**Riccardo Lencioni** (Ed.)

# Enhancing the Role of Ultrasound with Contrast Agents

Springer

**Editor**
**Prof. Dr. Riccardo Lencioni**
Division of Diagnostic and Interventional Radiology
Department of Oncology, Transplants
and Advanced Technologies in Medicine
University of Pisa
Pisa, Italy

**CD-ROM edited by**
**Prof. Dr. Riccardo Lencioni**
**Dr. Clotilde Della Pina**
Division of Diagnostic and Interventional Radiology
Department of Oncology, Transplants
and Advanced Technologies in Medicine
University of Pisa
Pisa, Italy

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# Preface

The introduction of microbubble contrast agents and the development of contrast-specific scanning techniques have opened new prospects in ultrasound. The advent of second-generation agents – that enable real-time contrast-enhanced imaging – has been instrumental in improving the acceptance and the reproducibility of examinations. Contrast ultrasound substantially improves detection and characterization of focal liver lesions with respect to baseline studies, and has already been introduced in international guidelines for the diagnosis of liver tumors. The role of contrast agents in vascular ultrasound is also established, and several new clinical applications are emerging.

This book, written by the leading experts in the field, provides an up-to-date overview on the clinical value of contrast agents in ultrasound. The volume moves from a background section on technique and methodology to the main sections on the clinical application of contrast ultrasound in the liver and in vascular diseases. A final section discusses results and prospects of contrast ultrasound modality in the other fields. A selection of relevant clinical cases is presented in the enclosed CD-ROM.

This volume is the result of the efforts of several world-renowned experts. I am greatly in debt to them for their enthusiastic commitment and support. Also, I would like to thank my mentor, Professor Carlo Bartolozzi, for his precious advice, as well as Drs. Dania Cioni, Laura Crocetti, and Clotilde Della Pina for their contribution to this editorial project. I sincerely hope that the book fulfils the expectations of my colleagues – radiologists, clinicians, and surgeons – who are interested in the very exciting field of contrast ultrasound.

Pisa, March 2006 **Riccardo Lencioni**

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## SECTION IV – New Prospects in Clinical Application of Contrast Ultrasound

# Contributors

**Luca Aiani**
Department of Radiology
Ospedale Valduce
Como, Italy

**Thomas Albrecht**
Department of Radiology
and Nuclear Medicine
Campus Benjamin Franklin
Charité – Universitätsmedizin Berlin
Berlin, Germany

**Eva Bartels**
Department of Clinical
Neurophysiology
Georg-August-University
Göttingen, Germany

**Carlo Bartolozzi**
Division of Diagnostic and
Interventional Radiology
Department of Oncology,
Transplants, and Advanced
Technologies in Medicine
University of Pisa, Italy

**Claudia Borghi**
Department of Radiology
Ospedale Valduce
Como, Italy

**Molly Brewer**
Department of Gynecology Oncology
University of Arizona
Arizona Cancer Center
Tucson, USA

**Concepcio Bru**
Diagnostic Imaging Center
Hospital Clínic
Barcelona, Spain

**Dania Cioni**
Division of Diagnostic
and Interventional Radiology
Department of Oncology,
Transplants, and Advanced
Technologies in Medicine
University of Pisa
Pisa, Italy

**Roberta Chersevani**
Department of Diagnostic Imaging
General Hospital
Gorizia, Italy

**David Cosgrove**
Imaging Sciences Department
Imperial College
Hammersmith Hospital
London, UK

**Laura Crocetti**
Division of Diagnostic
and Interventional Radiology
Department of Oncology,
Transplants, and Advanced
Technologies in Medicine
University of Pisa
Pisa, Italy

**Stefan Delorme**
Department of Radiology
German Cancer Research Center
Heidelberg, Germany

**Clotilde Della Pina**
Division of Diagnostic and
Interventional Radiology
Department of Oncology,
Transplants, and Advanced
Technologies in Medicine
University of Pisa
Pisa, Italy

**Christoph F. Dietrich**
Department Innere Medizin 2
Caritas Krankenhaus
Bad Mergentheim, Germany

**Dirk W. Droste**
Department of Neurology
Centre Hospitalier de Luxembourg
Luxembourg
Department of Neurology
University of Münster
Münster, Germany

**Robert Eckersley**
Imaging Sciences Department
Imperial College
Hammersmith Hospital
London, UK

**Thomas Fischer**
Department of Radiology
Charité
Universitätsmedizin Berlin
Berlin, Germany

**Ferdinand Frauscher**
Department of Radiology II
Medical University Innsbruck
Innsbruck, Austria

**Paul G. Horgan**
Senior Lecturer and Hon Consultant
Surgeon,
University of Glasgow
Glasgow, UK

**Andrea S. Klauser**
Department of Radiology II
Medical University Innsbruck
Innsbruck, Austria

**Martin Krix**
Department of Radiology
German Cancer Research Center
Heidelberg, Germany

**Tiziana Ierace**
Department of Radiology
General Hospital
Busto Arsizio, Italy

**Nathalie Lassau**
Ultrasonography Unit
and Imaging Laboratory
of Small Animal
Imaging Department
Institut Gustave-Roussy
Villejuif, France

**Jérome Leclère**
Ultrasonography Unit
Imaging Department
Institut Gustave-Roussy
Villejuif, France

**Edward Leen**
Consultant Radiologist
Hon Senior Lecturer
University of Glasgow
Glasgow, UK

**Riccardo Lencioni**
Division of Diagnostic
and Interventional Radiology
Department of Oncology,
Transplants, and Advanced
Technologies in Medicine
University of Pisa
Pisa, Italy

**Henri Marret**
Department of Gynecology,
Obstetrics, Fetal Medicine
and Human Reproduction
Bretonneau Hospital
CHRU Tours
Tours, France

**Alberto Martegani**
Department of Radiology
Ospedale Valduce
Como, Italy

**Susan Moug**
Research Fellow
Department of Surgery
University of Glasgow
Glasgow, UK

**Carlos Nicolau**
Diagnostic Imaging Center
Hospital Clínic
Barcelona, Spain

**Anders Nilsson**
Department of Radiology
Ultrasound Unit
Uppsala University Hospital
Uppsala, Sweden

**Pierre Péronneau**
Imaging Laboratory
of Small Animal
Imaging Department
Institut Gustave-Roussy
Villejuif, France

**Giorgio Rizzatto**
Department of Diagnostic Imaging
General Hospital
Gorizia, Italy

**Stéphane Sauget**
Department of Gynecology, Obstetrics,
Fetal Medicine and Human Reproduction
Bretonneau Hospital
CHRU Tours
Tours, France

**Luigi Solbiati**
Department of Radiology
General Hospital
Busto Arsizio, Italy

**Massimo Tonolini**
Department of Radiology
General Hospital
Busto Arsizio, Italy

**Lars Thorelius**
Head of Ultrasound Section
Department of Radiology
Linköping University Hospital
Linköping, Sweden

**François Tranquart**
Department of Medical Imaging,
Nuclear Medicine and Ultrasound
Bretonneau Hospital
CHRU Tours
Tours, France

# Section I

# Technique and Methodology of Contrast Ultrasound

# I.1

# Contrast-Enhanced Ultrasound: Basic Physics and Technology Overview

David Cosgrove and Robert Eckersley

## Introduction

Microbubbles represent an entirely new class of materials that are mainly used as intravascular contrast agents for US, though they can also be instilled into the urinary bladder to look for ureteric reflux [1] and into the uterus to check tubal patency [2]. Their effect depends on the compressibility of gases, which is markedly different from the near-incompressibility of tissue. Exploiting this difference has led to the development of several multipulse sequences that cancel tissue signals and emphasise those from the microbubbles, thus improving the contrast-to-tissue signal ratio. Overlay or side-by-side displays allow the agent image to be viewed along with the grey-scale image to facilitate locating the region of interest.

