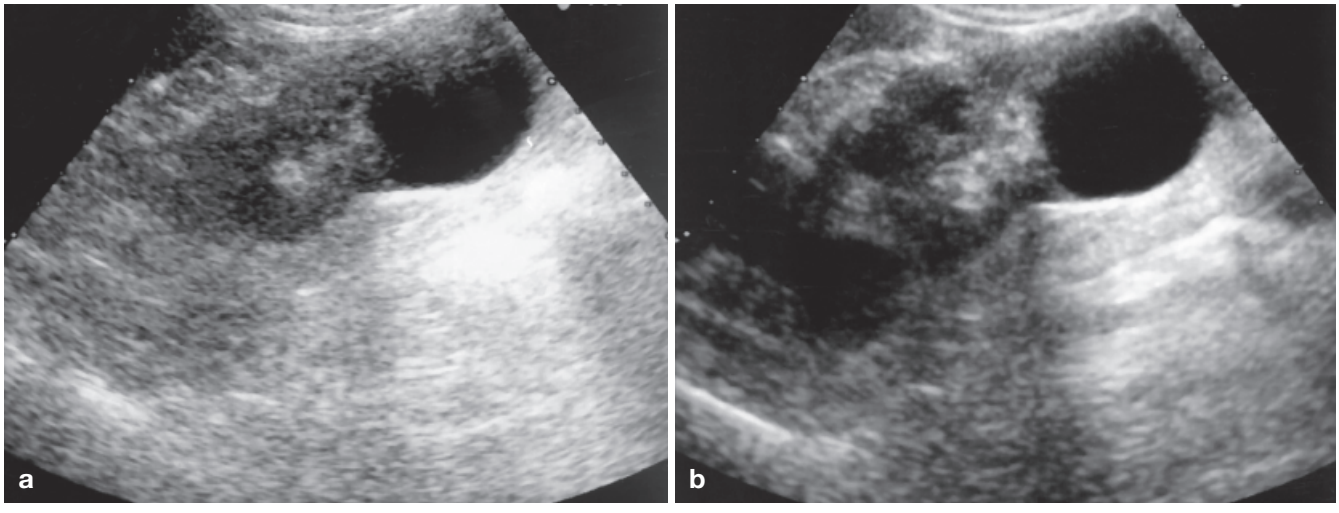
since 10–20 dB of signal strength are lost using harmonic imaging, a wide dynamic range is needed to preserve signal-to-noise ratio and to image this relatively weak signal. Secondly, the transmitter must emit a smooth pulse, with frequencies contained in a narrow band with very little energy at the harmonic frequency. Since US beam width is the principal determinant of lateral resolution, the reduced width of the US beam in tissue harmonic imaging improves lateral resolution [5]. This narrow band pulse must be coupled with a sharp receiver filter, so that only the harmonic frequencies are passed to the demodulator (Fig. 2). When all these technical requirements are met, the improvement in image quality provided by tissue harmonic imaging can be substantial [5].

Fig. 3a, b Renal cyst. **a** Conventional US image in fundamental mode is degraded by reverberation and side-lobe artifacts. **b** In tissue harmonic imaging, the overall image quality is improved and cystic content is blackened

Clinical applications

Tissue harmonic imaging has been shown by many authors to improve image quality by increasing signal-to-noise ratio – as a result of the reduction of side-lobe artifacts – and enhancing lateral resolution owing to the narrower width of the US beam with respect to that of the fundamental beam [5, 6, 7]. These properties enable tissue harmonic imaging to obtain an image in which tissues appear brighter and cavities appear darker and to resolve small anatomic structures and details [7, 8]. Reverberation and side-lobe artifacts commonly degrading the assessment of fluid-containing lesions, particularly in obese patients, are substantially reduced, and posterior acoustic enhancement is more easily demonstrated, allowing accurate characterization (Figs. 3, 4). Tissue harmonic imaging has also been shown to increase lesion conspicuity, improving delineation of low-contrast solid lesions (Figs. 5, 6). Moreover, elimination of reverberation artifacts and diminished defocusing effects of ample subcutaneous fat in tissue harmonic imaging permit more reliable demonstration of acoustic shadowing from stones or calcifications (Fig. 7) [7].

The advantages of tissue harmonic imaging over fundamental mode US have been shown mainly in the evaluation of abdominal pathologic findings. Shapiro et al. [5] performed a prospective analysis in 89 patients to compare the image quality of tissue harmonic imaging with that of fundamental mode in abdominal US. For tissue harmonic imaging, the transmitted frequency was 2.0 MHz, and the received frequency was 4.0 MHz. Tissue harmonic imaging outperformed fundamental mode for penetration, detail, and total image quality in the large majority of the examinations. Choudhry et al. [8] confirmed that tissue harmonic imaging was superior to conventional US with regard to lesion visibility and diagnostic confidence, especially for depicting cystic le-



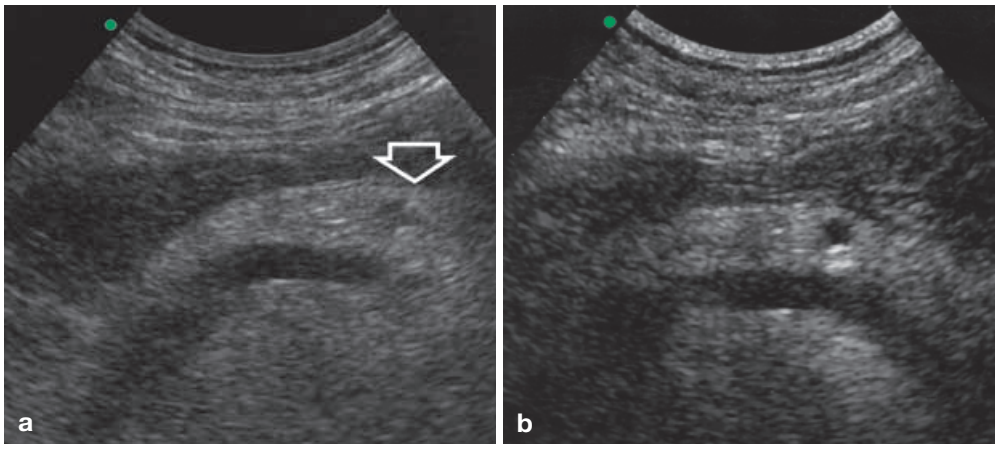


Fig. 4 a, b Small pancreatic cyst. **a** On the conventional US image, the tiny focal lesion of the pancreatic body *(arrow)* has solid-like appearance. **b** Tissue harmonic image shows its cystic nature by demonstrating anechoic content with posterior acoustic enhancement

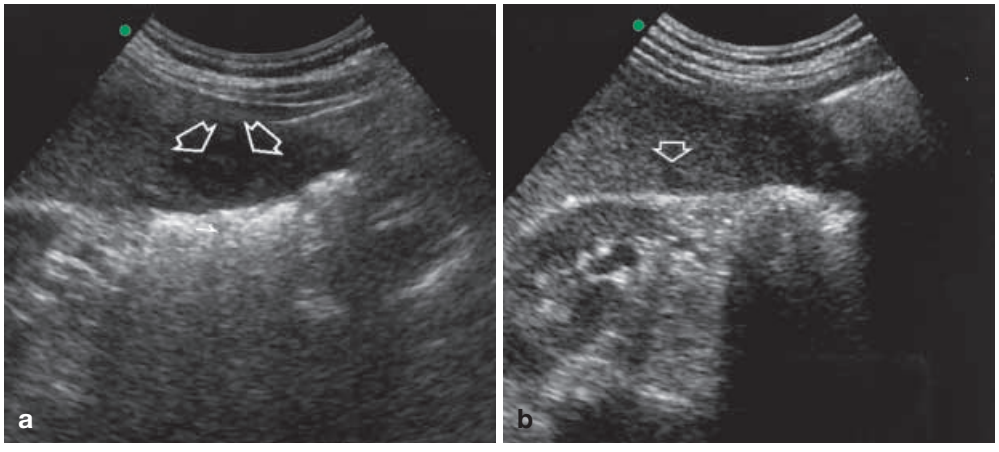


Fig. 5 a, b Hepatocellular carcinoma. **a** Conventional US shows small hepatocellular carcinoma in cirrhotic liver *(arrows)*. **b** Tissue harmonic image better delineates the lesion and shows a tiny satellite nodule *(arrow)* that was not prospectively called in fundamental mode

sions and those containing echogenic tissues such as fat, calcium, or air.

In the series of Cioni et al. [9], 68 abdominal or pelvic abnormalities – including nodular lesions of liver, pancreas, or spleen; abdominal or pelvic lymphoadenopathies; cystic lesions of liver, kidney, pancreas, or ovary; and biliary or renal stones – were examined with both conventional US and tissue harmonic imaging. Masked images were then reviewed, and overall image quality, lesion conspicuity, quality of lesion delineation, and confidence in diagnosis were assessed. The overall image quality of tissue harmonic imaging was shown to be better than that of conventional US, with the highest difference observed in cystic lesions and stones. Lesion conspicuity and quality of lesion delineation also reached a higher score in tissue harmonic than in conventional US images, although the confidence in final diagnosis was not significantly increased.

