

The Second Symposium on Ultrasound Contrast
for Radiological Diagnosis:

Bubbles in Radiology- The State of the Art

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Programme Directors:
Peter N Burns PhD and Stephanie Wilson, MD
University of Toronto



E-mail: Burns@sten.sunnybrook.utoronto.ca

Website: <http://www.sunnybrook.utoronto.ca/bubble>

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Bubbles in Radiology - The State of the Art

Symposium held at the University of Toronto

23-24 October 2000

FOREWORD

Microbubble contrast agents for ultrasound have finally arrived after years of research and development. But what will they be good for? Our intention in these two days in Toronto was to try to define the role that these agents will play in radiological diagnosis. An international faculty of leaders in the field presented both their investigations and their clinical experience with the present and future generations of agents and described the exciting new imaging techniques that have grown with them. Their conference notes are presented in this document.

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Peter N Burns
Bubbles in Radiology Symposium
Professor of Medical Biophysics and Radiology, University of Toronto

Bubbles in Radiology - The State of the Art

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Programme Directors:

Peter N. Burns PhD
Professor of Medical Biophysics & Radiology
University of Toronto
Sunnybrook & Women's College Health
Sciences Centre

Stephanie R. Wilson MD
Professor of Radiology
University of Toronto
The Toronto Hospital

Guest Faculty:

| | |
|----------------------------|--|
| Thomas Albrecht MD | Klinikum Benjamin Franklin, Berlin, Germany |
| Richard Barr MD | North Eastern Ohio Universities College of Medicine, USA |
| Martin Blomley MD | Hammersmith Hospital, University of London, UK |
| Roland Brassard MD | Montreal Neurological Institute, McGill University, Canada |
| Rodolfo Campani MD | University of Pavia, Italy |
| Jean-Michel Correas MD PhD | Necker Hospital, University of Paris, France |
| David Cosgrove MD | Hammersmith Hospital, University of London, UK |
| Marcus Dill-Macky MD | University of Melbourne, Australia |
| Rob Eckersley PhD | Hammersmith Hospital, University of London, UK |
| Ethan Halpern MD | Thomas Jefferson University, Philadelphia, USA |
| Edward Leen MD | Glasgow University, UK |
| Alberto Martegani MD | Como, Italy |
| Jeff Powers PhD | Product Generation, ATL Ultrasound, USA |
| Harry Rakowski MD | University of Toronto, Canada |
| Michelle Robbin MD | University of Alabama, USA |
| Annette Schmider MD | Charité Hospital, Berlin, Germany |
| Luigi Solbiati MD | Milan, Italy |
| Evan Unger MD | University of Arizona & ImaRx Pharmaceutical Corporation, USA |
| Hanspeter Weskott MD | University of Hanover, Germany |

Bubbles in Radiology - The State of the Art
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CONTRAST AGENTS AND BUBBLE BEHAVIOUR

Peter N. Burns

Depts Medical Biophysics and Radiology
University of Toronto

Introduction

Contrast agents are a routine part of clinical X-ray, CT, MR and radionuclide imaging. Yet in spite of the importance of the vascular component of an ultrasound examination, the modality has yet to exploit the potential benefit of contrast enhancement. Why? A common response from ultrasonographers is that intravascular injections would detract from one of ultrasound's major attractions, that it is noninvasive. Yet this is a sentimental point of view: if it could be shown that the additional diagnostic information obtained with contrast enhancement will spare the patient from a more invasive procedure, we would be doing them no favor to deny them a painless intravenous injection. Another explanation appeals to the ultrasound image itself. Looking at an image of a blood vessel, it is apparent that tissue gives rise to a naturally high contrast between blood and solid tissue. It could be argued that we do not need a subtraction method to see blood. Furthermore, if we are interested in seeing where the blood flows, the Doppler shift associated with its rather weak echoes can be used to add to this contrast, as evidenced routinely by a color Doppler imaging. Doppler also provides quantitative information about, for example, the direction and velocity of flow. In the early days of echocardiography a B-mode image could be used to see blood in a cardiac chamber but not whether it was flowing across a septal defect. Bubbles were injected into the cavity and their trajectory imaged: this was really the first use of a contrast agent in ultrasound. Yet this invasive procedure, which required catheterization, is no longer necessary; color Doppler imaging has largely supplanted the intra-arterial injection of bubbles in cardiac diagnosis. In spite of this, the most recent developments in ultrasound contrast for cardiac diagnosis are redefining the capabilities of echocardiography and, we argue here, are likely to do the same for ultrasound imaging and Doppler in radiology.

The Need for Contrast Agents in Ultrasound

The major motivation for the current rapid rate of development of contrast agents for ultrasound lies in the nature of the current performance limits of ultrasound imaging and Doppler. Consider the bifurcation structure of the vascular system: as ultrasound is used to investigate more peripheral vessels, so the spatial resolution of imaging and the sensitivity of Doppler systems limit the minimum size of detectable vessels. At present, most duplex and color Doppler imaging systems are capable of detecting flow from vessels whose lumina lie below the resolution of the image. The detection of such 'unresolved' flow using Doppler systems can be demonstrated simply by using a duplex scanner to create a power Doppler image of the kidney, in which vessels that are not seen on the greyscale image become visible using the Doppler mode. These vessels are the arcuate and interlobular branches of the renal artery, whose diameter is known to be less than 100 μ m and therefore below the resolution limit of the image. However, as we progress distally in the arterial system, the blood flows more slowly as the rate of

bifurcation increases, giving lower Doppler shift frequencies, and the quantity of blood in a given volume of tissue also decreases, weakening the backscattered echo. Eventually, a point is reached at which the vessel cannot be visualised and the Doppler signals cannot be detected.

Two factors determine where that point will lie: Doppler shift frequency and echo strength. First, the velocity of blood must be sufficient to produce a Doppler shift frequency that is distinguishable from that produced by the normal motion of tissue, and second, the received intensity of the backscattered ultrasound must provide adequate signal strength for detection by the transducer above the acoustic and electrical noise of the system. Using a higher ultrasound frequency helps in both respects: the Doppler shift corresponding to a given flow velocity increases in proportion to the transmitted sound's frequency and the backscattered intensity increases with the fourth power of transmitted frequency, as predicted by the Rayleigh relationship. In practice, of course, the penetration of the sound through tissue places an upper limit on ultrasound frequency to be used. For deeper vessels of the abdomen, ultrasound frequencies above 5MHz produce blood echoes whose amplitude at the skin surface is too small for detection by most current systems. In this, and many other applications involving small vessels, it is the strength of the backscattered echo, rather than the Doppler shift frequency, that defines the smallest vessels from which Doppler signals can be detected. This limit of echo strength presents a barrier to the Doppler detection of flow in the vessels and organ parenchyma the abdominal and pelvis, as well as the breast, testes and extremities. It also defines the scale of the vasculature that it is possible to detect in a neovascularized mass or those collateral to a vascular occlusion. For the Doppler detection of blood flow in larger vessels, the effect of increasing the echo from blood is to enhance the signal-to-noise ratio, which again determines detectability in such vessels as the renal artery or the middle cerebral arteries approached transcranially. There is, then, clear clinical potential for a method capable of enhancing the echo from moving blood, especially in the systemic arterial system.

Contrast Agents For Ultrasound

The principal requirements for an ultrasound contrast agent are that it should be easily introducible into the vascular system, be stable for the duration of the diagnostic examination, have low toxicity and modify one or more acoustic properties of tissues which determine the ultrasound imaging process. Although it is conceivable that applications may be found for ultrasound contrast agents which will justify their injection directly into arteries, the clinical context for contrast ultrasonography requires that they be capable of administration intravenously. As we shall see, these constitute a demanding specification for a drug, one that has only been met recently.

Contrast agents might act by their presence in the vascular system, from where they are ultimately metabolized ('blood pool' agents) or by their selective uptake in tissue after a vascular phase. Of the properties of tissue that influence the ultrasound image, the most important are backscatter coefficient, attenuation and acoustic propagation velocity (1). Most agents seek to enhance the echo by increasing the backscatter of the tissue that

bears them as much as possible, while increasing the attenuation in the tissue as little as possible.

Blood Pool Agents

The concept of an ultrasound contrast agent to enhance the blood pool echo was first introduced by Gramiak and Shah in 1968 (2), who injected saline into the ascending aorta during echocardiographic recording. The saline gave rise to strong echoes within the normally echo free lumen of the aorta and the chambers of the heart, which were subsequently shown to be the result of free bubbles of air which came out of solution either by agitation or by cavitation during the injection itself. To tackle the natural instability of free gas bubbles, various attempts have been made to encapsulate gas within a shell so as to create a more stable particle. In 1980 Carroll et al (3) encapsulated nitrogen bubbles in an 80 μm gelatin shell, whose size, however, precluded administration by an intravenous route. Now, a burgeoning number of manufacturers have since produced forms of stabilized microbubbles that are currently being assessed for use as intravenous contrast agents for ultrasound. Several have passed through "Phase 3" clinical trials and gained regulatory approval in both Europe and North America. Levovist® (SH U 508A, Schering AG, Berlin, Germany), is a stable mixture consisting of 99.9% specially manufactured microcrystalline galactose microparticles, and 0.1% palmitic acid. Upon dissolution and agitation in sterile water for injection, the galactose disaggregates into microparticles which provide an irregular surface for the adherence of microbubbles 3 to 4 μm in size. Stabilization of the resulting air microbubbles takes place as they become coated with palmitic acid, which separates the air : liquid interface and slows their dissolution (4). These microbubbles are highly echogenic and are sufficiently stable for transit through the pulmonary circuit. The estimated median particle size is 1.8 μm and the median bubble diameter approximately 2 μm with the 97th centile approximately 6 μm (5).