Not only can major vessels be displayed, but microbubbles within the microvasculature are also detected (though the capillary bed itself cannot be resolved anatomically), because these methods do not depend on microbubble flow but merely on their presence. In practice, these specific modes have found major clinical application in the liver for the detection and characterisation of focal lesions, although the same principles apply also in the kidneys and spleen. They are also very widely used in echocardiography, for endocardial border detection and to evaluate myocardial perfusion, applications that are beyond the remit of this article.

Conventional Doppler methods also work well and contrast is helpful in peripheral vascular disease (notably for transcranial Doppler, carotid stenosis and in renal artery stenosis) when the unenhanced Doppler signals are too weak to be clinically useful.

Microbubbles are eminently suitable for use as tracers because of the small volumes injected. This has been exploited in the liver to identify conditions characterised by arterio-venous shunting such as cirrhosis and metastases - an early hepatic vein transit time indicates a haemodynamic abnormality.

## Microbubble Contrast Agents

Unlike all other imaging technologies, until recently US lacked agents that could be administered to patients to improve or enhance the diagnostic information available. However, this has changed with the recent introduction of microbubble contrast agents [3]. The field is a dynamic one, with many new agents being developed along with new ways to exploit the opportunities they offer [4-8]. Microbubbles are made small enough to cross capillary beds (the pulmonary capillaries have the smallest calibre in the body at 7μm) and are usually given as an intravenous injection (Fig. 1). The discovery of their striking enhancement of US signals dates from a chance observation by a cardiologist, Dr Claude Joyner, who noticed an increase in the signal intensity on an M-mode study of the aortic root each time an injection of an iodinated contrast medium was made for an angiographic cardiac study [9]. Further investigation revealed that the effect was not specific to the X-ray contrast agent, but occurred with any injected liquid and, importantly for the ongoing development of these agents, it was enhanced by first drawing up some of the patient's blood into the syringe. Dr Steve Feinberg found that this improvement resulted from the stabilising effects of serum albumen, and that the enhancement resulted from gas bubbles in the injectate, demonstrated by the fact that the effect was suppressed if the fluid was subjected to high pressure in the syringe. The

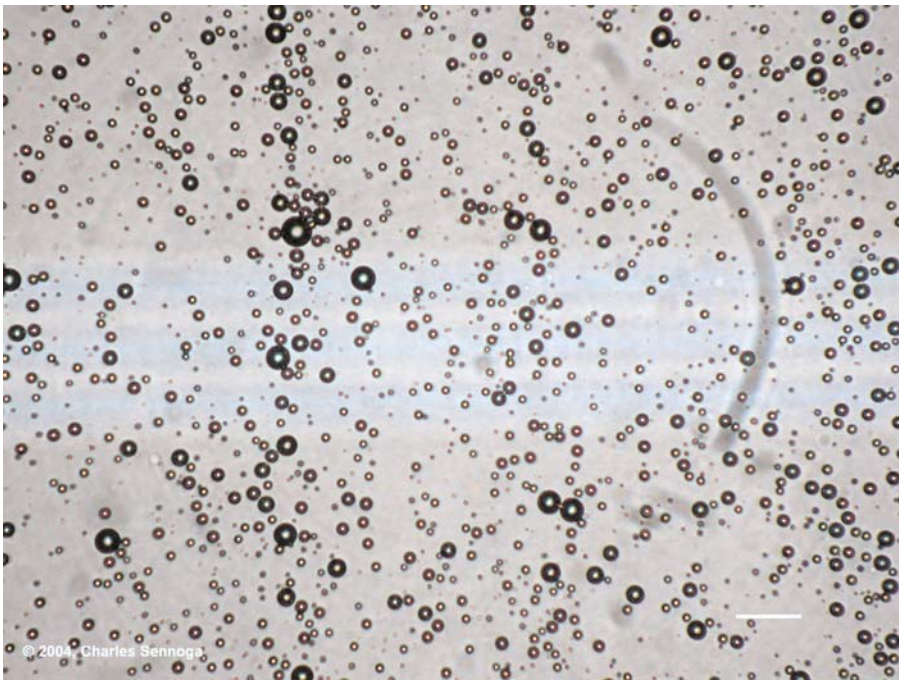


**Fig. 1.** Light microscope view of a phospholipid-shell microbubble. The size distribution and spherical form of these microbubbles is shown in this micrograph. The scale represents 25µ. (Image courtesy of Dr. Charles Sennoga, Imaging Sciences Department, Imperial College, London)

first clinical applications of the phenomenon used "shaken saline", which was actually injected rapidly from one syringe into another via a three-way tap so that traces of air were incorporated as bubbles, often stabilised by a small amount of the patient's blood. This was used to reveal right-to-left intracardiac shunts, seen as echoes in the left atrium or ventricle following intravenous (iv) injection using M-mode or B-mode scanning. The size of the bubbles thus produced could not be controlled and unfortunately, some serious complications occurred, notably paradoxical cerebral emboli, producing transient or prolonged ischaemic attacks and even an associated death. The development of colour Doppler obviated the need for shaken saline injections for the detection of these shunts. The line of research eventually led to the commercialisation of Albunex, which is produced by high intensity sonication of an albumen solution.

A different line of work cumulated in the development of disaccharide-based microbubbles such as Echovist and Levovist [10, 11]. Development of these agents resulted from the hypothesis that pressure changes in the left ventricle could be estimated by measuring the resonant frequency of microbubbles in the blood because the change in mean diameter would correspond to a change in the acoustic resonance. Many means of making microbubbles were evaluated, including using the ability of rough surfaces to act as nidation sites, much as irregularities on the inside of a glass trigger the formation of bubbles from a carbonated drink. Amongst the materials tried, sugar crystals seemed promising, as they are non-toxic and well characterised. They were ground into a fine powder to produce a matrix of microcrystals. The size of the resultant microbubbles depended on the dimensions of the spaces between the microcrystals. Unfortunately, measuring intracardiac pressure turned out to require an extremely tight microbubble size-range and the original goal was never achieved. However, the microbubbles produced gave strong enhancement. The first agent to enter clinical trials, Echovist, had only a short life in the circulation after iv injection because it was not stable enough to survive cardio-pulmonary transit and thus it could only be used for intracardiac shunt detection. Though safer than shaken saline (because of the controlled microbubble sizes), it too was replaced by colour Doppler for this purpose, though it is still used in US contrast salpingography. The addition of traces of a surfactant (palmitic acid) greatly improved stability so that the bubbles could survive cardio-pulmonary passage to give left-sided heart enhancement, hence its designation as Levovist. It also enhanced the signals from the peripheral vasculature, and was licensed for Doppler enhancement of vessels such as the carotid arteries and the portal system. Unfortunately, it turned out that the enhancement it gave necessitated disruption of the microbubbles (in fact, only the free air bubbles liberated in this process were responsible for the US signals) and therefore, only

short duration studies could be performed: the act of imaging these fragile microbubbles destroyed them.