Two recent studies compared tissue harmonic imaging with conventional US in the study of focal liver lesions. In one series [10] tissue harmonic imaging provided the same information as conventional US in 71 % of patients but added information in the remaining 29 %. In addition, 17 % of patients had lesions revealed by tissue harmonic imaging only, and 10 % of patients had a change in therapeutic approach based on tissue harmonic imaging findings. In another study [11], a total of 15 reviewers reviewed 100 randomly arranged liver images, a fundamental gray-scale and a tissue harmonic image of each of 50 patients taken from the same section. The overall accuracy for detecting lesions was significantly better with tissue harmonic imaging than with fundamental mode, with the highest difference found in patients with liver cirrhosis. In this group the correct diagnosis of hepatocellular carcinoma was achieved at a higher rate with tissue harmonic imaging than with fundamental US.

Ortega et al. [12] compared tissue harmonic imaging with conventional US of the biliary tract in 80 patients. Final diagnoses included malignant biliary obstruction, choledocholithiasis, sclerosing cholangitis, normal or nonobstructed ducts, and miscellaneous conditions. Similar images were taken with each technique in terms

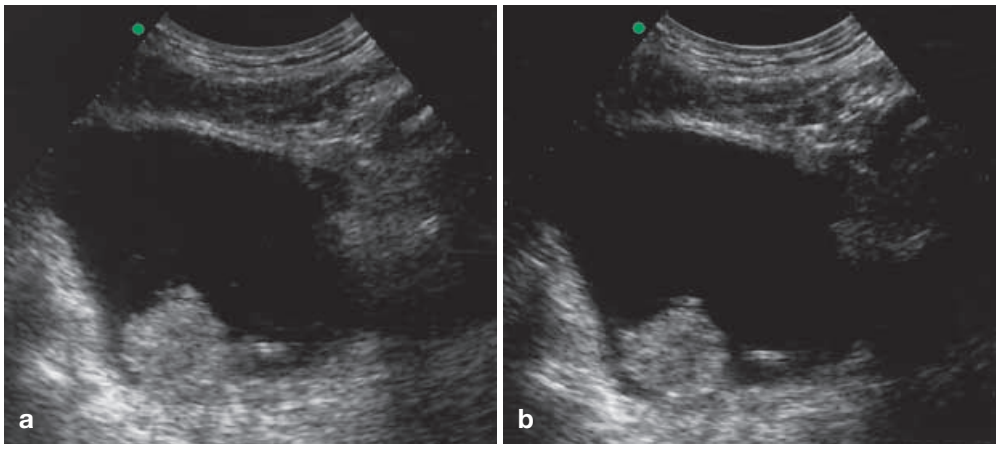


Fig. 6 a, b Bladder carcinoma. **a** In the conventional US image, the relationship of the tumor with the bladder wall is unclear. **b** Tissue harmonic image shows pedunculated lesion

of projection, field of view, focal zone selection, and evidence of disease. Two separate observers blinded to patient data and technique reviewed and graded images individually for the appearance of the lumen of the bile ducts, the length of the visible duct, the appearance of the duct wall, the presence of any intraluminal masses, and the appearance of associated acoustic shadows. The median of tissue harmonic images was one grade higher than the median for the corresponding conventional images. Improvements with tissue harmonic imaging included better sharpness of the duct walls, a clearer lumen, identification of a longer length of the common bile duct, and improved detection of intraluminal masses. Acoustic shadows were better defined and blacker with tissue harmonic imaging.

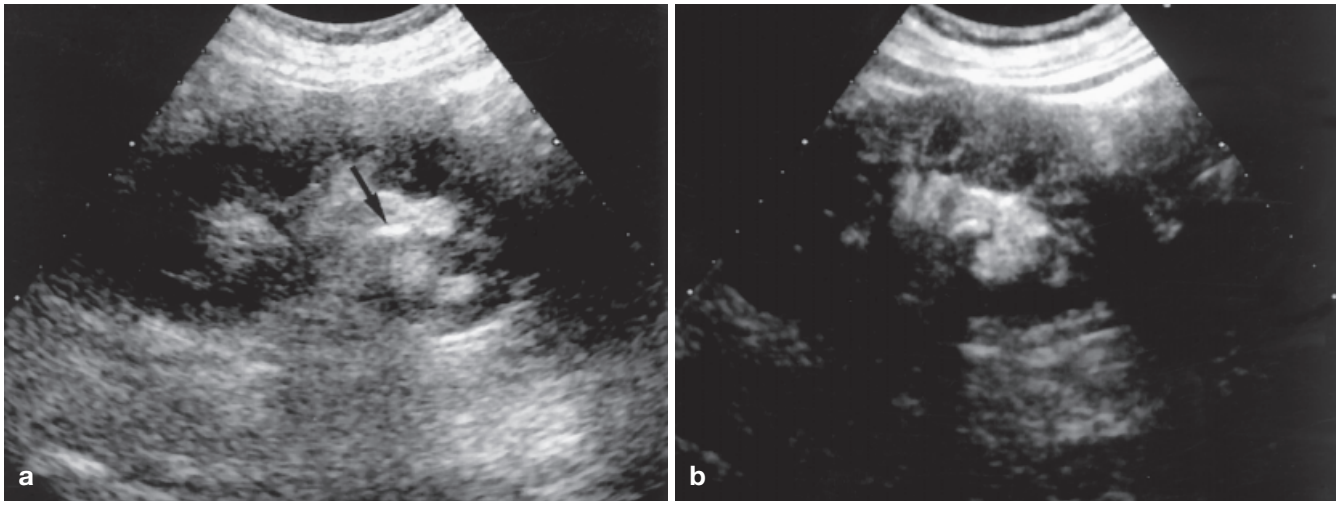
Fig. 7 a, b Renal stone. **a** On the conventional US image, the stone *(arrow)* does not show acoustic shadowing. **b** Tissue harmonic image delineates the stone much better and clearly outlines acoustic shadowing

These early experiences show that tissue harmonic imaging can substantially improve image quality in abdominal US, particularly in those instances in which conventional US is not fully satisfactory, and are prompting the routine use of tissue harmonic imaging in the examination of several abdominal districts [12, 13, 14, 15].

Contrast-specific imaging

Physical principles

Stabilized microbubbles act as effective vascular echo enhancers due to the large difference in acoustic impedance compared with the surrounding blood, and were, in fact, primarily designed as blood pool agents. Microbubble contrast agents contain either air ("first-generation contrast agents": Levovist, Schering, Berlin, Germany; and Albunex, Mallinckrodt, St. Louis, Mo.) or other gases ("second-generation contrast agents"),



including sulfur hexafluoride (SonoVue, Bracco, Milan, Italy) and perfluorocarbons (Optison, Mallinckrodt/Nycomed Amersham, Oslo, Norway; Echogen, Sonus/Abbott, Bothell, Wash; Definity, Du Pont Merck, North Billerica, Mass.; and others) [16, 17, 18, 19, 20, 21]. Second-generation contrast agents have been shown to have prolonged longevity due to the lower solubility of their gases with respect to air. Another new class of contrast agents, which includes Sonazoid (Nycomed Amersham), Sonovist (Schering), and BR14 (Bracco), is characterized by reticuloendothelial uptake, which follows the vascular phase [21, 22, 23, 24, 25, 26].

For several years the major diagnostic objective in using US contrast agents has been to detect flow in the circulation at a lower level than that otherwise possible in Doppler techniques; however, it turned out that enhanced color and power Doppler could provide fine details on small and deep vessels, but were not sensitive enough to detect contrast agents in the microcirculation [27, 28]. New US techniques based on specific properties of US contrast agents have therefore been introduced. These contrast-specific imaging techniques can display microbubble enhancement in gray-scale, thus maximizing contrast and spatial resolution, and enabling evaluation of the microcirculation. As a matter of fact, these techniques were instrumental in permitting the evolution of contrast US from vascular imaging to the imaging of perfused tissues.

The novel gray-scale contrast-dedicated techniques are based on the complex interactions between microbubbles and US beam. The major determinant of this interaction is the peak negative pressure of the transmitted US pulse, grossly reflected in the mechanical index (MI) [29]. By scanning with a low peak pressure (0–100 kPa), microbubbles behave as linear backscatterers, alternatively contracting and expanding according to the positive and negative pressures of the sinusoidal sound waves. This results in simple reflection of US echoes. As the incident peak pressure increases (100 kPa to 1 MPa), however, microbubbles begin to show nonlinear characteristics and start emission of harmonics. Harmonics are created, since on the negative portion of the sound waves, microbubbles can become quite large, but on the positive portion there is a limit to which they can contract: This asymmetry is what constitutes the harmonic emission. With further increase in the peak pressure of the incident US field, although still within accepted limits for diagnostic imaging, the shell of the microbubbles is disrupted: During this process a transient, strong, nonlinear echo is emitted [21, 29].