The "shells" which stabilize the microbubbles are extremely thin and allow a gas such as air to diffuse out and go back into solution in the blood. How fast this happens depends on a number of factors which have been seen to vary not only from agent to agent, but from patient to patient. After venous introduction, however, the effective duration of the two agents described above is of the order of a few minutes. Because they are introduced as a bolus and the maximum effect of the agent is in the first pass, the useful imaging time is usually considerably less than this. Newer (sometimes referred to as 'second generation') agents, designed both to increase backscatter enhancement further and to last longer in the bloodstream are currently under experimental investigation. Instead of air, many of these take advantage of low solubility gases such as perfluorocarbons, the consequent lower diffusion rate increasing the longevity of the agent in the blood. Optison ® (Mallinckrodt Inc) is a perfluoropropane filled albumin shell with a size distribution similar to that of Albunex. The stability of the smaller bubbles in its population is the probable cause of the greater enhancement observed with this agent. Echogen® (Sonus Inc, Bothell WA) is an emulsion of dodecafluoropentane droplets which undergo a phase change in the blood, literally boiling at body temperature. Recent reports suggest that this agent produces visible enhancement to the renal parenchyma on conventional greyscale imaging (6). Yet another agent is Definity®, a perfluoropropane

microbubble coated with a bilipid shell (DMP115, Du Pont Merck Inc, Boston MA) which also shows improved stability and high enhancements at low doses (7).

Mode of Action

The interaction of an ultrasound beam with a population of bubbles is a process that is increasingly recognised as being subtle and complex. Yet understanding what happens when a contrast agent is exposed to ultrasound is the key to understanding - and developing - new clinical methods for contrast imaging and thus the key to interpreting a clinical contrast echocardiographic study.

A sound field comprises a train of travelling waves, much like ripples on a pond. The fluid pressure of the medium (in this case tissue) changes as the sound propagates through it. A gas bubble is highly compliant and hence is squashed when the pressure outside it is raised and expanded when the pressure is lowered. At a typical clinical frequency of 2MHz, for example, a bubble sitting in an acoustic field undergoes this oscillatory motion two million times per second. As it moves in this way, the bubble becomes a source of sound that radiates radially from its location in the body, as would ripples on a pond from a small object moving at a point on its surface. The sound that reaches the transducer from this bubble, combined with that from all of its neighbours, is what constitutes the *scattered* echo from a contrast agent. Characterising this echo so that it can be differentiated from those of ordinary tissue such as the cardiac muscle is the basis of contrast specific imaging modes such as pulse inversion Doppler or harmonic imaging.

Unlike tissue, a bubble does not scatter in the same way if it is exposed to weak (that is low amplitude) sound, than to strong, high amplitude sound. Instead, there are three broad regimes of scattering behaviour that depend on the peak pressure of the incident sound field produced by the scanner (Figure 1). These are used in different ways in perfusion imaging of the heart. Looking at Figure 1, we see that at low incident pressures (corresponding to low transmit power of the scanner), the agents produce linear backscatter enhancement, resulting in an augmentation of the echo from blood. As the pressure increases beyond about 100kPa, which is still below the level used in most diagnostic scans, the contrast agent backscatter begins to show such nonlinear characteristics as the emission of harmonics, which form the basis of harmonic and other nonlinear imaging techniques. Finally, as the peak pressure passes about 1Mpa, many agents exhibit transient scattering, which forms the basis of most strategies for triggered detection of myocardial perfusion. We now examine each of these patterns of behaviour in turn.

1. Low incident pressure: Linear Backscatter Enhancement

Although a bubbles of a typical contrast agent are smaller than red blood cells, and their volume concentration in the blood following intravenous injection is a fraction of a percent, the echo from the microbubble agent eclipses that of the blood itself. This is because the weakness of the echo from blood originates from the cells themselves, which are poor scatterers of ultrasound. Their acoustic impedance is almost identical to that of the surrounding plasma. A bubble containing compressible gas, on the other hand,

presents a strong discontinuity in acoustic impedance and hence acts as a strong reflector. Size for size, a bubble is about one hundred million million times more effective at scattering ultrasound. Thus the injection of a relatively sparse population of bubbles into the bloodstream results in a substantial enhancement of the blood echo.

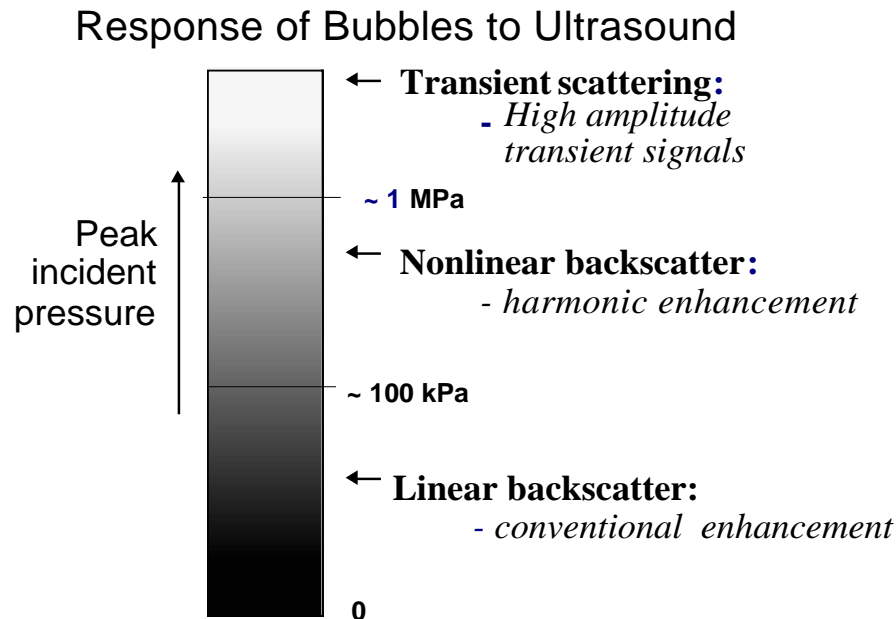


Figure 1: The three broad regimes of scattering behaviour of microbubbles depend on the peak pressure of the incident sound field.

In a Doppler examination, the arrival of the contrast agent, some seconds after peripheral venous injection, in the portion of the systemic vasculature under interrogation is marked by a dramatic increase in signal strength. In spectral Doppler this is seen as an intensifying of the greyscale of the spectrum. For a given vessel, this enhancement is related to dose. For spectral Doppler examinations that fail because of lack of signal strength, the effect of the contrast agent is to ‘rescue’ the examination, rendering otherwise undetectable signals clearly.

In the past few years, many studies have been carried out in which the sole indication for contrast agents has been a nondiagnostic Doppler examination. These have demonstrated the capacity of contrast agents to increase the technical success rate of Doppler ultrasound. In clinical echocardiographic studies, trials have shown that a single injection of an IV contrast agent can improve the technical success rate in quantitative Doppler assessment of aortic stenosis, mitral regurgitation and pulmonary venous flow, in which latter case it rose from 27% to 80% (8).

There have been extensive studies in other organ sites. For example, in clinical transcranial Doppler studies of the middle cerebral artery, administration of up to 10ml of Levovist resulted in dose-dependent increases in both signal intensity and duration of

enhancement (9). At a concentration of 400mg/ml, Levovist increased Doppler signal by approximately 25dB. The time to peak enhancement was between 30 and 60 seconds and the duration of enhancement was reported to be sufficiently long to be clinically useful. In another transcranial study the agent reduced the technical failure rate by 80% (10, 11). In radiological studies, the enhanced detection of small vessel flow by spectral and colour Doppler flow with contrast agents has been demonstrated in a number of clinical studies. These include flow in the renal parenchyma (12), in tumours of the breast (13, 14), prostate (15) and liver (16). The effect of the agent may be considered in other ways too. For example, given a satisfactory transthoracic colour Doppler study, one use of the agent might be simply to enable a higher ultrasound frequency to be used, exploiting the agent to counter the higher tissue attenuation. In such a case, the contrast enhancement translates into higher spatial resolution. Alternatively, the colour system may be set to use fewer pulses per scan line (that is a lower ensemble length) while still achieving the same sensitivity to blood flow by means of the contrast enhancement. The agent will then provide the user with a higher frame rate.

With these agents, enhancement is not usually seen on the greyscale image of the blood vessel lumen or in the vascular parenchyma of organs such as the heart, even though the agent is present and enhancing the Doppler signal. This is because the 10-20dB of enhancement provided by the agent still leaves the blood echo some 10-20dB below that of the echogenic tissue of the heart wall. In order to enhance the visible grey level, either higher concentrations of bubbles must be achieved or specific, new imaging strategies employed.