Levovist was found to have one very useful property: it is taken up by the reticulo-endothelial system in the liver and spleen where it persists for several minutes after it has been cleared from the circulating blood pool [12]. This property, which was a chance discovery, proved to be useful for the detection of focal lesions such as metastases that did not contain Kupffer cells, even when they were undetectable on unenhanced scans because of their small size or because of lack of contrast with the surrounding liver (so-called isoechoic metastases). Tissues that were comprised of functioning liver, such as focal fatty change, regenerating nodules and focal nodular hyperplasia, shared this property with the surrounding liver and so disappeared in this late phase rather than becoming prominent as defects against the enhanced liver background, as was the case with metastases. Newer microbubbles share this property to some extent, though it seems likely that they are not specifically taken up by phagocytes, but instead simply float in the high capacity vasculature of the sinusoids in the liver and spleen. The importance of this late sinusoidal phase for the detection of malignancies, first discovered with Levovist, also applies to the newer agents.

As well as reflecting incident US, microbubbles respond to the alternating compression and rarefaction cycles of the US wave by changing diameter, since their gas content is much more compressible than tissue. These oscillations are typically asymmetrical (the gas tends to resist compression but expands more easily) and this "non-linear response" alters the character of the returned signals. Signal processing to select these signals and separate them from tissue echoes has been developed and it permits continuous real-time imaging of the contrast agent as it arrives at a region of interest and then washes out over 2-5 minutes. This allows the arterial and venous phases to be studied. In addition, many agents show an affinity for the liver and spleen, persisting in normal sinusoids for many minutes. This parenchymal 'sinusoidal' or 'late' phase highlights masses that do not contain normal liver tissue and has proven to be particularly valuable for detecting metastases, while the arterial phase helps characterise focal lesions in the liver (and to a lesser extent, in the spleen) by virtue of their vascular supply.

In addition to their value in imaging, microbubbles may be used as tracers to provide information on haemodynamics. An example is the ability to time the delay from their first appearance in the hepatic artery until they arrive in hepatic vein branches. Normally this takes 12 seconds or more, but when there is arterio-venous shunting in the liver this time is greatly shortened. This simple test has been shown to be able to discriminate mild from severe forms of viral hepatitis and to separate both from frank cirrhosis [13, 14]. It seems also to be capable of detecting micrometastases in colorectal cancer [15].

## Microbubble Properties

The microbubbles used as contrast agents for US are made to be smaller than 7 μm in diameter so that they can cross capillary beds. When administered intravenously, these agents flood the blood pool and remain within the vascular compartment unless there is active bleeding. Thus, they serve as blood pool agents and their contrast effects are closer to those of the labelled red blood cells used in nuclear medicine studies than to the ionic agents used in X-ray and MR studies; in particular, microbubbles do not cross the endothelium and therefore do not have the interstitial phase that is typical of the conventional contrast agents. Microbubbles do not seem to affect blood flow and generally behave in the same way as red blood cells (with the important exception of when they are phagocytosed by the reticulo-endothelial system, which treats some microbubbles as foreign particles – see below).

They must survive passage through the cardio-pulmonary circulation to produce useful systemic enhancement. Both the gas they contain and their stabilising shell are critical to their effectiveness as contrast agents and to rendering them sufficiently stable, allowing them to persist for several minutes after injection. The first agent for cardiac use, Albunex, has a shell of denatured albumen and contains air. The first widely used agent, Levovist (Schering, Germany), is made of galactose microcrystals whose surfaces provide nidation sites on which air bubbles form when they are suspended in water; they are stabilised by a trace of palmitic acid which acts as a surfactant. An improved version of Albunex, Optison (GE, UK), is filled with perfluoropropane while a family of perfluoro gas-containing agents such as SonoVue (Bracco, Italy) and perflutren (Definity, Bristol Meyers Squibb, USA) that use phospholipids as the membranes are important in clinical practice.

High molecular weight gases are chosen for their slow diffusion through the membrane, prolonging their effective life in the circulation, where they are subject to mechanical trauma by the action of heart valves and by the US beam.

Obviously, the gas should be biologically inert and for this reason, the perfluoro gases have been chosen, in which a carbon chain has its hydrogen atoms completely replaced by elements such as fluorine or sulphur. The perfluorocarbons such as perfluoropropane (a three carbon chain compound used in Definity) have excellent durability and provide strong enhancement. SonoVue contains sulphur hexafluoride, which is characterised by very low solubility in blood and is also a very effective contrast agent.

The membrane is also critical for both longevity and resonance and, naturally, must also be biologically inert. Denatured human serum albumen (HSA) is used in several contrast agents (Albunex and Optison are available for clinical use, but Quantison has been withdrawn from further development). Denatured HSA is sufficiently flexible to allow the bubbles to respond to the acoustic pressure changes when insonated. In general, it is biologically inert, with the denaturation neutralising any immunogenic potential. However, there have been theoretical concerns over the possibility of transmitting viral or prion diseases because of the human origin of the protein. While this is unlikely because of the denaturing process to which the albumen is subjected, there remains a risk, so ethics committees have insisted that the origin of the shell be clearly labelled in patient information sheets, which has resulted in a few patients refusing to have this type of contrast agent.

Many microbubbles use phospholipid-shells. The bubbles are not present in the lyophilised powder that is presented in the vial, but are formed during agitation after the diluent (usually normal saline) has been added. The size of the resulting microbubbles and their shell thickness are critically determined by the exact mixture of the different phospholipids and the associated surfactants. The consistency of the resulting microbubbles is remarkable. Again, the shell properties are important for both the stability and response of the microbubbles.

## Microbubble Behaviour

While the classical specific acoustic impedance change between plasma and gas is sufficient to produce strong signals from microbubbles, just as from any other gas, the effect is weak *in vivo* because of the high dilution (for example, a dose of 5mL in around 5 litres of blood, i.e. 1:1000 or more). On intravital microscopic views after injection of clinically equivalent doses, one sees one microbubble pass between several hundred red cells. Using conventional B-mode scanning, the effect can be discerned in large blood spaces such as the cardiac chambers, but not in smaller vessels. Doppler is more sensitive and can be used in clinical practice. The effect is useful for improving the signal-to-noise ratio of studies in situations where the signals are attenuated. This application, known as 'Doppler rescue', is important for transcranial Doppler where the skull attenuates the signals from intracranial arteries. Contrast agents are widely used in neurology centres where monitoring vasospasm after cerebrovascular events is important. It is also useful in otherwise difficult radiological studies, for example, for renal artery stenosis and for Tips shunts in the liver, both of which are situations where attenuation of the Doppler signals frequently impedes unenhanced studies. In 'Doppler rescue', important as these studies may be, essentially no new principles are involved, the microbubbles simply boost the signals from blood.

However, the behaviour of microbubbles in a US field is unique and this can be exploited in entirely new ways to give information of a new order, in particular in detection of the microcirculation. This unique phenomenon results from the high compressibility of the gas of microbubbles: whereas tissue is virtually incompressible (the molecules moving only a few Angstroms as the ultrasonic compression and rarefaction wave passes), microbubbles expand and contract markedly. The extent of the change depends on the acoustic power applied, but at diagnostic levels they may halve and double in size.