The novel gray-scale contrast-enhanced techniques use the microbubbles nonlinear and transient scattering properties to enhance signals from the contrast agents over the tissue. Different strategies were followed to cancel fundamental linear components and highlight the nonlinear harmonic components. In the methods termed “pulse or phase inversion” (ATL-Philips, Bothell, Wash.; Siemens Medical Systems, Issaquah, Wash.) [30, 31, 32, 33], two identical but inverted pulses are transmitted down each ray line: any linear target which responds equally to positive and negative pressures will reflect back to the transducer equal but opposite waveforms. These are then added in the beamformer and all linear targets cancel. Microbubbles respond differently to positive and negative pressures and do not reflect identical inverted waveforms; hence, when these waveforms are added, they do not cancel completely, and the harmonic components add, giving strong signal. Alternate methods, such as the “C-cube mode” (Esaote Biomedica, Genoa, Italy) rely on the transmission of two identical pulses down each ray line and on the comparison of returning signals: This enables identification of the nonlinear response.

These techniques offer high sensitivity either to microbubble movement or to microbubble collapse in dependence of the level of the applied acoustic peak pressure. At low acoustic peak pressure levels (low MI), the microbubble destruction can be reasonably neglected and the microbubble movement effect (due to blood circulation) is predominant. Conversely, when increasing the acoustic peak pressure levels (high MI), the destruction phenomena become most important: The signal, in fact, is produced by microbubble collapse, and is related to microbubble concentration and not to blood flow velocity. As a consequence, very slow blood flow in microcirculation and stationary blood (i.e., in hepatic sinusoids) can be detected (Figs. 8, 9) [34].

Technical issues

In gray-scale US, contrast agents cannot be depicted in the microcirculation and do not create enhancement of liver parenchyma when the fundamental mode is used. The echo from the tissue is still far too strong, compared with that from the small volume of contrast agent in the microcirculation of the tissue itself. The simplest method for displaying microbubbles over tissue is to destroy the microbubbles themselves by using a contrast-specific technique with high US power (high MI). Destruction of the microbubbles results in a strong, transient effect that may be seen as intense enhancement only on the first frame of the US scan: The interval between frames, in fact, is far too short to allow replenishment of the scanning plane with new microbubbles. It is therefore essential to wait a minimum time interval of 10–15 s between each sweep to have replenishment of new microbubbles: This is why the method is known as “interval delay” or “intermittent” imaging.

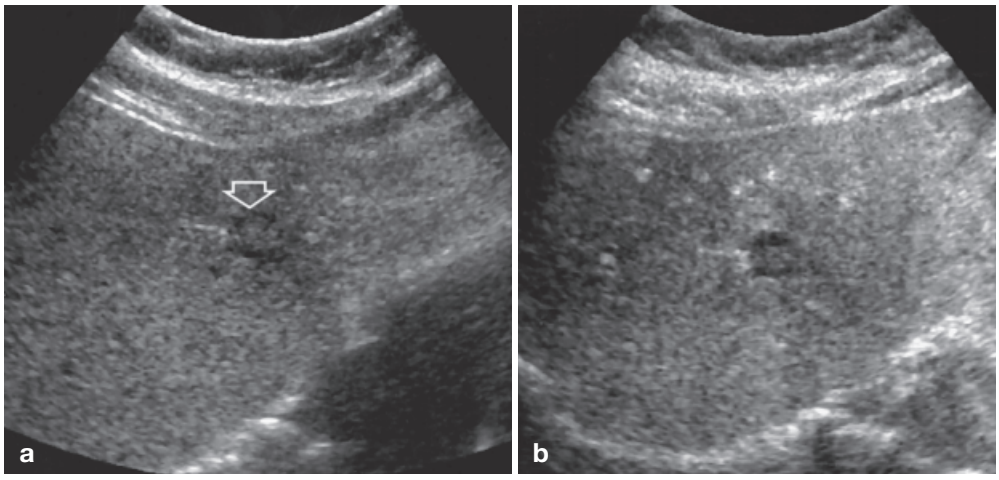


Fig. 8 a, b Pulse inversion imaging of the liver. **a** Ultrasound in fundamental mode hardly shows focal lesion *(arrow)* that is isoechoic with respect to liver parenchyma. **b** In the pulse inversion image obtained in the late phase following contrast injection, liver parenchyma is enhanced due to destruction of microbubbles in hepatic sinusoids. As a result, contrast between lesion and liver is increased

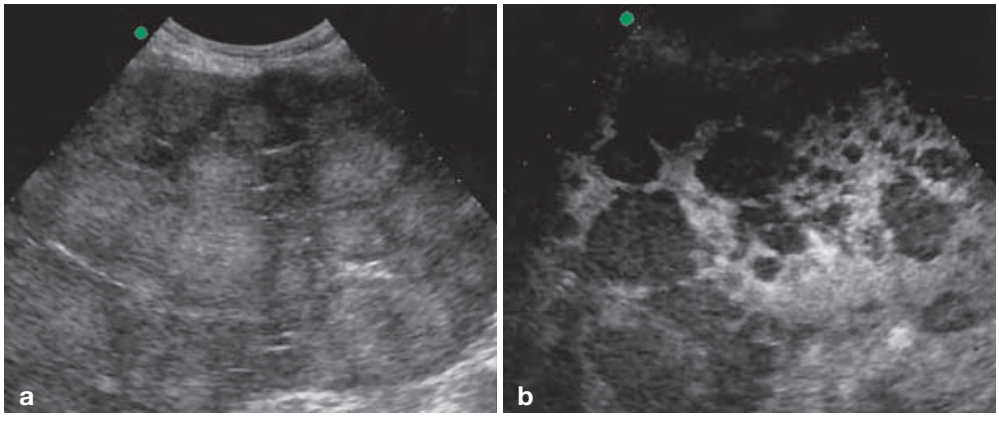


Fig. 9 a, b C-cube imaging of the liver. **a** Ultrasound in fundamental mode shows multiple focal lesions that are hyperechoic with respect to liver parenchyma. **b** In the C-cube image obtained in the late phase following contrast injection, destruction of microbubbles in hepatic sinusoids results in strong enhancement of liver parenchyma, with focal lesions remaining unenhanced. Numerous additional small nodules become visible

This method has found interesting application in the study of the liver. Interval delay or intermittent imaging, in fact, can nicely depict the differential perfusion kinetics between normal liver tissue and tumors, thus improving lesion detection and characterization. It can be performed by using either first- or second-generation US contrast agents [35, 36, 37].

When the US examination is aimed at lesion detection, scanning is typically started in the late liver parenchymal phase. This usually means a delay of 2–5 min from the injection of the contrast agents to scanning, a time interval that allows microbubbles to replenish hepatic sinusoids and possibly be phagocytosed by Kupffer cells. The key of the method is to perform a series of sweeps that could enable adequate coverage of the whole liver parenchyma, avoiding microbubble destruction in the liver volume adjacent to the one under interrogation. Cine loops of each sweep are then reviewed, and liver tumors, appearing as focal defects, are assessed (Fig. 10) [30, 31, 33, 34, 35].

If the US examination is aimed at lesion characterization, the best scan plane to image the lesion is selected, and intermittent images are acquired following bolus injection of the contrast agents. It is important to obtain images reflecting the arterial phase (15–25 s after the start of the injection), the portal in-flow phase (40–55 s), the full portal phase (70–85 s), and the delayed phase (180–200 s) to have a precise assessment of the lesion enhancement pattern. Since focal hepatic lesions show, to some extent, differences in blood supply, it is possible with this technique to characterize benign and malignant tumors (Fig. 11) [36, 37, 38, 39].