Quantitative measures of enhancement

In order to use an agent clinically, we need tools not only to detect and display its presence in tissue, but also to measure it. At present echocardiography analysis software provided in many ultrasound scanners is being extended to satisfy the requirements of contrast echo. In addition certain fundamental questions must be answered about the behaviour of a contrast agent in tissue. How does the dose concentration and injected volume relate to the effect on the echo? How long does the agent last? Does it

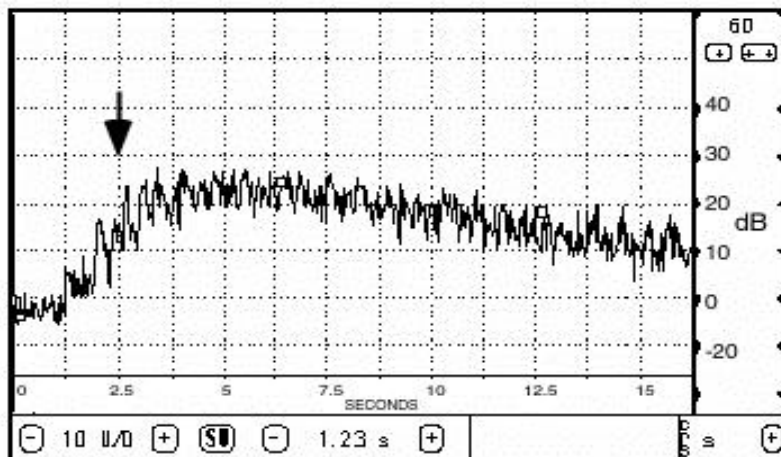


Figure 2: Results of frequency domain analysis of amplitude of blood echo following injection. Arrow indicates arrival of contrast bolus

recirculate? Is it affected by the heart and lungs or the mesenteric vasculature? Does absence of agent in a vessel mean absence of flow? Quantitative measurement of the effect of an agent is not only of practical importance: as will be seen, it also provides important insight into

the mechanism of bubble contrast.

To date, ultrasound contrast agents have been detected using conventional B-scan imaging and Doppler methods. Quantitation relies on video presentation of the echo data and in general has borrowed methods of video densitometric analysis (17-20). The relationship between video level on a B-scan display and received echo intensity is, however, complicated by technical factors such as dynamic range compression and non-linear processing, so that quantitative interpretation of these images is unreliable, or even misleading. Similarly, colour Doppler imaging systems provide video data which bear a nonlinear relationship not only between backscattered power of moving targets and video level, but also to different Doppler shift frequencies and such machine settings as the

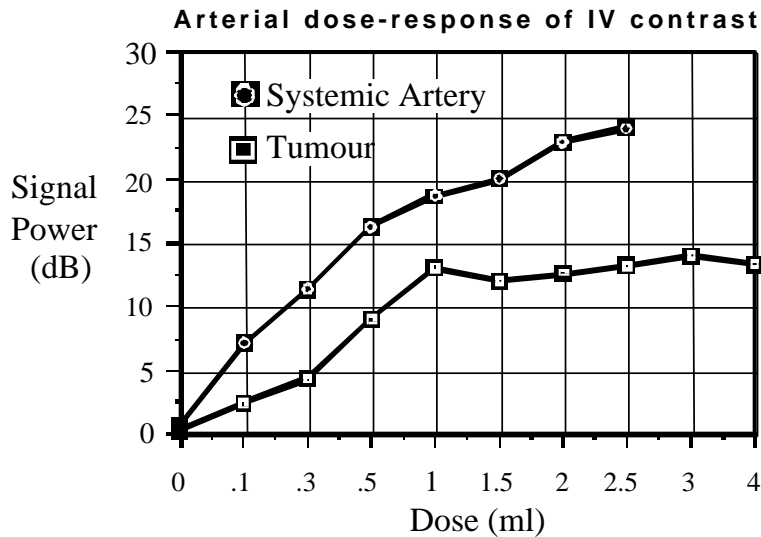


Figure 3: Contrast agent dose-response measured by the Doppler method

receiver gain and filter (21, 22). Relying on such methods, we can only obtain a qualitative assessment of the agents' effect. By exploiting the Doppler signal itself, however, it is easy to develop a quantitative method to measure the relative backscatter intensity of the RF signal and hence the enhancement due to the agent. The outline is described more completely elsewhere (23). The principle relies on the fact that,

for sufficiently small Doppler sample volumes, the power of the Doppler signal is proportional to the power of the echo that gives rise to the Doppler shift. By careful processing of the Doppler signal, the large power components due to the movement of solid structures (the clutter) can be eliminated and the contribution to the echo for which the contrast agent is responsible measured (Figure 2).

This method allows both the degree of enhancement and the time course of the enhancement to be quantified and thus opens the way to measuring dose-response in different vessels (Figure 3) as well as indicator-dilution applications of ultrasound contrast (24). These measurements can be carried out using a dedicated Doppler probe positioned on, say, the femoral artery of a patient; a curve obtained from a brachial artery after a slow injection of Levovist shows an enhancement of 15dB effectively lasting for more than 9 minutes. In the near future, machines are likely to be able to present time-enhancement curves from regions of interest selected in the image, so as to enable quantitative comparison of enhancement patterns in different locations from the same injection.

2. Nonlinear Backscatter: Harmonic Imaging

In many of the potential applications of contrast agents, one might ask whether it is possible to continue to increase the amount of agents injected and obtain progressively stronger echoes from blood; to the point, for example, where the myocardium becomes visible on a greyscale image. Unfortunately, attenuation of the ultrasound beam by the agent in the cavity also increases with bubble concentration, with the result that shadowing occurs distal to the agent and the myocardium disappears altogether. Because of this limitation of the useable concentration of the agent, we are generally left with small enhancements in the myocardium echo that must be identified against the strong background echo from the solid tissue itself. X-ray angiography, which is faced with a similar problem after dye is injected into the bloodstream, deals with these 'clutter' components of the image by simple subtraction of a pre-injection image. What is left behind reveals flow in individual vessels or the 'blush' of perfusion at the tissue level. If, however, we subtract two consecutive ultrasound images of a solid organ, we get a third ultrasound image of the same organ, produced by the decorrelation of the speckle pattern between acquisitions. In order to show parenchymal enhancement due to the agent, speckle variance must first be reduced by filtering, with a consequent loss of spatial or temporal resolution. Even if the speckle problem could be overcome, subtraction would still be poorly suited to the dynamic and interactive nature of ultrasound imaging. In Doppler modes, the problem of the moving wall interference (the clutter) prevents the smaller echo from the blood itself being detected.

How then might contrast agents be used to improve the visibility of blood in moving vascular structures such as the myocardium? Clearly, a method that could identify the echo from the contrast agent and suppress the echo from solid tissue would provide both a real time 'subtraction' mode for contrast-enhanced B-mode imaging, and a means of suppressing Doppler clutter without the use of a velocity-dependent filter in spectral and colour modes. Harmonic scattering is the signature that is unique to the contrast agent echo, and harmonic imaging is the method that provides the means for detection of flow in smaller vessels than is currently possible.

Harmonic Scattering

Examining the behaviour of contrast-enhanced ultrasound studies reveals two important pieces of evidence. First, the size of the echo enhancement at very high dilution following a small peripheral injection (7dB at a dose of 0.01 ml/kg of Levovist®, for example (25)) is much larger than would be expected from such sparse scatterers of this size in blood. Second, investigations of the acoustic characteristics of several agents (26) have demonstrated an approximately linear dependence of backscattered coefficient on numerical density of the agent at low concentrations, as expected, but a dependence of attenuation on ultrasound frequency different to that predicted by the Rayleigh law,

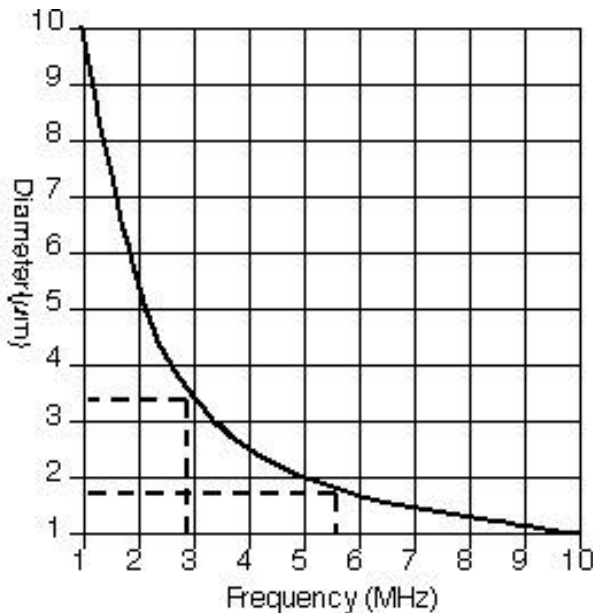


Figure 4: Microbubbles resonate in a diagnostic ultrasound field. This graph shows that the resonant - or natural - frequency of oscillation of a bubble of air in an ultrasound field depends on its size. For a 3.5µm diameter, the size needed for an intravenously injectible contrast agent, the resonant frequency is about 3MHz

which describes how the echogenicity of normal tissue changes with frequency. Instead, peaks exist which are dependent on both ultrasound frequency and the size of the microbubbles, suggestive of resonance phenomena. This important observation suggests that the bubbles resonate in the ultrasound field. What this means is that the bubbles get bigger and smaller in sympathy with the oscillations of pressure caused by the incident sound. Like vibrations in other structures, these radial oscillations have a natural - or resonant - frequency of oscillation at which they will both absorb and scatter ultrasound with a peculiarly high efficiency. Considering the linear oscillation of a free bubble of air in water, we can use a simple theory (1) to predicts the resonant frequency of radial oscillation of a bubble of 3µm diameter, the median diameter of a typical transpulmonary microbubble agent. Figure 4 shows that it is about 3MHz, approximately the center frequency of ultrasound used in a typical abdominal scan. This extraordinary - and quite fortuitous - coincidence explains why ultrasound contrast agents are so efficient and can be administered in such small quantities. It also predicts that bubbles undergoing resonant oscillation in an ultrasound field can be induced to nonlinear motion, the basis of harmonic imaging.