Bubbles have a natural oscillation frequency, or resonance, depending on their size. When the driving pressure changes of the US are matched to this resonant frequency, the bubbles become extremely efficient at translating the sound energy from the propagating sound wave into scattered signals. The frequencies used for abdominal US imaging are in the 3-5 MHz range. This corresponds to the resonant frequencies of 3-5 μ microbubbles (5 μ SonoVue microbubbles, for example, resonate at around 5 MHz and those of 2 μ resonate at 7 MHz). This is a coincidence, because different considerations determine the bubble size and the acoustic wavelength needed, but it accounts for the exceptionally strong response of US contrast agents (Fig. 2).

In addition to the resonance effect, the response of the microbubbles to the US wave becomes non-linear at higher acoustic pressures. This behaviour results from the fact that the bubbles are able to expand more easily than they contract because the increasing bubble pressures at smaller volumes resist further compression due to their stiffness, whereas further expansion

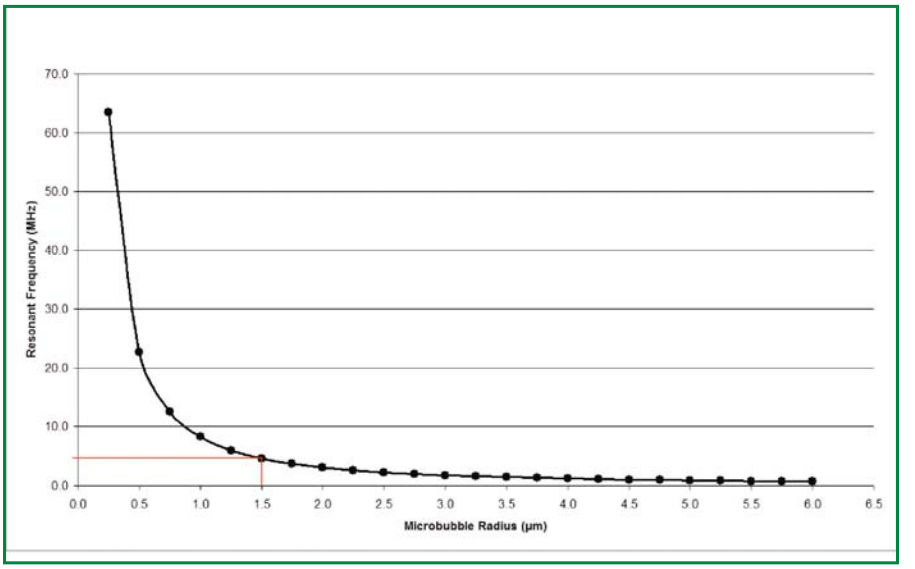


**Fig. 2.** The calculated resonance frequency plotted against the radius of a microbubble. The chart shows the resonant frequency for the microbubble Sonazoid plotted against the bubble's radius at rest. The red lines indicate that at 5 MHz the resonance corresponds with the median diameter of 5 K. (Figure kindly supplied by Kevin Chetty, Imaging Sciences Department, Imperial College, London)

consumes less energy. This means that their diameter change is asymmetrical about the radius at equilibrium, with larger increases in the rarefaction phase than contractions in the compression phase. Thus, the signals returning from the bubbles are a distorted version of the insonating wave; this is known as a non-linear response. At still higher pressures, the bubble oscillations become more complex, diverging from simple spherical changes and further increasing the non-linear properties of the scattered US.

These non-linearities appear as harmonics (or overtones) of the original transmitted US frequency and can be seen on a frequency spectrum of the returning signals. Usually these are higher frequencies (most prominently the second harmonic at twice the transmitted or fundamental frequency); higher third or fourth order harmonics are typically outside the range of sensitivity of the US transducer and so are undetectable. For higher frequency applications, the second harmonics are very strongly attenuated and often lie outside the band pass of the transducer. An alternative approach to harmonic imaging is to use microbubbles with a second harmonic frequency tuned to the transducer centre frequency and detect the subharmonic signals from the microbubbles, i.e., at half the transmitted frequency. There are also other sources of non-linear behaviour, which occur because of the pressure dependent behaviour and the inertia of the bubble shell. For example, if two similar pulses with differing amplitudes are incident on the microbubbles, the scattered response will not be directly proportional to the difference in the incident pressures. This asymmetrical response is not associated with a change in frequency and these non-linear responses at the fundamental frequency are important because the transducer is at its most sensitive at this point and pulses can be designed to use the full bandwidth of the transducer most efficiently [16]. In practice, a combination of these two sources of non-linearity is employed by modern US scanners to generate contrast-specific images.

A mathematical description of the non-linear response of a microbubble has been devised and is commonly referred to as the modified Rayleigh-Plesset equation. A number of researchers have developed variations on this model, an example described by de Jong is presented in equation (1) [16a]. This model describes the bubble radius as a function of time and accounts for the physical properties of the bubble shell and the effect of the gas within the bubble. The model takes the form of a second order differential equation:

*(1)*

$$\rho R\ddot{R}+\frac{3}{2}\rho\dot{R}^{2}=p_{go}\left(\frac{R_0}{R}\right)^{3\Gamma}+p_v-p_{lo}-\frac{2\sigma}{R}-2S_p\left(\frac{1}{R_0}-\frac{1}{R}\right)-\delta_{Tot}\omega\rho R\dot{R}-p_{ac}$$

Where R is the instantaneous bubble radius; $\rho$ is the density of the surrounding medium; $P_{go}$ is the initial gas pressure inside the bubble; $P_v$ is the vapour pressure; $P_{lo}$ is the hydrostatic pressure; $\sigma$ is the surface tension coefficient; $\delta_{Tot}$ is the total damping coefficient due to sound radiation, liquid viscosity, heat transport and the viscous resistance of the encapsulating shell; $\omega$ is the angular frequency of the incident sound field; $P_{ac}$ is the time varying pressure of the acoustic field; $S_p$ is the shell stiffness parameter and $\Gamma$ is the polytropic exponent of the gas. ´•´ denotes differenti-

ation with respect to time. The model is only applicable for low acoustic driving pressures, when the bubble oscillations remain radially symmetrical. At higher pressures, the bubble behaviour becomes more complex and the bubbles will ultimately be disrupted. This disruption can cause fragmentation into smaller bubbles or the release of free gas bubbles; in both cases, the resulting bubbles then dissolve more rapidly.

## Methods for Detection of Microbubble Signals

The change in density at the surface of a bubble in plasma represents a major impedance mismatch and the echogenicity this produces is exploited in the uses of microbubbles to improve Doppler studies, so-called 'Doppler rescue'. The increase in the reflectivity of blood thus produced is obvious and useful for Doppler but is not visible on conventional grey-scale imaging because the microbubble concentration is too low.

The harmonics emitted by microbubbles, as described above, can be used to image US contrast agents by tuning the receiver to the harmonic signal (e.g. the second harmonic at double the fundamental frequency), so that the harmonics can be separated from the fundamental signals from tissue.