A limitation of this destructive high-MI method is that it only produces a transient display of contrast agent. This may cause inhomogeneity of enhancement, especially in the near field, as well as shadowing artifacts from larger vessels carrying high concentration of the contrast agent. Moreover, this method has limited penetration. Newer techniques based on the higher harmonic emission capabilities of second-generation contrast agents, such as “contrast tuned imaging” (Esaote Biomedica), overcome some of these limitations. Their main advantage is that a lower, nondestructive US

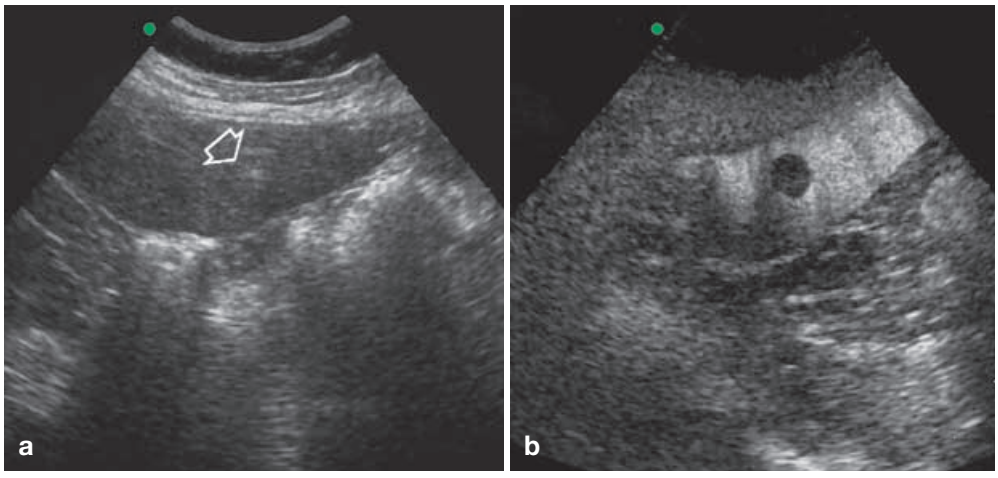


Fig. 10 a, b Late-phase scanning of liver parenchyma aimed at lesion detection. **a** Conventional US detects a questionable small focal lesion *(arrow)* at the anterior margin of the liver. **b** In the C-cube image obtained 3 min after contrast injection liver parenchyma is homogenously enhanced, and focal lesion, corresponding to liver metastasis, is easily detected

power (very low MI) is used, which enables continuous imaging without the need for time intervals between scans for contrast replenishment [40].

The continuous scanning permitted by very low-MI techniques facilitates examination of liver parenchyma and improves detection of focal lesions, including hypervascular tumors. Nevertheless, for lesion characterization based on accurate assessment of tumor vascularity and neoangiogenesis, the interval-delay modality at high MI described above can be combined [39, 40], and offer advantages. Ultrasound imaging strategy may consist of preliminary continuous scanning at low MI to detect contrast arrival and evaluate the tumoral vascular morphology. An interval-delay scan at high MI may then be performed during the arterial as well as the portal venous phase to accentuate the differential vascularity between tumor and normal liver tissue. It has to be considered, however, that for lesions with very slow internal flow, such as liver hemangiomas, the protocol needs to be tailored to demonstrate the centripetal progression of the peripheral nodular enhancement by increasing the interval delays to allow for the accumulation of agents throughout the lesion. For contrast agents with liver-specific parenchymal phase, a late sweep over the lesion is performed using any of the nonlinear imaging modality either at low MI for second-generation contrast agents or high MI for first-generation contrast agents.

Clinical applications

Liver lesion detection

Worldwide, US is the most commonly used imaging modality to screen for focal liver disease. Populations at high-risk to develop either primary or secondary hepatic tumors, such as patients with liver cirrhosis and those with an history of extrahepatic malignancy, are usually followed-up with US examination performed every 6 months in order to detect malignant lesions at an early stage. Early detection of primary and secondary liver malignancies, in fact, greatly enhances the possibility of curative surgical resection or successful percutaneous ablation. Unfortunately, US has a limited sensitivity in the detection of small tumor nodules, especially in the setting of cirrhotic patients or oncology patients undergoing chemotherapy. Spiral CT and MR imaging offer higher sensitivity than conventional US but are not suitable for most screening programs.

The goal of improving lesion detection with contrast-specific US techniques has been initially pursued by imaging with high MI (destructive mode) in the late phase following the administration of either first-generation or second-generation contrast agents. Dalla Palma et al. [30] evaluated the capability of contrast US, performed by using pulse inversion harmonic imaging, to detect liver metastases in comparison with fundamental B-mode US and spiral CT. Thirty-six consecutive patients with known malignancies and proved or suspicious liver metastases on baseline US were examined with contrast US performed 2, 4, and 6 min following bolus administration of 2.5 g Levovist at the concentration of 300 mg/ml. The optimum parenchymal enhancement and contrast difference between liver and metastases was observed during the 2-min measurements. Contrast US revealed more lesions than fundamental B-mode US in 56 % of patients, and more lesions than spiral CT in 22 % of patients; however, in 5 % of patients with lesions mainly located in anterior superficial and deep liver areas spiral CT showed more lesions than contrast US. Harvey et al. [31] investigated 20 consecutive patients with known liver malignancies that underwent US in conventional B mode and in pulse inversion mode in the late parenchymal phase after intravenous administra-

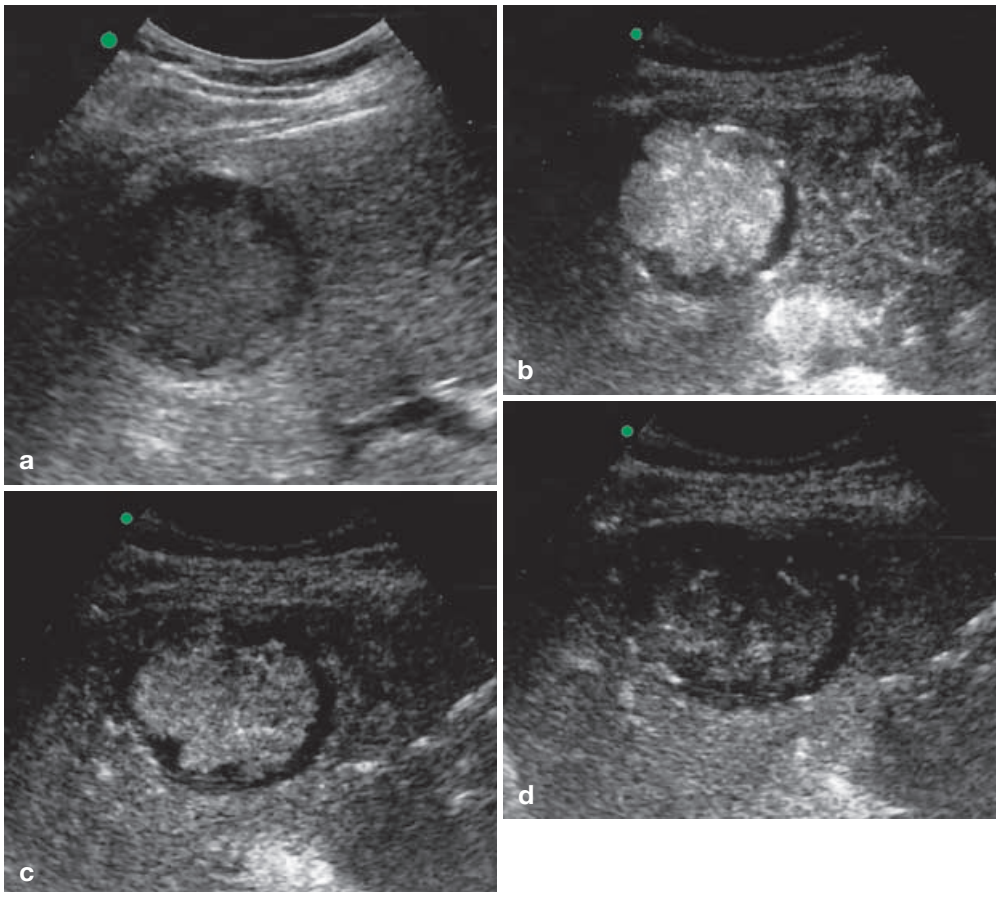


Fig. 11 a–d Interval-delay scanning of focal liver lesion aimed at tumor characterization. **a** Baseline US shows solid hypoechoic tumor. **b** Contrast-enhanced C-cube image obtained in the arterial phase shows strong and homogeneous enhancement of the tumor. **c** In the portal phase, tumor starts wash-out of contrast, and liver parenchyma is enhanced, resulting in better delineation of hypoechoic tumor capsule. **d** In the delayed phase, the lesion is hypoechoic to liver. Findings at the US study are consistent with hepatocellular carcinoma

tion of Levovist. Subjective and objective lesion conspicuity were improved with pulse-inversion mode, and smaller lesions were depicted with pulse-inversion mode than with conventional B mode, improving the detection of metastases less than 1 cm in size. Kim et al. [32] and Albrecht et al. [33] confirmed that delayed contrast US was superior to unenhanced imaging in terms of lesion detection rate, lesion conspicuity, and lesion-to-liver contrast. The superiority of contrast US was especially manifest in the depiction of very small lesions, down to 2 mm in size.

From these experiences, it appears that the US sensitivity in lesion detection can be substantially increased with the use of contrast-specific imaging. Improved detection of small hypovascular metastases with respect to baseline US studies, in particular, has been confirmed by different authors who used different contrast agents and scanning techniques (Figs. 12, 13). In addition, the possibility to perform a reliable and comprehensive continuous real-time examination of the liver parenchyma, by using techniques at very low MI in combination with second-generation contrast agents, seems to enable even better lesion detection, especially in the case of hypervascular tumors.