One consequence of this extraordinary coincidence is that bubbles undergoing resonant oscillation in an ultrasound field can be induced to nonlinear motion. It has long been recognised (27) that if bubbles are 'driven' by the ultrasound field at sufficiently high acoustic pressures, the oscillatory excursions of the bubble reach a point where the

alternate expansions and contractions of the bubble's size are not equal. Lord Rayleigh, the originator of the theoretical understanding of sound upon which ultrasound imaging is based, was first led in 1917 to investigate this by his curiosity over the creaking noises that his tea-kettle made as the water came to the boil. The consequence of such nonlinear motion is that the sound emitted by the bubble, and detected by the transducer, contains harmonics, just as the resonant strings of a musical instrument, if plucked too vigorously, will produce a 'harsh' timbre containing overtones. Figure 5 shows the frequency spectrum of an echo produced by a microbubble contrast agent following a 3.75MHz burst. This particular agent is Levovist, though many microbubble agents behave in a similar way. Ultrasound frequency is on the horizontal axis, with the relative amplitude on the vertical axis. A strong echo, at -13dB with respect to the fundamental, is seen at twice the transmitted frequency, that known as the second harmonic. Peaks in the echo spectrum at sub- and ultraharmonics are also seen. Key factors in the harmonic response of an agent, which varies from material to material, are the incident pressure of the ultrasound field, the frequency, as well as the size distribution of the bubbles and the mechanical properties of the bubble capsule (a stiff capsule, for example, will dampen the oscillations and attenuate the nonlinear response).

A real-time imaging and Doppler method has been based on this principle, which is known as harmonic imaging (28) The system transmits normally at one frequency, but when in harmonic mode receives echoes only at double that frequency. A broadband digital colour flow system can be modified so as to transmit at between 1.5 and 4 MHz and to receive at the second harmonic, between 3 and 8 MHz. Harmonic imaging uses

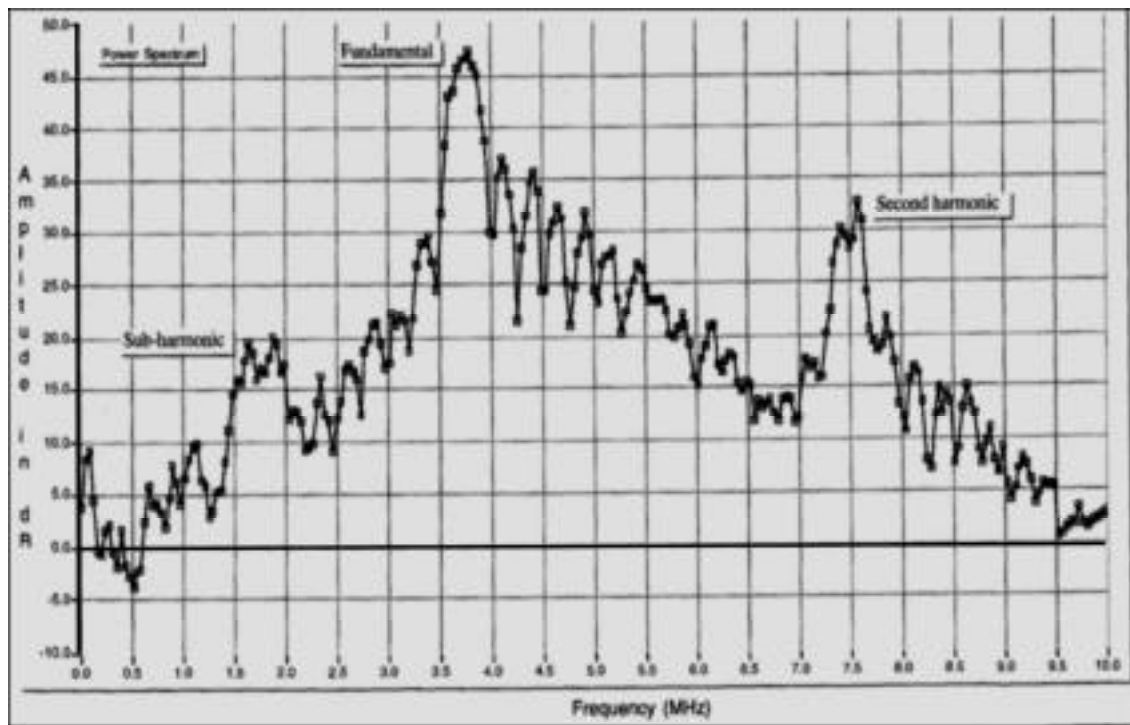


Figure 5: Graph showing the response of the agent Levovist to a 3.75MHz pulse of sound. Note that there is an echo at 3.75MHz – the fundamental – but also one at 7.5MHz – the second harmonic.

the same array transducers as conventional imaging and for the ATL ultrasound system involves only software changes. In harmonic mode, echoes from the contrast agent are received preferentially by means of a bandpass filter whose center frequency is at the second harmonic. Echoes from solid tissue, as well as red blood cells themselves, are suppressed. Real-time harmonic imaging, Doppler and colour Doppler modes have now been implemented experimentally on a number of commercially available systems. Clearly, an exceptional transducer bandwidth is needed to operate over such a large range of frequencies. Fortunately much effort has been directed in recent years towards increasing the bandwidth of transducer arrays because of its significant bearing on conventional imaging performance.

Harmonic imaging is a method that succeeds in identifying microbubble contrast agent in the tissue vasculature by means of its echo 'signature'. In doing so it helps tackle some old problems in ultrasound, such as the rejection of the tissue echo in Doppler modes designed to image moving blood, and creating a 'subtraction' mode without sacrificing the real time nature of the examination. Figure 16 summarizes graphically the effect of contrast agents and harmonic detection on the Doppler process. In conventional Doppler, the signal from blood is larger than the clutter signal from tissue. In contrast enhanced Doppler, the signal from blood is raised, sometimes to near that of tissue. With harmonic mode, the signal from blood is raised but that from the tissue reduced, so reversing the contrast between tissue and blood. Another way of looking at the harmonic method is that, because of its greater sensitivity to small quantities of agent, a given level of enhancement due to a bolus will last longer (Figure 17). This is the reason that a number of investigators have found that cardiac contrast imaging of small vessels such as those in the myocardium, is more effective in harmonic mode (29, 30).

Harmonic imaging demands exceptional performance from the transducer array and system beamformer. Its implementation forces implicit compromises between, for example, image resolution and rejection of the non-contrast agent echo. It places unusual demands on the bandwidth performance of transducers, as well as the flexibility of the architecture of the imaging system. The ease with which it has been developed on modern instruments, however, reflects this flexibility and augers well for the future of the method. More significantly, contrast agents are now being developed specifically with nonlinear response as a design criterion. Entirely new agents present opportunities for entirely new detection strategies (31). Our laboratory measurements show that some new contrast agents are capable of creating an echo with more energy in the second harmonic than at the fundamental: that is, they are more efficient in harmonic than conventional mode. With such agents, nonlinear imaging should become the preferred clinical method for their detection.