However, in order to perform a good separation of the harmonic microbubble signals from the linear scattered tissue signals, a longer, narrow band US pulse must be used. This trade-off results in a compromise between sensitivity and specificity to microbubbles versus image resolution. Furthermore, tissues also produce harmonics, especially when higher acoustic powers are used and these contaminate the signals from microbubbles. Tissue harmonics result from distortion of the acoustic wave as it propagates through the tissue and arise because the wave travels slightly faster through the higher density tissue at the high pressure phase of the wave than in the lower density tissue in the rarefaction phase (sound velocity depends on the density of the medium). Thus, the wave of the transmitted pulse is gradually distorted as it propagates through the tissue, i.e. US conduction is non-linear, and this implies that harmonics are added to the signal. The higher the acoustic power, the greater the effect. Tissue harmonics are exploited in conventional B-mode imaging to reduce sidelobe and reverberation artefacts but contaminate the non-linear signals from microbubbles and appear as noise in contrast-only images.

Distinguishing between tissue and microbubble harmonics is challenging; in practice, when using many of the simple contrast modes available, the two signals are inextricably mixed together. The fact that tissue harmonics are stronger when a higher acoustic power is used underlies the use of a low mechanical index (MI) for contrast studies. This has the important added benefit of minimising bubble destruction and so allowing continuous real-time scanning. Inevitably, low MIs are associated with reduced signal-to-noise ratios so the contrast images may become noisy and a compromise may have to be reached.

The goal of separating tissue from microbubble harmonics completely can be achieved in two ways. In the first to be discovered, a high MI beam is used and the microbubbles are deliberately disrupted. Using a multipulse sequence such as standard colour Doppler, the sudden disappearance of a signal from its previous location (loss of correlation between sequential echoes) is seen as a major Doppler shift and is registered as colour signals [12]. This method works well for the more fragile air-based agents such as Levovist, and modified colour Doppler signal processing has been developed to optimise the display, particularly by improving spatial resolution. This approach, often known a Stimulated Acoustic Emission (SAE), is particularly successful in the late phase of contrast agents that show liver/spleen tropism a few minutes after injection. Lesions that do not contain functioning tissue, such as malignancies, are shown as voids in the colour map, because Levovist highlights the normal liver and spleen. This method has the advantage of high sensitivity (it can probably detect a single microbubble being disrupted) and of showing the microbubble signature exclusively in the colour layer of the registered image with the conventional grey-scale image as an underlayer or on a split screen display for navigation purposes. However, because the contrast agent is rapidly destroyed, real-time scanning cannot be used, so a rather clumsy sweep-and-review approach has to be adopted [17].

The alternative approach, and now the mode of choice for contrast studies, relies on the fact that newer microbubbles, particularly those with phospholipid-shells, can be driven to generate non-linear signals at much lower acoustic powers than those necessary to generate tissue harmonics [18, 19]. Thus, if a very low acoustic power can be used without the image disappearing into noise, only the microbubbles produce non-linear signals and these can be separated from the tissue signals. An important step in the progress to this ideal was the development of the pulse inversion mode (PIM), which evolved from the de-

sire to detect microbubble harmonics but to avoid frequency filtering [20] (Fig. 3). In this mode a pair of pulses is sent sequentially along each scan line, the second being inverted in polarity from the first. The returning echoes from the pair are summed so that the linear echoes cancel because they are completely out-of-phase, leaving only non-linear components for image formation. Spatial resolution is not impaired because the transmitted pulses are the same as those used for conventional imaging. PIM gives excellent quality images in both vascular and late phases and, like the high MI modes, detects the presence of microbubbles without relying on their motion. Thus, both these modes can detect contrast within the microcirculation though, of course, vessels smaller than the resolution limit of US – some 200 μm at best – cannot be resolved as discrete structures.

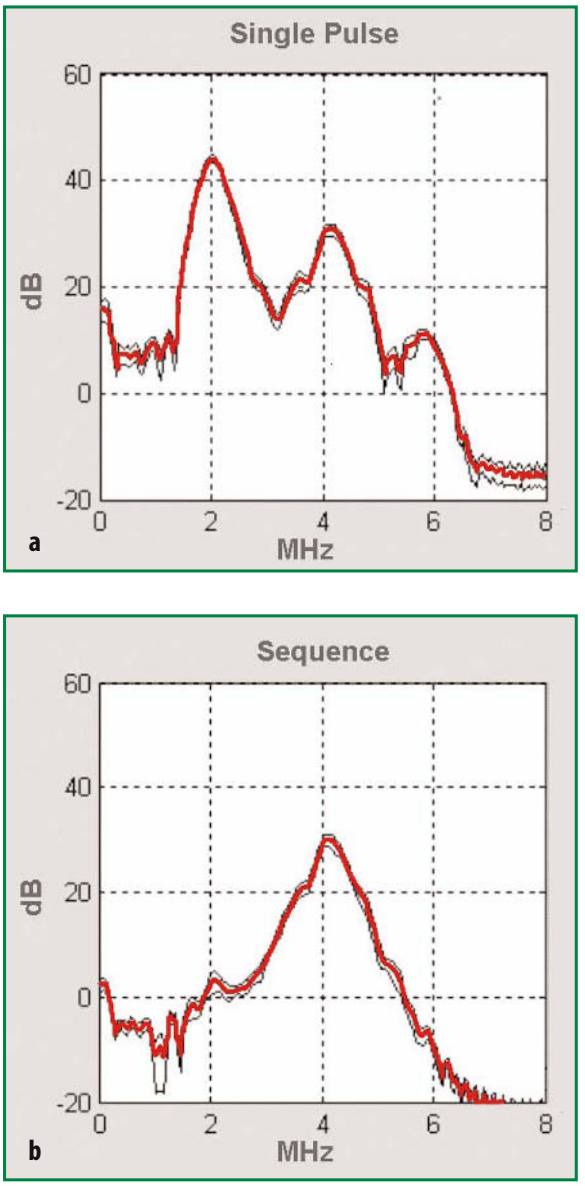


**Fig. 3.** *Frequency Spectra of Microbubbles.* In these charts the frequencies emitted by the microbubbles in a water tank is plotted on the X-axis with the signal strength on the Y-axis. **a** Shows the spectrum using a single pulse of 2 MHz frequency, as used in conventional B-mode imaging. The fundamental signal at 2 MHz is dominant but there are also second and third harmonics at 4 and 6 MHz. In **b**, a phase inversion sequence was used: the fundamental (and the third harmonics) have been suppressed, leaving only the second harmonic. This approach allows the microbubble signals to be separated from tissue signals. (The *red lines* are the averages of repeated measurements and the *black lines* indicate the standard deviation of the measurements) (Modified from [16])

In its initial implementation, PIM deployed relatively high MIs, resulting in contamination of the microbubble signals by tissue harmonics. Special approaches are required to operate at the low powers needed to avoid tissue harmonics without too much image degradation. One method is to send a stream of alternating phase inverted pulses and use colour Doppler circuitry to pick out the harmonics; essentially this method (known as Power Pulse-Inversion, PPI) exploits the high sensitivity of Doppler to overcome the signal-to-noise limitation [20]. By using the Doppler circuitry to depict the microbubble signature, the B-mode tissue image can be displayed as a background image, just as in colour Doppler. In a similar approach to PPI, the direction of flow of the microbubbles (and therefore of blood) in larger vessels is detected with low MI velocity Doppler, while slow moving and stationary microbubbles are shown in green using a pulse inversion sequence. This combined mode, known as Vascular Recognition Imaging (Toshiba), also allows the microbubble signature to be displayed separately from, or combined with, the B-mode and provides additional information on the flow direction in larger vessels.