Liver lesion characterization

In focal liver lesions, US findings are often nonspecific, as there is enough variability and overlap in the US appearance of benign and malignant tumors to make a definite distinction problematic. Spiral CT and MR imaging, with use of either extracellular or tissue-specific contrast agents, are commonly used to clarify questionable US findings. With the introduction of the newer US techniques, interest has been focused on the evaluation of the microcirculation of focal liver lesions, with the aim to improve lesion characterization (Figs. 14, 15, 16, 17) [36, 39, 40].

Kim et al. [36] evaluated contrast agent-enhancement patterns of hepatic lesions at pulse-inversion harmonic US using a first-generation contrast agent. Twenty hepatic hemangiomas and 41 malignant hepatic tumors (33 metastases and 8 hepatocellular carcinomas) were evaluated. Ultrasound images were obtained before injection and every 10–15 s after injection of a 4-g bolus (300 mg/ml) of Levovist for 5 min. Of 20 hemangiomas, 19 revealed peripheral enhancement, which was globular in 14 (70%) and rim-like in 5 (25%), with centripetal fill-in; the remaining one (5%) showed ho-

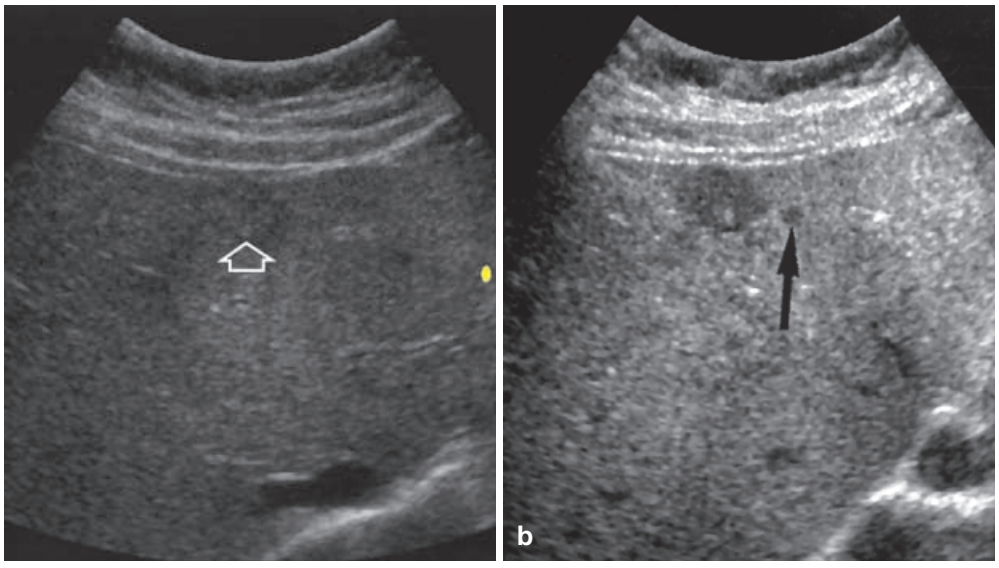


Fig. 12 a, b Improved detection of focal liver lesions with use of contrast-enhanced pulse inversion imaging. **a** Ultrasound in fundamental mode detects a questionable focal lesion *(arrow)*. **b** Pulse inversion image obtained in the late phase after contrast injection clearly delineates the small metastasis and shows an additional 3-mm metastatic deposit *(arrow)*

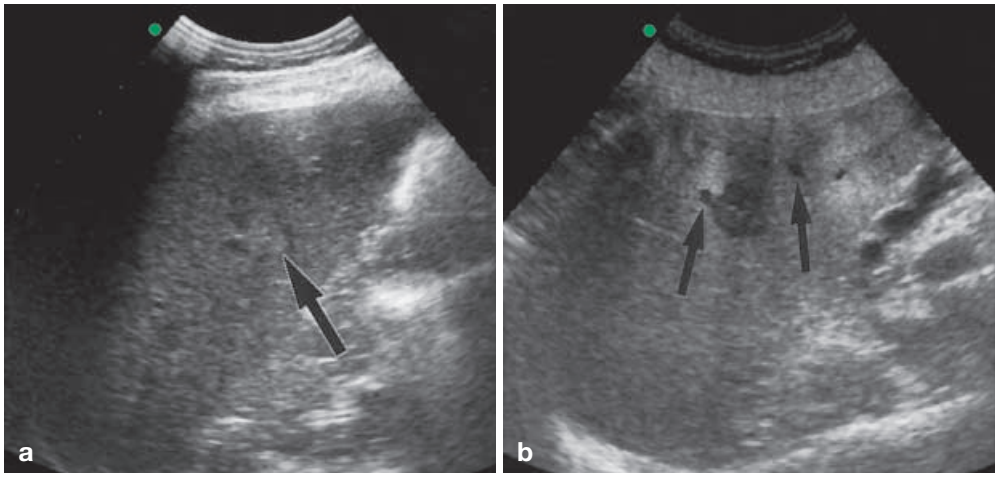


Fig. 13 a, b Improved detection of focal liver lesions with use of contrast-enhanced C-cube imaging. **a** Conventional US detects a small solitary metastasis *(arrow)*. **b** C-cube image obtained in the late phase after contrast injection clearly delineates the lesion and shows two additional 3-mm metastatic nodules *(arrows)*

mogeneous enhancement. In 33 metastases, the enhancement was rim-like in 16 (48%), homogeneous in 7 (21%), and stippled in 2 (6%); in the remaining 8 metastases (24%), no enhancement was seen. Of 8 hepatocellular carcinomas, 4 (50%) showed homogeneous enhancement and the remaining 4 (50%) showed heterogeneous enhancement. Centripetal fill-in of lesions with intratumoral enhancement was not seen in any malignancy. Bertolotto et al. [37] evaluated the capabilities of pulse inversion harmonic imaging in characterization of unifocal liver lesions in 46 consecutive patients. Images were acquired before and 30 s, 2 min, and 4 min after bolus administration of Levovist (2.5 g, 300 mg/ml). Hepatocellular carcinoma was hyperechoic on 30-s scan, and hypoechoic ($n = 5$) or isoechoic ($n = 2$) on 2-min scan. Cholangiocarcinoma had inhomogeneous persistent enhancement. Focal nodular hyperplasia was hyperechoic ($n = 5$) or isoechoic ($n = 2$) on 30-s scan, and hyperechoic ($n = 4$), isoechoic ($n = 2$) or slightly hypoechoic ($n = 1$) on 2-min scan. Large hemangioma revealed peripheral enhancement on 30-s scan which extended centripetally on 2-min scan. Small hemangioma appeared isoechoic on 2-min scan in all but 2 cases in which they were hypoechoic on 2-min scans and hyperechoic on 4-min scan. Metastasis was hypoechoic on all scans, 70% with rim enhancement.

Numata et al. [38] compared findings at contrast-specific gray-scale US imaging, performed with use of wideband harmonic mode after intravenous bolus injection of Levovist, with those at spiral CT. In this paper, 57 of 61 hepatocellular carcinomas included in the study showed hypervascular enhancement, with intratumoral vessels depicted in 40 of 57 lesions: Spiral CT revealed

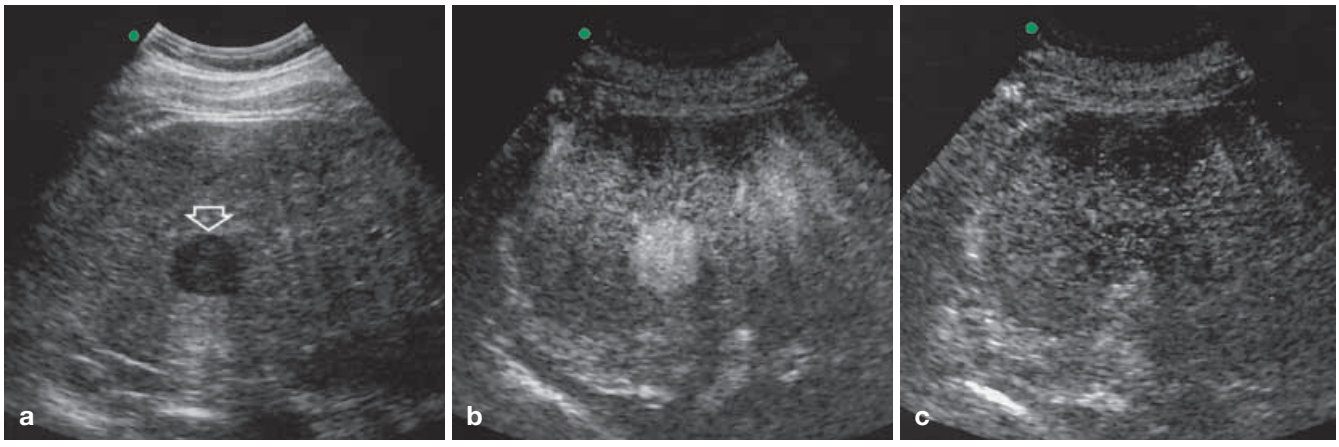


Fig. 14 a–c Hepatocellular carcinoma. **a** Baseline US detects a small hypoechoic lesion *(arrow)*. The tumor shows clear-cut homogeneous enhancement in **b** the arterial phase C-cube image, followed by **c** wash-out in the late phase