3. Transient Scattering: Intermittent nonlinear echoes

Following the introduction of contrast agents, it was soon discovered that by pressing the 'freeze' button on a scanner for a few moments, and hence interrupting the acquisition of ultrasound images during a contrast study, it was possible increase the effectiveness of a contrast agent. So dramatic is this effect that it can raise the visibility of a contrast agent in the myocardial circulation to the point that it can be seen on a B-mode image, above

the echo level of the normal heart muscle (32). It is now understood that the ultrasound field, if its peak pressure is sufficiently high, is capable of disrupting a bubble shell and hence destroying it (33, 34). During this destruction, a strong, brief and highly nonlinear echo is emitted. By setting a scanner to detect this nonlinear echo, much higher contrast can be achieved of the tiny myocardial blood pool over the muscle tissue. This pool is, however, moving very slowly, and has not had time to reperfuse the bed before the next ultrasound frame is created. The beam for this frame destroys only the minute quantity of bubbles that have washed in between frames, so no contrast is seen. It is therefore necessary to interrupt the scanning process for a sufficient time to allow the agent to wash in to the myocardial bed, a period usually somewhere between two and six heartbeats. For this reason, it is normal to use triggered imaging for myocardial perfusion studies. This imaging strategy is sometimes referred to as 'intermittent' or 'transient' imaging. We (35) and others (29) have subsequently found that with harmonic imaging, this single acquisition imaging gives even more contrast. By combining intermittent harmonic imaging with power Doppler, one can assemble the most sensitive imaging mode so far available for contrast agents, with which we have been able to demonstrate the first Doppler images of myocardial perfusion (35, 36). It is now commonplace to image a single frame over one, two, or up to five heartbeats, so as to allow the agents time to refill the smallest vessels of the myocardium, into which the blood flows at only about 1mm/s.

Summary

The three regimes of behaviour of bubbles in an acoustic field depend on the strength of the transmitted ultrasound beam. At low intensities, the bubbles act as simple, but powerful echo enhancers. At higher intensities (those most commonly used diagnostically), the bubbles emit harmonics as they go into nonlinear oscillation. Finally, at the highest intensity setting of the machine used in routine scanning, the bubbles are disrupted, emitting a strong transient echo, sometimes referred to as "stimulated acoustic emission".

Modern agents for the production of ultrasound contrast offer the prospect that extremely small, harmless injections of a solution into a peripheral vein will produce spectacular improvements in the detection of blood filled structures and blood flow velocity in echocardiography. Clinical applications include the imaging of the endocardial border, the detection of low volume jets, of flow in smaller vessels such as the coronary arteries and of flow in moving parenchyma such as that of the myocardium. They also offer the potential measurement of relative blood flow rates and vascular volume using indicator dilution methods. Harmonic imaging gives new capability to imaging and Doppler systems, effectively allowing real-time 'subtraction' imaging of flowing blood with ultrasound and the detection of flow in at the perfusion level of the myocardium. These, together with newer methods such as pulse inversion Doppler, all rely on the fascinating behaviour of a bubble in an acoustic field.

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CONTRAST IMAGING METHODS

Jeff Powers

ATL Ultrasound, Bothell, Washington

Ultrasound contrast agents have been under development for many years. The first agents to be reliably detected in the systemic arterial circulation following an intravenous injection have recently received regulatory approval in the United States and Europe. This has led to increased interest on the part of the ultrasound equipment manufacturers to develop improved methods of visualizing them. Consequently the past few years have witnessed a dramatic improvement in the effectiveness of the contrast agents, which has been largely led by improvements in the imaging equipment itself. This, in turn, was led by an improved understanding of the behavior of the microbubble contrast agents themselves.

Background

As contrast agents were being developed, the only imaging methods available were those designed for tissue, therefore all clinical trials were done using standard imaging techniques. While the newer imaging modalities to be discussed here have, in some cases, significantly increased contrast agent effectiveness, in many cases standard imaging methods may be used with some minor adjustments of conventional controls. The imaging methods discussed here utilize unique properties of the microbubbles to help distinguish them from tissue. To understand these imaging methods, a brief review of microbubble physics is essential.

Microbubble Physics

Microbubble contrast agents are inherently nonlinear reflectors, or scatterers of ultrasound energy. This means that they return sound at frequencies that were not in the transmitted waveform. This sound is primarily at multiples, or harmonics of the transmitted sound wave.

An acoustic wave generated by an ultrasound system consists of alternating high and low pressures. These occur at the transmitted ultrasound frequency, from about 1.5 to 10 MHz. When an acoustic wave encounters a microbubble, it alternately compresses the microbubble on the positive pressure, and expands it on the negative pressure. This is illustrated in Figure 1 for a low amplitude acoustic wave.

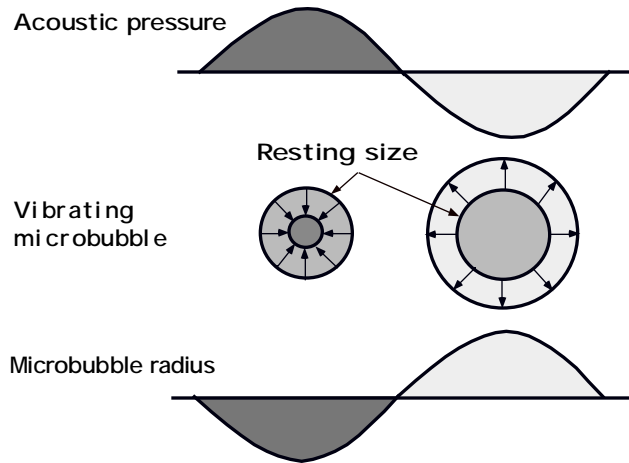


Figure 1. Microbubble response to low amplitude acoustic wave. On the positive pressure the bubble shrinks, on the negative pressure it expands compared to its resting size.

As the sound field becomes more intense, as happens when the power is increased, the microbubbles can only become so small. However, on the negative portion of the sound wave they can become quite large, as illustrated in Figure 2.¹ Instead of producing a nice sinusoidal wave with a clean frequency spectrum, it produces an odd looking waveform with non-symmetrical top and bottom. It is this asymmetry which produces harmonics.

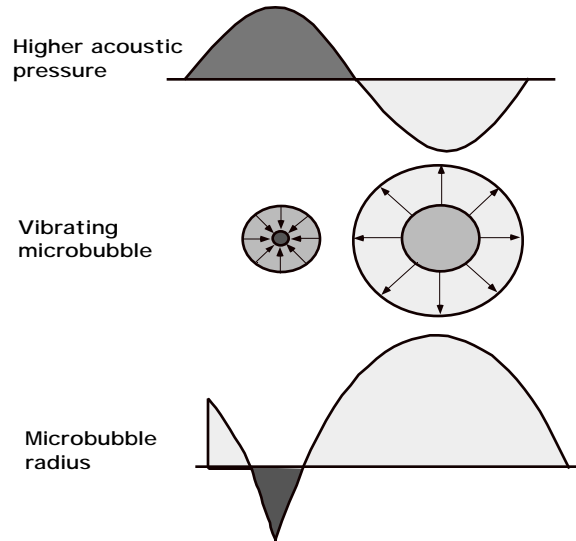


Figure 2. Microbubble response to a higher amplitude acoustic wave. On the positive pressure the bubble can only shrink so far, but on the negative pressure it can expand quite large.

¹ This is a transient phenomenon and only lasts a few tenths of a microsecond as the acoustic wave passes by. It does not lead to the development of larger bubbles in vivo.

Harmonic Imaging

The first imaging method to utilize microbubble nonlinear properties was harmonic imaging. In this technique, the ultrasound beamformer transmits at one frequency and receives at twice that frequency. Since the contrast agent microbubbles generate far more harmonic energy than tissue does, harmonic imaging enhances the signals from contrast over those from tissue. This has been shown to be very effective and has become the standard technique for B-mode contrast imaging.

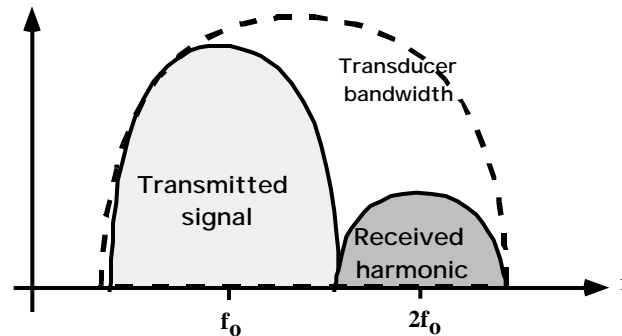


Figure 3. Harmonic transmit and receive frequencies.

One thing that will be noticed from Figure 3 is that both the transmit and receive portions of the spectrum must fit within the transducer bandwidth. This requires them are relatively narrow to prevent part of the transmitted signal leaking into the received signal, which reduces resolution in a harmonic image.

Harmonic Doppler

This same harmonic signal can be used with Doppler signal processing to produce harmonic Doppler images. In this case, the frequency of the received harmonic signal determines the Doppler shift attained. Therefore, harmonic Doppler signals are at twice the frequency of the fundamental signals for the same velocity of blood. Harmonic Doppler techniques can be used when moving tissue obscures the response from the contrast agent.

One harmonic Doppler method that has been particularly effective is Power Harmonics™, in which the power of the harmonic Doppler signal is displayed, rather than its frequency. This has some of the same sensitivity advantages as conventional power Doppler imaging with the added advantage of reducing the motion artifacts from moving tissue.

Transient Echo Imaging

Most microbubbles consist of a gas bubble surrounded by a thin stabilizing shell. These shells are very thin and break easily when the microbubble expands under insonation, allowing the gas to diffuse more rapidly into the blood. This destruction of

microbubbles, was first seen as a major drawback, but has since been shown to aid in their detection.