Another approach also uses a series of pulses, though only three per line, but as well as inverting the phase, the amplitude of the pulses is changed and this preserves more of the non-linear content of the received signals and thus improves sensitivity [21, 22]. Implemented as Contrast Pulse Sequences (CPS, Siemens Acuson), the non-linear microbubble signals are displayed in a colour tint over the B-mode picture or as a split screen so that both can be viewed as required.

All of these modes operate at very low transmit powers (MI <0.2) and, as well as not producing tissue harmonics, this has the important advantage that bubble destruction is minimised so that real-time scanning can be used. This makes contrast studies much easier to perform since no special scanning techniques are required.

## Functional Studies

The use of microbubbles for functional studies employs analytical techniques originally developed for nuclear medicine, CT and MRI [23]. Essentially, following a bolus injection, the arrival and wash-out of the microbubbles is tracked in a region of interest, for example, a major vessel or a tissue region. The way the contrast signal changes with time is plotted as a time-intensity curve from which a variety of features can be extracted. These divide into two families, those relating to the timing of the changes and those relating to the amount of enhancement: some features such as the full-width-at-half-maximum value, incorporate both temporal and quantitative values. The quantitative features depend on a fixed relationship between the amount of microbubbles in the region of interest and their signal strength [24]. This has been demonstrated to be largely true for Levovist under clinically relevant concentrations using the spectral Doppler signal strength [25] and with colour Doppler for other microbubbles [26], but not for non-linear modes with newer types of microbubble. It seems certain that this predictable relationship will breakdown when the concentration of the microbubbles is high because they self-shadow, an effect that is obvious in clinical studies of the left ventricle of the heart. It is also likely that the relationship varies with the region's depth in the body, because of simple attenuation, so that comparison for regions at different depths may not be valid.

However, these limitations do not apply to the temporal features, which are therefore likely to be more robust. Examples of these are the time from injection to arrival or to peak enhancement (e.g., in a tumour) and the delay between the appearances in related vessels (e.g., the hepatic or renal artery and vein).

Measurement of the signal strength can be made off-line and much pioneer work in this field was carried out with the audio output from spectral Doppler, which can easily be digitised by a computer equipped with a sound card. The two-dimensional data can also be analysed, either using digital cine clips, despite the fact that they are usually too short, or from videotaped sequences. Software to perform the analyses on this data has been developed by several academic laboratories and is frequently found built-in to modern clinical US scanners. This has the advantage of allowing the data used for analysis to be corrected for scanner settings such as gain and TGC. In practice, these applications offer a tool with which a series of regions of interest can be drawn on screen, and offer a selection of other analytical tools. Some also incorporate motion tracking [22], which helps to correct for the effects of respiratory motion. For cardiac use, ECG gating is essential.

The raw time-intensity curves are usually noisy, with cardiac fluctuations superimposed on the slower wash-in and wash-out data (as well as other forms of noise and clutter) and usually require smoothing before they can be analysed. If only the initial wash-in data is required (e.g., for arrival time measurements), the curve does not need further manipulation, but the effects of recirculation must be corrected in order to analyse the decay parts of the curve. This is usually done by applying a $\gamma$–variate fit which essentially strips out late surges in the signal strength caused by recirculation [27].

The best features for discriminating between various pathologies, such as benign from malignant, are still being worked out. In some applications, such as the hepatic vein transit time, normal values and the effects of using different contrast agents have been established and simple, practicable methods for clinical implementation have been developed. This method is particularly promising for the grading of changes in the liver in viral hepatitis and deterioration to cirrhosis; it may be possible to reduce dependence on biopsies to assess the best time to offer antiviral therapy. Another promising application of the hepatic vein transit time is in the detection of occult metastases.

Another way to use the dynamic data from tissue regions is to assemble the image sequence into functional (parametric) images, for example depicting the time to arrival of the contrast agent [28]. This approach is particularly promising in tumours, since it could be expected to highlight chaotic flow in malignancies.

A unique way to obtain excellent bolus arrival characteristics exploits the fragility of many types of microbubble in the sound field. First developed for the myocardium [29], this method uses a high MI beam to destroy the microbubbles within a slice of tissue. As the contrast refills the slice that has been cleared, the signals build exponentially, as described by the equation:

$$I = A(1 - e^{-\beta t})$$

where I = signal intensity and t is the pulsing interval. The slope of the exponent ($\beta$) is proportional to the speed of flow into the slice while the peak signal strength, A, is proportional to the fractional vascular volume. The product of these two parameters is an indirect measure of tissue perfusion. This provides the opportunity to compare perfusion in adjacent tissue regions.

A practical way to generate reperfusion curves is to switch the scanner to a high MI

mode (often this uses colour Doppler beams) for a second or so and then to a low MI contrast mode to observe the tissue refill in real-time. Assumptions involved include the supposition that the low MI beam is non-destructive (probably this is unachievable), that microbubble destruction is complete within the slice, and that the circulating concentration is steady. It also assumes that the tissue does not move across the scanned beam.

Reperfusion Kinetics using this technique have been applied to the heart [29] and the brain [30] and the resulting perfusion overlay images are impressive, though this has not yet become accepted as a clinical tool. A small study in the kidney using the destruction-reperfusion method with Levovist infusion in normal volunteers showed a good correlation between cortical flow increases produced by administration of dopamine and total renal blood flow, measured as the clearance of radio-labelled paramino hippurate (PAH) clearance [31]. The fractional vascular volume did not change. Though this was a small study, it convincingly demonstrated that non-invasive renal blood flow measurements are feasible.

## Targeted Microbubbles

Despite the apparent simplicity of their shells, ligands can be attached to microbubbles to target them to specific cell membrane molecules for diagnostic or therapeutic purposes [32, 33]. A common way to effect this is to exploit the biotin-avidin conjugation system. Avidin, found in egg albumen, has high affinity for biotin, a vitamin, and proteins bind to the complex [34, 35]. Antibodies or protein ligands for example, can be attached to activated endothelium in this way. Microbubbles modified in this way selectively bind to e-selectin that is expressed on endothelium that has been activated (for example, by inflammatory processes including atheroma), but not on normal endothelium. They remain acoustically active and emit harmonics when insonated. Thus, they can be used to detect activated endothelium. Other potentially useful ligands include monoclonal antibodies directed against malignant cells and against activated platelets that are found in fresh thrombus [33, 36].

## Drug Delivery

The potential for drug and gene delivery using US and microbubbles is a research arena of great interest [37]. The process is twofold: US, even at diagnostic power levels, produces transient gaps in cell membranes that facilitate ingress of molecules present in the interstitial fluid [38]. This process is known as sonoporation. The cell wall defects close spontaneously within hours or minutes of turning off the US field and, provided the acoustic power is low, do not seem to cause permanent damage to the cells. This enhancement of incorporation of ambient molecules is improved if microbubbles are also present, presumably because of the mechanical effects of the microbubble oscillation as they resonate in the sound field. Thus, microbubbles can be used to potentiate incorporation of molecules into cells. Even large molecules such as genetic material can be incorporated in this way, a process known as transduction. Furthermore, genetic material incorporated in this way remains active and can synthesise copies of functioning enzymes [39]. A hope is that further selectivity can be achieved by using targeted microbubbles.