hyperattenuation in 54 of the 61 lesions, whereas the other lesions showed an equivocal attenuation area. Wilson et al. [39] used a second-generation US contrast agent to characterize blood flow in focal hepatic tumors: Lesion enhancement was assessed with continuous, harmonic gray-scale imaging performed with a low MI after injection of Optison, and brief, high-MI interval-delay imaging, was associated to help determine tumor vascular volume. In this series, contrast-specific US imaging enabled accurate assessment of tumor vascularity. The contrast agent with late reticuloendothelial phase Sonazoid was used by Leen et al. [41] to characterize focal liver tumors: The large majority of malignant lesions, including all of the metastases and most hepatocellular carcinomas, showed no contrast uptake in the reticuloendotelial phase and appeared as complete filling defects. In contrast, benign lesions, such as focal nodular hyperplasia and hemangiomas, showed definite contrast uptake. A minority of hepatocellular carcinomas, however, also showed contrast uptake in the late phase: This behavior was believed to be associated with well-differentiated tumors containing Kupffer cells.

Other areas of investigation

Besides liver lesions, the areas of potential clinical interest for contrast-enhanced gray-scale US are numerous. However, as the technique is at a very early stage of

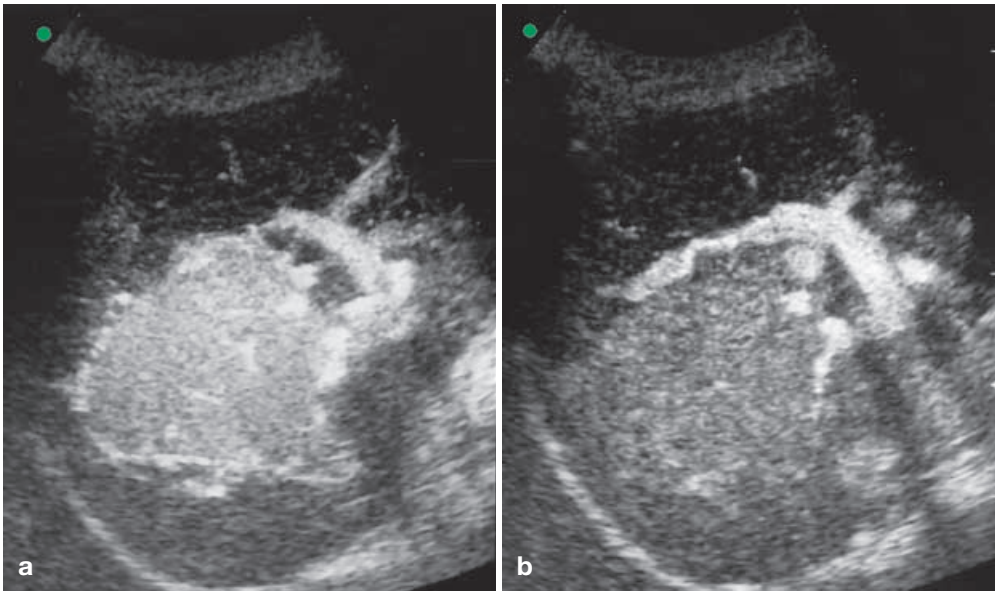


Fig. 15 a, b Focal nodular hyperplasia of the liver. **a** The lesion shows marked homogeneous enhancement in the arterial phase C-cube image, and **b** remains slightly hyperechoic with respect to liver parenchyma in the portal phase

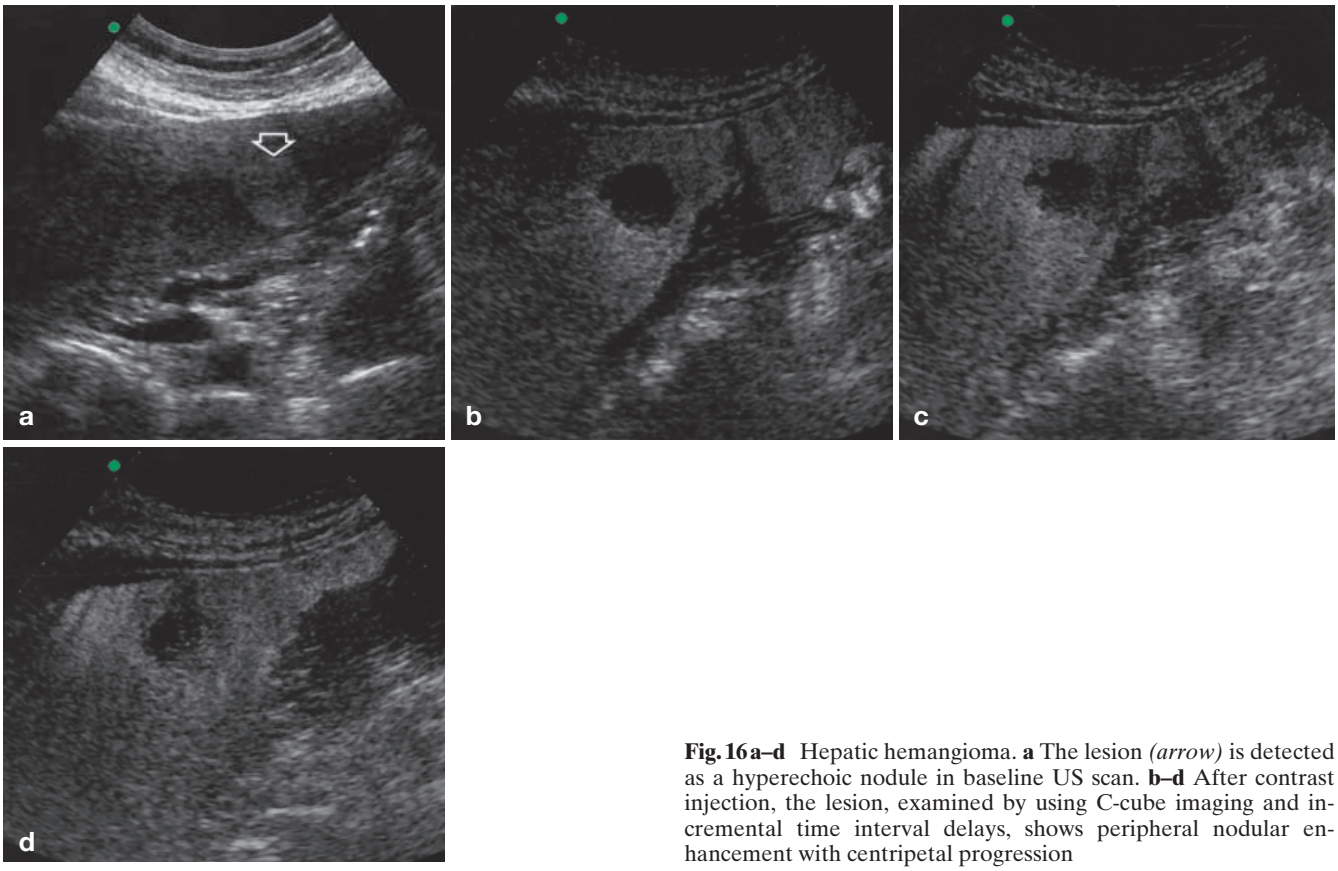


Fig. 16 a–d Hepatic hemangioma. **a** The lesion *(arrow)* is detected as a hyperechoic nodule in baseline US scan. **b–d** After contrast injection, the lesion, examined by using C-cube imaging and incremental time interval delays, shows peripheral nodular enhancement with centripetal progression

development, few reports, excluding those related to cardiological applications, especially with regard to the evaluation of myocardial perfusion [42, 43, 44, 45], have been published.

Interest has been attracted by vascular applications of the new contrast US techniques [46]. Sirlin et al. [47] compared contrast-enhanced gray-scale US with color and power Doppler US angiography in the assessment of atherosclerotic lesions of rabbit aortas. In addition, four replicas of diseased human carotid arteries were immersed in a tissue-mimicking phantom and imaged with gray-scale and color and power Doppler US before and after the administration of the contrast agent. Radiopaque plastic casts of the rabbit aortas and contact radiographs of the plastic replicas served as standards. Although color and power Doppler US allowed immediate localization of the lumen, precise estimation of stenoses and reliable visualization of surface irregularities were not possible. After contrast administration, angiogram-like images of the lumen were created with gray-scale US, allowing rapid assessment of the entire vessel lumen and wall. Consequently, luminal stenoses were more accurately measured than with unenhanced gray-scale US or Doppler. In addition, plaques and ulcerations were visible only with contrast-enhanced gray-scale US.