Transient echo imaging, also known as Stimulated Acoustic Emission (SAE), utilizes this destruction of microbubbles to form an image. Doppler techniques, whether harmonic or conventional, look for changes in the sound wave returning to the transducer over multiple pulsing intervals. When that motion is regular and in the same direction over several pulse intervals, the ultrasound system is able to determine the approximate velocity and direction of the moving particles. Then the velocity can be displayed as color flow, or the amplitude as a power Doppler image.

When contrast agents are destroyed, the resulting changes in the returned ultrasound signal are often more chaotic than those from RBC's. While the ultrasound system can tell that something changed, if the change is due to the microbubble disappearing, it is impossible to assign a meaningful direction or velocity. In color flow mode, the resulting image has a random mottled red / blue appearance. In Doppler power mode it will have a more uniform appearance, since the amplitude, not the frequency is being displayed.

This technique can be used to detect virtually stationary blood, such as in the microvasculature. In some cases, the color flow image is preferred, as the mottled red / blue makes very clear what is contrast and what is moving tissue. For extra sensitivity the power Doppler image might be preferred, which averages several frames together, but is more motion sensitive.

Another effect of microbubble destruction is that contrast agent in small vessels with slowly moving blood is destroyed during real time scanning. For example, the blood in the microvasculature moves on the order of 1 mm/sec. If the scanplane is on the order of 5 mm thick, it will take approximately 5 sec. for the blood to fully reperfuse the scanplane. Thus, imaging of perfusion of the microvessels requires triggering, or very low frame rate imaging.

Pulse Inversion Imaging

Pulse Inversion Imaging is the newest contrast specific imaging method. As described above, in "conventional" harmonic imaging, the bandwidth of both the transmitted and received signals must be restricted to ensure that the received harmonic signal can be separated from the transmitted signal. If the frequency spectra of the transmitted signal overlaps that of the harmonics of interest, they cannot be completely separated. This is illustrated in Figure 4.

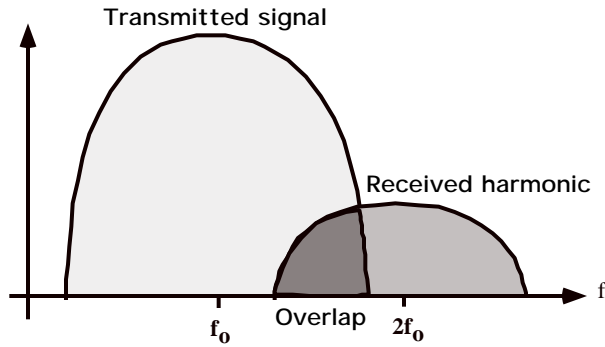


Figure 4. “Conventional” harmonic spectrum showing the potential for overlap of transmitted fundamental and received harmonic energy.

Pulse Inversion Imaging avoids these bandwidth limitations by utilizing characteristics specific to microbubble vibrations to subtract rather than filter out the fundamental. This allows the use of broader transmit and receive bandwidths for improved resolution, and increases sensitivity to contrast.

In Pulse Inversion Imaging two pulses are transmitted down each ray line, instead of only a single pulse as is done with conventional or harmonic B-mode. The first is a normal pulse, the second is an identical copy of the first, but inverted, so wherever there was a positive pressure on the first pulse there is an equal negative pressure on the second. Any linear target that 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, as shown in Figure 5a.

Microbubbles respond differently to positive and negative pressures and do not reflect identical inverted waveforms. When these waveforms are added, they do not cancel completely. The harmonic component adds, giving twice the harmonic level of a single waveform. This is illustrated in Figure 5b.

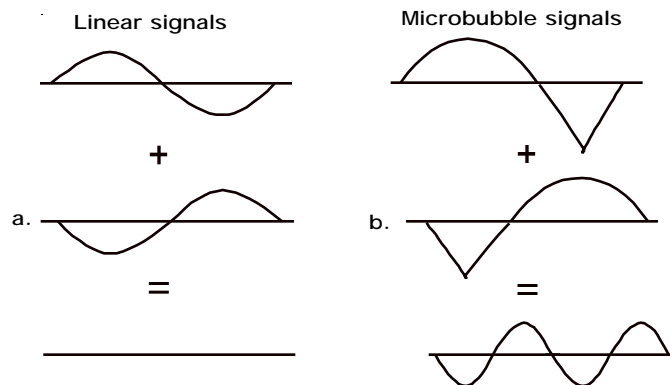


Figure 5. Pulse Inversion Harmonic imaging. a. Cancellation of linear components. b. Enhancement of harmonic component from microbubbles.

The fact that two pulses are used to form each ray line leads to other effects. Anything that moves between the two pulses is not completely cancelled, which leads to some

tissue motion artifact. This is similar to color Doppler motion artifacts, but since Pulse Inversion is a greyscale mode, the effect is to simply brighten the greyscale image slightly. This has a beneficial effect on microbubbles, though, similar to transient echo imaging described above. Since this is a differencing technique, any bubble that was in the sound field on the first pulse, but gone, or significantly changed on the second, will lead to a strong signal in Pulse Inversion Imaging.

Since two pulses are used, the frame rate will be less than normal real time frame rates. Again, this has its upside also. Contrast is destroyed by realtime scanning, which has led to the use of ECG triggering to visualize perfusion in cardiology. The reduced frame rate with Pulse Inversion Imaging, combined with the increased sensitivity to contrast may lead to the ability to image perfusion in real time, though at a reduced frame rate.

Summary

The recent availability of contrast agents has led to the rapid development of new imaging methods. The ones currently available are harmonic imaging, Power Harmonics™, and Pulse Inversion Imaging. Each of these has its advantages and drawbacks, and is useful in certain situations. Further investigations into the clinical utility of ultrasound contrast agents will help identify which imaging mode should be used in a given clinical situation. Also, as more is learned about the physics of microbubbles and their interaction with ultrasound, improvements will be made on these imaging methods, as well as new ones being developed.

QUANTITATIVE METHODS IN CONTRAST RADIOLOGY

Robert Eckersley

Hammersmith Hospital, University of London, UK

Background

Quantitative measurement of radiological images can provide detailed and useful diagnostic information. This is possible because a quantitative approach can reveal subtle information about the underlying physiology that is hard to determine by qualitative assessment of the images. Such information can be important in both the detection of disease and in monitoring patient response to therapy. In other modalities such as MRI, CT and nuclear medicine quantitative approaches have already gained widespread acceptance and are in routine use.

By measuring, or quantifying, the strength of the echo enhancement in a region perfused with blood carrying microbubbles it is possible to estimate the number of bubbles present. Due to signal attenuation and other technical factors the absolute concentration of the microbubbles is not obtainable. However, this relative measurement can still amount to a powerful tool.

The contrast agents used in ultrasound are typically confined to the blood pool this is unlike those generally used in the other modalities that have a more systemic effect, leaking from the blood into interstitial spaces. This difference together with the more general advantages of ultrasound examinations makes quantitative ultrasound imaging an exciting prospect.

In echocardiography such quantitative measurement techniques are already gaining clinical acceptance and these techniques are crossing over into more general radiological applications.

What Can We Measure?

There are wide ranges of possible quantitative measurements. These are each closely related to the mode of data acquisition.

The most basic measurement is that of the echo strength (figure 1). Experiment has shown that this can be related to bubble concentration. It should be noted however a number of factors can confound this relationship. Some of these are out of the control of the operator but artefacts such as blooming, bubble destruction, or shadowing can be minimised and a judicious choice of scanning mode and contrast agent can help to improve the measurements. These issues are discussed in more detail in the next section.

Providing the limitations of this technique are noted, it is possible to relate the quantitative measurement of bubble number to physiologically meaningful indices. For a fully perfused region of tissue the number of bubbles in the tissue can be related to the vascular volume of the tissue (figure 2).

By combining such measures with area measurement from the ultrasound image an estimate of fractional vascular volume can be obtained. Although such measurements have been possible previously with Doppler methods the use of microbubbles provides large improvements in sensitivity to both small vessels and slow flow.

Quantifying matching regions from a series of images allows dynamic, or kinetic, measurements to be made (figure 3). For example, if the agent is administered using a short bolus injection, it is possible to measure the rate of change of the echo strength after bolus injection. This can yield a relative measure of the flow rate into the region of interest. Alternatively the ultrasound beam can be used destructively to generate a short negative bolus in the tissue under investigation and the wash in or refill rate measured to give a measure of local flow. This, later destruction-reperfusion approach, is becoming widely used in echocardiography. The technique requires a study infusion of the agent to be established. Then using ECG-gated high MI imaging a series of images of the region of interest are obtained at different frame intervals. As the interval between frames is increased the number of bubbles reperfusing into the tissues is also seen to increase. The rate of this increase provides a measure of local blood flow rate.

Calculating the product of the flow rate and the fractional vascular volume gives an estimate of the local tissue perfusion.

A range of kinetic measurements is also possible after bolus administration. For example the arrival time, or time to peak and enhancement duration could all provide potentially useful clinical data.

Scanning Modes

It is possible to apply quantitative measurement across the range of ultrasound acquisition approaches. The key issues are: selecting an appropriate mode, the choice of contrast agent and its delivery (i.e. bolus or infusion), and the scanner settings.