## Safety of Microbubbles

The safety of microbubbles needs to be assessed both in terms of their effects on the body, in the same way as any administered substance, as well as for effects associated with their response to US energy [40, 41]. Overall, the fact that several microbubble preparations have gained approval by regulatory bodies following extensive phase three clinical trials underlies their safety in clinical practice. Minor adverse effects of contrast agents are reported in less than 5% of subjects and typically include transient discomfort at the injection site, taste aberrations and vaso vagal attacks. Some serious adverse events have been reported in post-marketing surveillance by manufacturers, including premature ventricular contractions (PVCs) during echocardiography [42]. These occur when US at high MIs is used at end systole (a particularly sensitive phase of the cardiac cycle) and the effect is reproducible in animal studies. It is increased when high doses of contrast are used, leading to the obvious recommendation to use low MIs and low doses for echocardiography.

Rare adverse events emerge for all drugs that are used extensively and, for US contrast agents, these include allergic or hypersensitivity reactions that cannot be predicted from the clinical trials. Three deaths have been reported following contrast echocardiograph studies, all in patients with severe coronary artery disease. Though the temporal relationship and the underlying clinical instability of these patients suggested that the deaths were not related to the contrast agent

used, the agent was withdrawn temporarily and caution was advised for patients with angina when it was later reintroduced.

Clinicians using microbubble contrast agents need to be trained to deal with such rare but serious adverse events, as recommended by the European Federation for Ultrasound in Medicine and Biology [43]: *"It is recommended that investigators wishing to undertake UCA examinations should gain experience by observing contrast studies being performed in a department with expertise in this area. They should also ensure that their equipment is optimized for contrast examination by discussion with their equipment manufacturers. It is also important that in their own department there are adequate numbers of examinations being performed and different types of pathological processes being observed to acquire and maintain their skills. Practitioners need to be competent in the administration of contrast agents, familiar with any contra-indications and be able to deal with any possible adverse effect, within the medical and legal framework of their country."*

*In vitro* studies show that US from clinical scanners produces effects such as sonoporation, detachment from glass substrates, haemolysis and cell death in the presence of microbubbles at diagnostic concentrations. These effects seem to be mechanical and are increased by using higher acoustic powers, by higher concentrations of microbubbles and by close proximity to cells, especially when they microbubbles have been phagocytosed. There does not seem to be a lower threshold for these effects.

Similar effects have been demonstrated *in vivo*, either using clinical scanners or with similar pulsing and power conditions and clinical microbubble concentrations. In particular, rupture of the microvasculature and intestinal peticheae have been observed. Though it is difficult to extrapolate these observations to human conditions, presumably these effects can also occur in humans. Their clinical relevance is unclear because capillary haemorrhage is commonplace under minor everyday stresses, including in the feet due to walking and in the lungs due to coughing, suggesting that they should not be of great concern. The possibility of damage to the cerebral microcirculation has been studied in human transcranial Doppler examinations and no effect has been found [44].

Microbubbles in the blood increase the vulnerability of many mammalian tissues and organs to damage from lithotripter fields *in vivo*. The increased vulnerability can persist for hours and this forms the basis for the advice not to use US contrast materials for 24 hours before lithotripsy.

Overall, though more work needs to be done, this class of contrast agents is very well tolerated and the agents may be used in patients with renal, cardiac and hepatic failure without concern. The restriction to avoid the use of SonoVue in patients with ongoing angina is the only safety-related limitation to have emerged in a period when microbubble agents have been used safely to benefit many thousand patients.

## Key Points

- The complex behaviour of microbubbles in the sound field leads to unique signatures that can be selectively displayed.
- Specific software can show the microbubble distribution alongside a conventional B-mode image in real-time.
- The use of low MIs minimises microbubble destruction and allows real-time observation for the duration of the microbubbles in blood, up to several minutes after injection.
- The exquisite sensitivity of the combination of microbubble-specific imaging modes and newer types of microbubble extends the abilities of ultrasound so that now the microcirculation can be studied as well as larger blood vessels.
- The use of microbubbles as tracers for ultrasound opens the way to functional studies.
- Ligands and large molecules can be attached to microbubbles, offering the possibility of targeted, molecular imaging and of drug and gene delivery using these tools.

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# Section II

# Clinical Application of Contrast Ultrasound in Liver Diseases

# II.1

# Characterisation of Benign Focal Liver Lesions with Contrast-Enhanced Ultrasound (CEUS)

Christoph F. Dietrich

## Introduction

Conventional B-mode ultrasound (US) allows for the definite diagnosis of common typical liver cysts by differences in echogenicity in comparison to the surrounding liver tissue using the following criteria: round, non-echogenic, smooth surface, sharp borders, lateral shadowing, posterior echo enhancement. Conventional B-mode US also allows for the definite diagnosis of calcifications due to their typical appearance: echo-rich with acoustic shadows [1].

Other focal liver lesions (FLL) are characterised sonographically, not only by analysis of differences in echogenicity from the surrounding liver tissue, but also by the detection of hyper- or hypovascularization (colour duplex US) and by changes occurring in inflow kinetics (enhancement) of contrast media. As a result of their double blood supply via both the portal vein and the hepatic artery, focal lesions in the liver often exhibit no sustained hyper- or hypoperfusion, but depending on the perfusion phase and the histology, present with a complex spatio-temporal picture of increased and reduced contrast-enhancement. Certain lesions display a characteristic vascular picture (e.g., wheel-spoke phenomenon in FNH) or a distinctive perfusion pattern (e.g., iris diaphragm phenomenon in haemangioma), allowing the lesions to be characterised. Unfortunately, contrast-enhancement does not always exhibit such typical and unique patterns. Multiple reviews and guidelines, as well as multicentre trials have been recently published using contrast-enhancing techniques [2-7].

The following chapter focuses on benign liver tumours and nodules, summarized in Table 1.

**Table 1.** Classification of liver tumours and nodular lesions (modified from [7a])

| Cell of origin | Benign liver tumour or nodular lesion (in parenthesis malignant transformation) | Abbreviation |
|---|---|---|
| Hepatocyte | Hepatocellular adenoma (→ hepatocellular carcinoma) | HCA (HCC) |
| | Focal nodular hyperplasia (→ fibrolammelar hepatocellular carcinoma) | FNH (FLHCC) |
| | Nodular regenerative hyperplasia | NRH |
| | Partial nodular transformation | PNT |
| | Macroregenerative nodule | MRN |
| Bile duct epithelium | Bile duct adenoma (→ combined hepatocellular and cholangiocarcinoma) | BDA (HCC/CCC) |
| | Bile duct cystadenoma (→ cystadenocarcinoma) | BDCA (BDCACa) |
| | Bile duct adenofibroma | BDAF |
| Mixed liver and bile duct cell | Mesenchymal hamartoma (→ angiosarcoma) | |
| Endothelial cell | Haemangioma | Haem |
| | Infantile haemangioendothelioma | |

## Differentiation of Benign and Malignant Lesions

Characterization of a liver lesion begins once an abnormality is found. An imaging procedure that is used to detect liver masses should also enable the examiner to differentiate between benign and malignant lesions, since benign and malignant lesions have been reported to vary in their uptake during the portal-venous and liver specific late phase after injection of Levovist. Homogeneous parenchymal Levovist uptake in the portal-venous and liver specific late phase seems to be indicative of benign focal liver lesions [8-13]. We propose that the non-enhancing defects of malignant liver lesions, and also abscesses, might be explained by the lack of liver specific tissue, such as portal veins, sinusoids and reticulo-endothelial cells. The extent of late phase contrast uptake by a lesion is mainly determined by the degree of similarity of the lesion to normal liver parenchyma, resulting in false positive findings and misinterpretation in patients with abscesses, necrosis, scars, calcifications, cysts, and thrombosis. As conventional B-mode US can correctly diagnose cysts and calcifications, it is therefore mandatory to perform a baseline scan before using US contrast agents. The results of a previously published study show that contrast-enhanced phase inversion sonography may discriminate between benign liver specific tissue and non-liver specific tissue - mainly malignant focal liver lesions in the portal-venous and liver specific late phase (Tables 2, 3) [13].