Concerning parenchymal organs, interesting studies focused on kidney and prostate abnormalities. Kim et al. [48] evaluated the ability of pulse inversion imaging in depicting renal parenchymal changes in acute pyelonephritis. The detection and conspicuity of renal parenchymal abnormalities in acute pyelonephritis observed with contrast-enhanced pulse inversion harmonic imaging were significantly better than those on conventional US. Halpern et al. [49, 50] assessed the detection of prostate cancer with contrast-enhanced transrectal US. Gray-scale imaging was performed in continuous mode and with intermittent imaging by using interscan delay times of 0.5, 1.0, 2.0, and 5.0 s. Sextant biopsy sites were scored prospectively as benign or malignant at baseline imaging and again during enhanced transrectal sonography. Baseline imaging demonstrated prostate cancer in 14 sites in 11 subjects. Enhanced transrectal US depicted prostate cancer in 24 sites in 15 subjects. The improvement in sensitivity from 38 % at baseline to 65 % with contrast enhancement was significant, and no significant loss in specificity was observed.

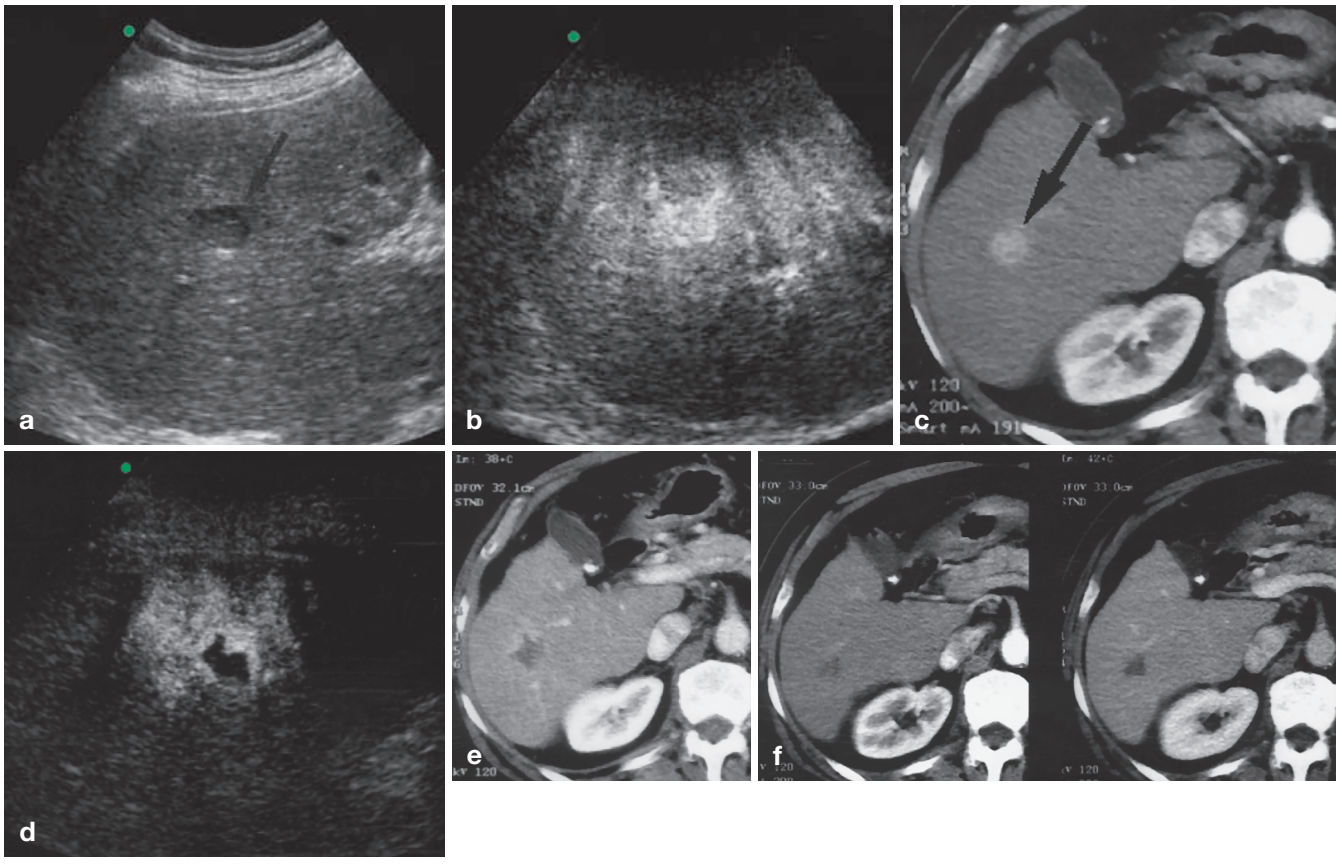


Fig. 17 a–f Hepatocellular carcinoma treated with radio-frequency thermal ablation. Before treatment, the lesion *(arrow)* is detected as a hypoechoic nodule in **a** baseline US scan with marked homogeneous enhancement on the **b** arterial phase C-cube image. **c** Spiral CT confirms hypervascular tumor *(arrow)*. **d** After the first radio-frequency treatment session, persistent peripheral enhancement is detected by C-cube image in the anterior aspect of the tumor. **e** Spiral-CT image confirms the presence of residual viable enhancing tumor tissue, matching the enhancing portions at contrast US. **f** After second treatment session performed with contrast US guidance, complete ablation is shown by dual-phase follow-up CT

Conclusion

Novel gray-scale US techniques, including harmonic imaging of native tissues and scanning methods based on nonlinear and transient acoustic effects of US contrast agents, has opened new prospects for US. Tissue harmonic imaging offers an opportunity for a more rapid, high-quality, and successful US study, and seems to have the potential to become a routine approach in most abdominal examination. Convincing reports have shown that contrast-specific US imaging can substantially improve US detection and characterization of focal liver lesions, and promising results have been reported in other areas of investigation. Some issues, however, are still unresolved. The standardization of contrast-specific US techniques is a mandatory step to allow a wide clinical use. The reproducibility and the comprehensiveness of the contrast US examination is still to be fully elucidated. Clinical applications outside the liver are currently at a very early stage, and further investigation is needed to confirm encouraging preliminary results. Further advances in current and future scanning methods will require a complete understanding of the specific properties of each new contrast agent and of its complex interaction with US. Nevertheless, it can be foreseen that, with continuous improvement in US technology and the optimal use of contrast agents, the new gray-scale US techniques will rapidly become a tool in numerous clinical scenarios.