The simplest 1D example is measuring the 'loudness' of the spectral Doppler signal. Figure (1) shows an example of the increase in both arterial and venous spectral Doppler signals after venous administration of a contrast agent. Quantitative measurement of the change in the audible signal output by the scanner can provide detailed information on the degree and timing of the enhancement.

Measuring the brightness of a standard fundamental grey-scale image can provide a similar 2D measure (figure 1).

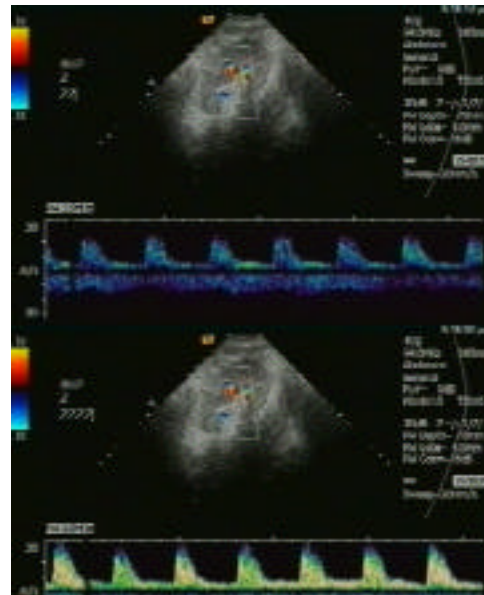


Figure 3. Example of in vivo spectral Doppler enhancement in the renal pedicle of a patient after Kidnet transplant

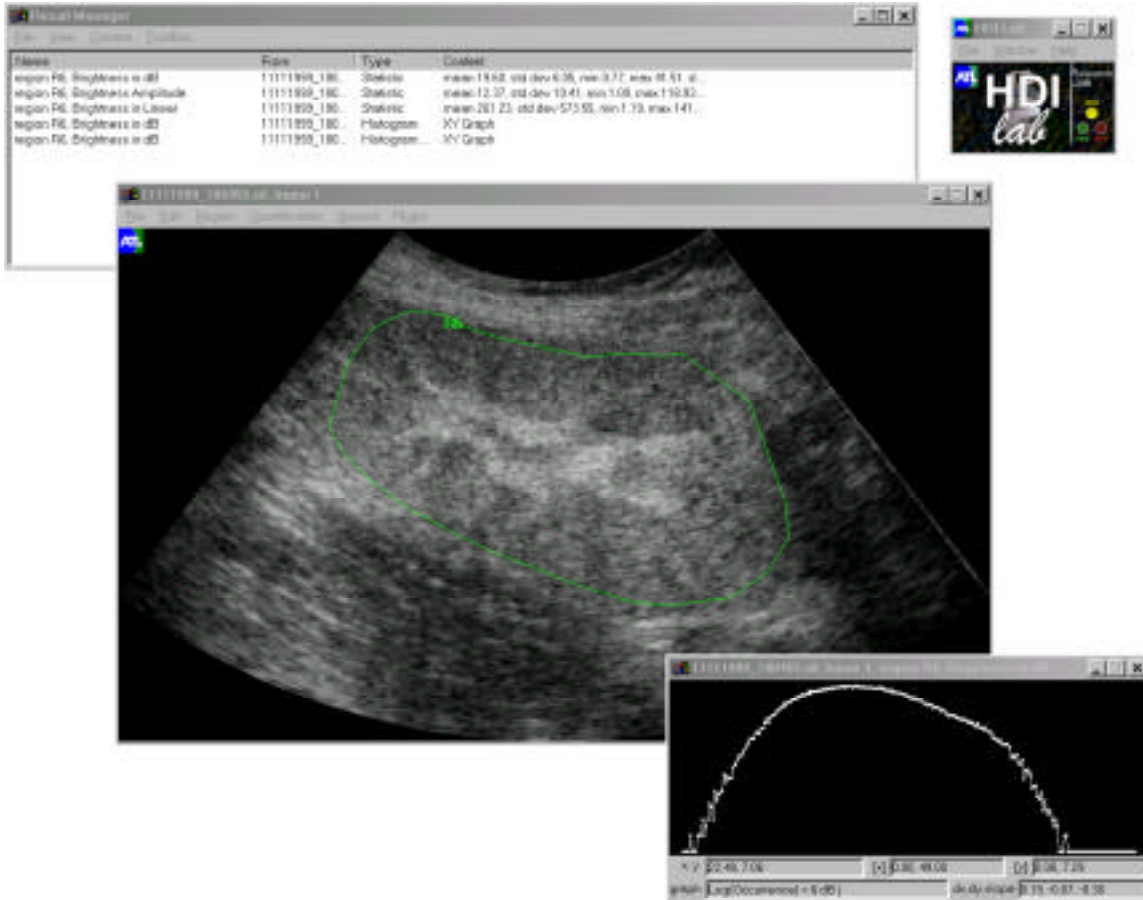


Figure 4. Example of single image quantitation with HDI Lab from ATL, the user simply selects a region of interest on the image and the software package calculates various statistics over the region. An estimate of fractional vascular volume can be arrived at by measuring the total enhancement over the region and divided by the area of the region. To perform this calculation a matching pre-contrast image is required as a baseline.

If quantitative measurement is performed over the entire duration of the contrast study a time-enhancement curve such as that presented in figure (3) can be obtained. This process is applicable to both 1D (spectral Doppler) measurements and 2D image sequences. Such data can provide kinetic information on the circulatory system and may be useful in the assessment of abnormal vasculature.

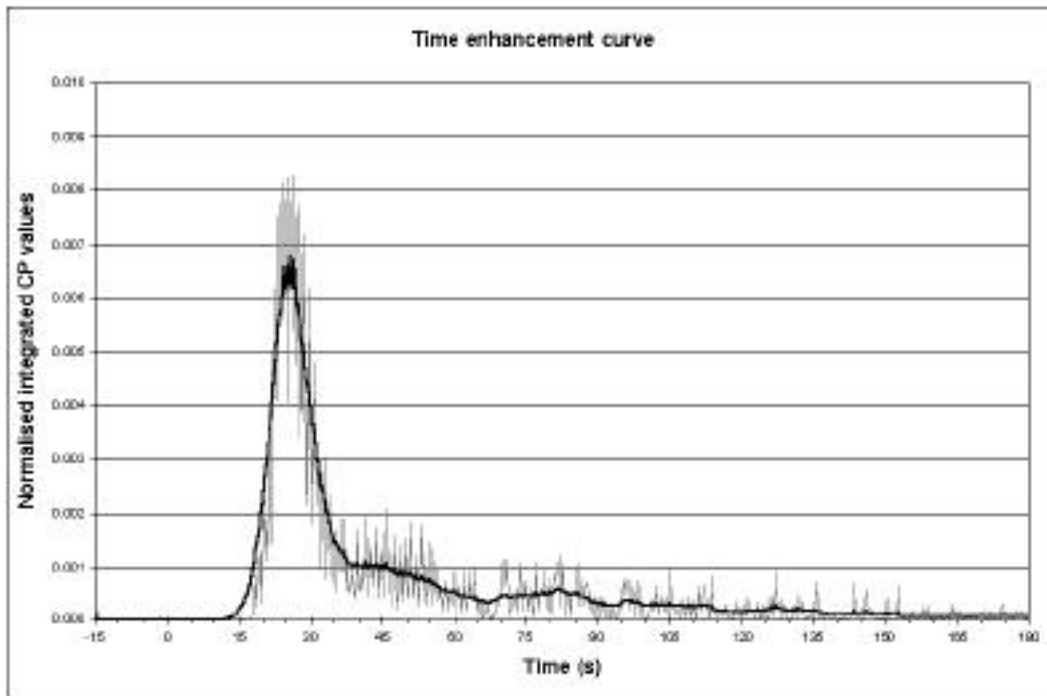


Figure 5. Example time enhancement curve, showing the change in colour power (CP) levels after bolus administration of Levovist. The vertical scale shows the CP values after anti-logging and normalisation by the size of the region of interest. Large variations arising from cardiac pulsatility are seen in the unsmoothed data (thin line). The thicker line shows the processed smoothed data used for subsequent calculations of the indices. These data were obtained using the CQ system from Kinetic Imaging Ltd.

There are a few important considerations when setting up the scanner for a quantitative study:

Care should be taken to ensure that the mode used provides a display of signal strength, rather than target speed or signal decorrelation such as in Colour Doppler mode.

The output power of the scanner should be adjusted to reduce artefacts due to bubble destruction. Depending on the contrast agent used and the tissue being studied an $MI < 0.6$ should be adequate.

Bubble specific modes, such as harmonic imaging or pulse inversion imaging should be used if available as these can improve the image quality at significantly lower output power.

The receive gain should be reduced compared to non-contrast imaging.

The dynamic range should be set as high as possible, although some compromise will be required to ensure that low signal areas are not lost as a side effect.

It is often essential to record an image series prior to administration of the contrast as a baseline measurement.

For kinetic measurements the time of injection should be clearly labelled on the image.

In many cases an initial contrast study will be required to enable the selection of the most appropriate scanner settings.