Only a few false positive findings were observed, mainly due to abscesses or necrosis, and in two liver tumours. Of these, one was a case of old focal nodular hyperplasia with predominantly scar tissue, and one was a case of an inflammatory pseudo-tumour of the liver, which was definitively diagnosed only by surgical exploration. The difficulties in diagnosing inflammatory pseudo-tumour of the liver is in accordance with current literature [14].

## Role of Contrast-Enhanced US in Clinical Practice

Contrast-enhanced US (CEUS) improves the detection rate of liver tumours in comparison to conventional B-mode US. CEUS was shown to be as effective as computed tomography (CT) and magnetic resonance imaging (MRI) in detecting neoplasia of the liver. We and others have obtained similar results when differentiating between benign and malignant lesions using

**Table 2.** Demographic profile and tumour size of patients with histologically proven liver tumours [13]

| Characteristics | |
|---|---|
| *Demography* | |
| No (M/F) | 80/94 |
| Mean age (yr)+ | 52 ± 15 [7 – 80] |
| | *Mean size [mm]*+ |
| *Benign liver lesions (n = 95)* | 41 ± 24 [ 8-130] |
| Focal nodular hyperplasia (n = 36) | 51 ± 18 [20-110] |
| Haemangioma (n = 31) | 37 ± 31 [ 8-130] |
| Adenoma (n = 10) | 40 ± 20 [15-80] |
| Abscess (n = 5) | 40 ± 24 [10-80] |
| Microhamartomas (n = 4) | 12 ± 2 [ 9-15] |
| Focal steatosis (n = 4) | 32 ± 5 [25-38] |
| Nodular regenerative hyperplasia (n = 1) | 40 |
| Hyperregenerative nodule (n = 1) | 35 |
| Focal biliary cirrhosis (n = 1) | 25 |
| Bacillary angiomatosis (n = 1) | 50 |
| Inflammatory pseudotumour (n = 1) | 40 |
| *Malignant liver lesions (n = 79)* | 40 ± 21 [ 7-120] |
| Metastatic liver tumour (n = 37) | 33 ± 12 [10-75] |
| Hepatocellular carcinoma (n = 33) | 42 ± 24 [ 7-120] |
| Cholangiocellular carcinoma (n = 4) | 59 ± 42 [40-100] |
| Lymphoma (n = 4) | 44 ± 17 [17-60] |
| Hemangioendotheliosarcoma (n = 1) | 100 |

+Mean ± standard deviation [range]

**Table 3.** Contrast-enhancement in 95 patients with histologically proven benign liver tumours or lesions [13]

| Liver tumour | Isoechoic contrast-enhancement |
|---|---|
| *Benign liver lesions (n = 95)* | 88/95 |
| Focal nodular hyperplasia (n = 36) | 35/36 |
| Haemangioma (n = 31) | 31/31* |
| Adenoma (n = 10) | 10/10 |
| Abscess (n = 5) | 0/5 |
| Microhamartomas (n = 4) | 4/4 |
| Focal steatosis (n = 4) | 4/4 |
| Focal biliary cirrhosis (n = 1) | 1/1 |
| Bacillary angiomatosis (n = 1) | 1/1 |
| Nodular regenerative hyperplasia (n = 1) | 1/1 |
| Regenerative nodule (n = 1) | 1/1 |
| Inflammatory pseudotumour (n = 1) | 0/1 |

*The vascular phase contrast-enhancement pattern of haemangiomas was variable. Characteristic was a progressive heterogeneous centripetal fill-in and considerable enhancement on liver specific late phase imaging, revealing diminished contrast to the surrounding liver parenchyma in all patients

SonoVue. Homogeneous uptake is indicative of benign focal liver lesions. The importance of this dual imaging approach (conventional B-mode US/contrast-enhanced ultrasonography) is highlighted by the recognition of the high prevalence of benign liver lesions in the adult population that do not require treatment. These include cysts, calcifications and most benign liver tumours. Therefore, correct characterisation by the ultrasonography as the primary imaging method is crucial for the further diagnostic, therapeutic and prognostic approaches.

## Hepatic Tumour and Liver Nodule Characterisation

### Liver Cyst

#### Conventional B-mode US

Liver cysts are a common sonographic finding. They are readily diagnosed using conventional B-mode US. The typical cysts are echo-free, round/oval, with well-defined borders with lateral shadowing and posterior echo-enhancement. Very early echinococcosis might be confused with atypical liver cysts.

#### Colour Doppler Imaging

Blood vessels have to be excluded by colour Doppler imaging ruling out arterio-portal-venous malformations with a cystic appearance.

#### Contrast-Enhanced US

Using CEUS, cysts show no contrast-enhancement at all. As they may be confused with metastases in CEUS, conventional B-mode US has to precede CEUS. CEUS is helpful in recognising echinococcosis at all stages.

#### Role of Contrast-Enhanced US in Clinical Practice

In clinical practice, CEUS has no role in routine diagnosis of cysts, since sensitive colour Doppler techniques differentiate between vascular abnormalities and typical and atypical liver cysts in almost all cases. CEUS can be helpful, however, in the differentiation of neoplastic nodules in atypical liver cysts, which is a very rare condition.

### Haemangioma

#### Conventional B-mode US

Most haemangiomas demonstrate typical sonomorphological features in conventional B-mode, including less than 3 cm in diameter, lobulated with a well-defined outline, located next to liver vessels, demonstrating an echo-rich texture and sometimes posterior acoustic enhancement due to blood filled capillaries (Table 4).

**Table 4.** Typical haemangioma, diagnostic criteria

| B-mode Criteria |
|---|
| Less than 3 cm in diameter |
| Echo-rich structure |
| Homogeneous interior |
| Round or slightly oval shape |
| Smooth outline |
| Absence of any halo sign |
| Possible detection of feeding and draining vessel |
| Absence of any signs of invasive growth |
| Dorsal through-enhancement |

#### Colour Doppler Imaging

Although haemangiomas are highly vascularised masses, from the histo-pathological point of view they consist essentially of a large number of capillary-sized vessels and so, even with the use of high-end machines, conventional colour Doppler US often detects little or no blood flow inside haemangiomas. This is due to the fact that the blood flow velocity in the capillary haemangiomas is too slow. The supplying and draining vessels ('feedings vessels') may be visualised (depending on the US system's peformance) (Table 5).