References

1. Liu DL, Waag RC (1997) Harmonic amplitude distribution in a wideband ultrasonic waveform after propagation through human abdominal wall and breast specimens. J Acoust Soc Am 101: 1172–1183
2. Thomas JD, Rubin DN (1998) Tissue harmonic imaging: Why does it work? J Am Soc Echocardiogr 11: 803–808
3. Whittingham TA (1999) Tissue harmonic imaging. Eur Radiol 9 (Suppl 3):323–326
4. Adler L, Heiderman EA (1962) Determination of the nonlinearity parameter B/A for water and m-Xylene. J Acoust Soc Am 34: 410–412
5. Shapiro RS, Wagreich J, Parsons RB, Stancato-Pasik A, Yeh HC, Lao R (1998) Tissue harmonic imaging sonography: evaluation of image quality compared with conventional sonography. Am J Roentgenol 171: 1203–1206
6. Ward B, Baker AC, Humphrey VF (1997) Non-linear propagation applied to the improvement of resolution in diagnostic medical ultrasound. J Acoust Soc Am 101: 143–154
7. Desser TS, Jeffrey RB, Lane MJ, Ralls PW (1999) Tissue harmonic imaging: utility in abdominal and pelvic sonography. J Clin Ultrasound 27: 135–141
8. Choudhry S, Gorman B, Charboneau W, Tradup DJ, Beck RJ, Kofler JM, Groth DS (2000) Comparison of tissue harmonic imaging with conventional US in abdominal disease. Radiographics 20: 1127–1135
9. Cioni D, Crocetti L, Lencioni R, Donati F, Franchini C, Perri M, Bartolozzi C (2001) Comparison between tissue harmonic imaging and conventional US in the evaluation of abdomino-pelvic pathologic findings. Eur Radiol 11 (Suppl 1):121
10. Hann LE, Bach AM, Cramer LD, Siegel D, Yoo HH, Garcia R (1999) Hepatic sonography: comparison of tissue harmonic and standard sonography techniques. Am J Roentgenol 173: 201–206
11. Tanaka S, Oshikawa O, Sasaki T, Ioka T, Tsukuma H (2000) Evaluation of tissue harmonic imaging for the diagnosis of focal liver lesions. Ultrasound Med Biol 26: 183–187
12. Ortega D, Burns PN, Simpson DH, Wilson SR (2001) Tissue harmonic imaging: Is it a benefit for bile duct sonography? Am J Roentgenol 176: 653–659
13. Tranquart F, Grenier N, Eder V, Pourcelot L (1999) Clinical use of ultrasound tissue harmonic imaging. Ultrasound Med Biol 25: 889–894
14. Hong HS, Han JK, Kim TK, Kim YH, Kim JS, Cha JH, Choi BI (2001) Ultrasonographic evaluation of the gallbladder: comparison of fundamental, tissue harmonic, and pulse inversion harmonic imaging. J Ultrasound Med 20: 35–41
15. Desser TS, Jeffrey RB (2001) Tissue harmonic imaging techniques: physical principles and clinical applications. Semin Ultrasound CT MR 22: 1–10
16. Schlief R, Schurmann R, Niendorf HP (1996) Blood-pool echo enhancement after intravenous injection of galactose-based microbubbles: results from European phase III clinical trials in Doppler sonography. Acad Radiol 3 (Suppl 2):S466–S467
17. Albrecht T, Urbank A, Mahler M, Bauer A, Dore CJ, Blomley MJ, Cosgrove DO, Schlief R (1998) Prolongation and optimization of Doppler enhancement with a microbubble US contrast agent by using continuous infusion: preliminary experience. Radiology 207: 339–347
18. Scheider M (1999) SonoVue, a new ultrasound contrast agent. Eur Radiol 9 (Suppl 3):347–348
19. Bokor D (2000) Diagnostic efficacy of SonoVue. Am J Cardiol (Suppl 1) 17: 19–24
20. Bokor D, Chambers JB, Rees PJ, Mant TG, Luzzani F, Spinazzi A (2001) Clinical safety of SonoVue, a new contrast agent for ultrasound imaging, in healthy volunteers and in patients with chronic obstructive pulmonary disease. Invest Radiol 36: 104–109
21. Harvey CJ, Blomley MJK, Eckersley RJ, Cosgrove DO (2001) Developments in ultrasound contrast media. Eur Radiol 11: 675–689
22. Schneider M, Broillet A, Bussat P, Giessinger N, Puginier J, Ventrone R, Yan F (1997) Gray-scale liver enhancement in VX2 tumor-bearing rabbits using BR14, a new ultrasonographic contrast agent. Invest Radiol 32: 410–417
23. Forsberg F, Goldberg BB, Liu JB, Merton D, Rawool NM, Shi WT (1999) Tissue specific US contrast agent for evaluation of hepatic and splenic parenchyma. Radiology 201: 125–132
24. Bauer A, Blomley M, Leen E, Cosgrove D, Schlief R (1999) Liver-specific imaging with SHU 563 A: diagnostic potential of a new class of ultrasound contrast media. Eur Radiol 9 (Suppl 3):349–352
25. Marelli C (1999) Preliminary experience with NC100100, a new ultrasound contrast agent for intravenous injection. Eur Radiol 9 (Suppl 3):343–346
26. Marelli C (2000) Preliminary clinical experience in cardiology with sonazoid. Am J Cardiol 86 (Suppl 1):10–13
27. De Jong N, Frinking PJ, Bouakaz A, ten Cate FJ (2000) Detection procedures of ultrasound contrast agents. Ultrasonics 38: 87–92
28. Leen E (2001) Ultrasound contrast harmonic imaging of abdominal organs. Semin Ultrasound CT MR 22: 11–24
29. Dalla Palma L, Bertolotto M (1999) Introduction to ultrasound contrast agent: physics overview. Eur Radiol 9 (Suppl 3):338–342
30. Dalla Palma L, Bertolotto M, Quaia E, Locatelli M (1999) Detection of liver metastases with pulse inversion harmonic imaging: preliminary results. Eur Radiol 9 (Suppl 3):382–387
31. Harvey CJ, Blomley MJ, Eckersley RJ, Cosgrove DO, Patel N, Heckemann RA, Butler-Barnes J (2000) Hepatic malignancies: improved detection with pulse-inversion US in late phase of enhancement with SH U 508A-early experience. Radiology 216: 903–908
32. Kim TK, Choi BI, Hong H, Choi BY, Han JK (2000) Improved imaging of hepatic metastases with delayed pulse inversion harmonic imaging using a contrast agent SH U 508 A: preliminary study. Ultrasound Med Biol 26: 1439–1444
33. Albrecht T, Hoffmann CW, Schettler S, Overberg A, Ilg M, Behren PL von, BauerA, Wolf KJ (2000) B-mode enhancement at phase-inversion US with air-based microbubble contrast agent: initial experience in humans. Radiology 216: 273–278
34. Harvey CJ, Blomley MJ, Eckersley RJ, Heckemann RA, Butler-Barnes J, Cosgrove DO (2000) Pulse-inversion mode imaging of liver specific microbubbles: improved detection of subcentimetre metastases. Lancet 355: 807–808
35. Albrecht T, Hoffmann CW, Schettler S, Overberg A, Ilg M, Wolf KJ (1999) Improved detection of liver metastases with phase inversion imaging during the liver-specific phase of the ultrasound contrast agent Levovist. Eur Radiol 9 (Suppl 3):388
36. Kim TK, Choi BI, Han JK, Hong HS, Park SH, Moon SG (2000) Hepatic tumors: contrast agent-enhancement patterns with pulse-inversion harmonic US. Radiology 216: 411–417

37. Bertolotto M, Dalla Palma L, Quaia E, Locatelli M (2000) Characterization of unifocal liver lesions with pulse inversion harmonic imaging after Levovist injection: preliminary results. Eur Radiol 10: 1369–137
38. Numata K, Tanaka K, Kiba T, Saito S, Ikeda M, Hara K, Tanaka N, Morimoto M, Iwase S, Sekihara H (2001) Contrast-enhanced, wide-band harmonic grey scale imaging of hepatocellular carcinoma: correlation with helical computed tomographic findings. Ultrasound Med 20: 89–98
39. Wilson SR, Burns PN, Muradali D, Wilson JA, Lai X (2000) Harmonic hepatic US with microbubble contrast agent: initial experience showing improved characterization of hemangioma, hepatocellular carcinoma, and metastasis. Radiology 215: 153–161
40. Lencioni R, Cioni D, Donati F, Crocetti L, Perri M, Franchini C, Grusti S, Bartolozzi C (2001) SonoVue – enhanced C3-mode US imaging in the detection and characterization of focal liver lesions: a pilot clinical study. Eur Radiol 9:C4
41. Leen E, Albrecht T, Harvey C et al. (2000) Multi-centre study of Sonazoid enhanced pulse inversion harmonic imaging in the characterization of focal hepatic lesions: preliminary results. Radiology 217 (P):458
42. Sieswerda GT, Kamp O, Visser CA (2000) Myocardial contrast echocardiography: clinical benefit and practical issues. Echocardiography 17: 25–36
43. Rovai D, Morales MA, Amyot R, L'Abbate A (2000) Clinical experience with SonoVue in myocardial perfusion imaging. Echocardiography 17: 17–23
44. Malhotra V, Nwogu J, Bondmass MD, Bean M, Bieniarz T, Tertell M, Conliss M, Devries S (2000) Is the technically limited echocardiographic study an endangered species? Endocardial border definition with native tissue harmonic imaging and Optison contrast: a review of 200 cases. J Am Soc Echocardiogr 13: 771–773
45. Sieswerda GT, Kamp O, van den Ende R, Visser CA (2001) Intermittent harmonic imaging and videodensitometry significantly enhance ability of intravenous air-filled ultrasonographic contrast agent to produce ventricular and myocardial opacification. J Am Soc Echocardiogr 14: 20–28
46. Grant EG (2001) Sonographic contrast agents in vascular imaging. Semin Ultrasound CT MR 22: 25–41
47. Sirlin CB, Lee YZ, Girard MS, Peterson TM, Steinbach GC, Baker KG, Mattrey RF (2001) Contrast-enhanced B-mode US angiography in the assessment of experimental in vivo and in vitro atherosclerotic disease. Acad Radiol 8: 162–172
48. Kim B, Lim HK, Choi MH, Woo JY, Ryu J, Kim S, Peck KR (2001) Detection of parenchymal abnormalities in acute pyelonephritis by pulse inversion harmonic imaging with or without microbubble ultrasonographic contrast agent: correlation with computed tomography. J Ultrasound Med 20: 5–14
49. Halpern EJ, Verkh L, Forsberg F, Gomella LG, Mattrey RF, Goldberg BB (2000) Initial experience with contrast-enhanced sonography of the prostate. Am J Roentgenol 174: 1575–1580
50. Halpern EJ, Rosenberg M, Gomella LG (2001) Prostate cancer: contrast-enhanced US for detection. Radiology 219: 219–225