Practical Approaches

Until recently quantitative measurements have only been possible in research orientated departments with in-house computer systems using bespoke applications. However a range of commercial products are now available, designed to bring quantitative techniques into more widespread clinical use. Broadly speaking these fall into two categories, offline post processing solutions available from third party vendors and built in systems that come as part of a package with your ultrasound system.

Even so quantification can still seem a daunting task. In most cases the process can be broken up into the following steps:

Data storage. For entirely practical reasons quantitation is usually performed after the ultrasound examination is over so some form of data storage is required. For single image quantitation, or short sequences such as pre and post contrast assessment this step is trivial. Simply saving the required images to the system disk or removable media is all that is required. For kinetic assessments the storage requirements are more demanding. To store an entire 5-minute study at 20 frames per second requires approx 7.5Gb of storage. The built in cineloops of most scanners are only able to store a few seconds worth of images and even with triggered acquisition the amount of data storage required is not usually available. The only practical solution at present is to videotape the study for subsequent offline assessment. The videotaped images these are redigitised at a reduced frame rate.

Image and region selection. It is important to ensure that images with artefacts such as flash or blooming are not included in the analysis. Regions of interest can then selected within the image, again avoiding areas with artefacts. For image sequences the position of the regions may require adjustment from one frame to the next and in some analysis packages this process has been automated.

Calculations. The quantitation software is now able to calculate area statistics for the regions chosen. Important at this stage is to ensure that your measurement is actually what you think it is. The best measure of relative bubble concentration over a region is the sum of the image pixels values, after they have been corrected for the compression and dynamic range used in the processing chain. For in built quantitation systems this is transparent step, however users of third party products will need to check that this step is performed correctly.

Kinetic measurements. Some packages are now available which provide basic tools for assessment of dynamic sequences, i.e. fitting a mono-exponential to a refill curve.

Future Application of Quantitative Measurement

Quantitative measurement is at present a research tool but in the very near future the results of this research will provide a suite of measurement opportunities available in real time on the ultrasound machine itself. It is of primary importance at this point for the radiological community to identify clinical applications for these tools and to clearly specify their measurement requirements.

Summary

Quantitative measurement provides a means to tweak out otherwise subtle information from the rich data provided by ultrasound contrast studies. There appear to be two principle areas where quantitative measurement can be applied. The first is to obtain meaningful measures of physiologically relevant indices, i.e. perfusion, or transit time. The second is to monitor changes in tissue characteristics over time, for example during therapy. In other radiological modalities such measurement approaches have become commonplace clinical tools. In the field of ultrasound quantitative measurement is now in a position to graduate from a research tool into a recognised clinical approach.

Further reading

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IMAGING THE BLOOD POOL IN THE LIVER

Peter N. Burns

Depts Medical Biophysics and Radiology
University of Toronto, Canada

Introduction

Although ultrasound imaging is used in the identification of lesions in abdominal organs such as the liver, its role is ultimately limited by the inherently low contrast differences that often exist between different tissue types. Doppler is frequently used as an adjunct to greyscale imaging to help identify blood flow signals associated with a lesion in the hope that these will, for example, betray a solid cancer by virtue of its vascularity. However, Doppler systems are limited in their ability to detect small blood vessels, the minimum size of which is determined by a number of technical factors in the scanning process. Two conditions must be met in order for a Doppler signal to be detected. First, the blood must be flowing sufficiently fast for a Doppler shift to be registered at all. Second, the echo from the small volume of blood in a microvessel must be sufficiently strong to be detectable at the transducer. These two criteria are set not only by instrument performance but also by the patient. Thus, motion of solid tissue produces a Doppler effect, and because the echo from solid tissue is so much stronger than that from blood, this often renders the low Doppler shift frequencies from slowly moving blood undetectable. Furthermore, small volumes of blood produce echoes which are so weak that they cannot be detected in all but the most superficial of structures using conventional scanners. The result of these limitations is the well known observation that the microvasculature of a tumour, which for example is seen to enhance upon contrast injection in CT or MR, remains invisible to ultrasound.

It is this challenge which the combination of contrast agents and new imaging technologies seeks to address. By combining new nonlinear imaging methods with careful contrast technique, we can show that by means of a simple intravenous injection, followed by either harmonic or pulse inversion imaging, we are able to produce greyscale ultrasound images that have characteristics which appear to be similar to those of a contrast enhanced CT scan.

Why Doppler Enhancement is Not Enough

Following the intravenous injection of a small volume of a typical modern ultrasound contrast agent, we can increase the echo from a volume of blood by a factor of between 100 and 1000. The result is that the echo level from, for example a cardiac cavity, becomes comparable to that of the echo from the heart muscle and blood becomes “visible” on a greyscale image. However, if we look at the parenchyma of an organ such as the liver, it is in general not possible to see any enhancement at the tissue level following a microbubble contrast injection, because the echo from the solid tissue is strong compared to that from the relatively small volume of blood lying within it. In these circumstances, it is natural to turn to Doppler as a better way of detecting flow within the organ following contrast enhancement. Of the two limitations described above, the

addition of a contrast agent mitigates only the second. Thus, the signals from hitherto undetectable vessels are pushed above the threshold of detection by enhancement of the echo when the microbubbles are present. However, Doppler is also sensitive to the movement of tissue and, especially in power modes, movement of the organs due to transmitted respiratory and cardiac motion will often swamp the signals from small blood vessels. Furthermore, a price is paid for the enhancement of the blood signal. The additional strength of the signal from enhanced blood results in extensive “blooming” of the colour Doppler image. This artifactual extension of the Doppler image in both lateral and axial directions degrades the resolution and creates an image that is neither pleasing nor diagnostic. Blooming can be reduced by reducing the gain, but this has the effect of negating the advantage of the contrast agent. Using the current generation of ultrasound scanners, contrast enhancement often results in a degraded, confusing image, in which it is hard to identify colour with real flow within an organ structure.

In order to overcome these problems, a method is first required in which the echo from the bubbles is distinguished from the echo from ordinary solid tissue, which can then be suppressed. Harmonic imaging provides this tool, but at a price. As discussed in the previous talks, harmonic images are made at the expense of spatial resolution, so it is quite difficult to make a harmonic image which is both sensitive to the microbubble oscillation described in the previous section and of acceptable resolution. In practice, harmonic imaging has sufficient sensitivity only to detect bubbles when their echo is strong, that is, when the incident energy (corresponding to the transmit power setting on the machine) is high. One unintended effect is that propagation nonlinearities of the ultrasound pulse tend to produce a harmonic image of tissue. Although this image has many desirable qualities, and is used in its own right in non-contrast imaging, it is effectively an artifact when trying to detect contrast agent, because it produces echoes from tissue that are hard to distinguish from bubble echoes. Furthermore, at high transmit powers (reflected by the Mechanical Index, or MI), bubbles are disrupted, making it impossible to image continuously.

Continuous, Low MI Imaging

Newer methods of nonlinear imaging, such as pulse (or “phase”) inversion, and coherent contrast imaging, have effectively overcome the limitations of simple harmonic modes. These methods offer higher bandwidth, so that image resolution is not compromised, and higher bubble sensitivity, so that a low, nondestructive level of insonation (typically with $MI < 0.2$) can be used continuously to demonstrate vascular structures. Because of the absence of a strong propagation harmonic at low MI, these modes are characterised by a very dark tissue background, over which the contrast is seen as a bright white blush.. Large vascular structures appear to be echogenic, allowing the blood in such vessels to be “seen”. The challenge is to be able to visualize the vessels when they are small - perhaps the perilesional vasculature of a hepatic metastasis - or when they are actually below the resolution limit of the image - such as the microvessels that result from angiogenesis. Whereas these low MI methods provide an excellent real-time tool, they are not as sensitive as methods which expose the bubble to more energy, and hence destroy it. A complementary method, which both relies on bubble destruction and exploits the consequent manipulation of the agent as a tracer, is therefore also helpful in the liver.

Interval Delay, High MI Imaging

One unique aspect of microbubble contrast agents, described earlier, is that their structure can be changed by the ultrasound imaging field itself. When bubbles are oscillating in an ultrasound field, their radius changes from small to large at a rate equal to the ultrasound frequency. When the amplitude of the sound (the “transmit power”) is increased, this excursion of radial size becomes greater. It is the reluctance of a bubble to compression and the ease with which it expands that creates the asymmetry in the bubble echo which contains the second harmonic signature. If a bubble is in harmonic oscillation and the incident amplitude is increased yet further - by increasing the transmit power of the ultrasound machine to near the maximum levels used routinely - the bubble can undergo such severe radial oscillation that it is actually disrupted. This disruption has two effects: first, it creates a single, transient but very strong echo which is rich in harmonics; and second, it causes the bubble to disappear. The fact that the bubble emits such a strong harmonic echo upon its disruption can be exploited as a way of detecting the microcirculation. The fact that it disappears so quickly after insonation can be exploited using the Doppler method, which is exquisitely sensitive to changes in the phase of sequential echoes from the same site. It was found in the early trials of harmonic imaging on the heart that the agent was visible in the cardiac chambers, but not in the myocardial muscle. However, when imaging acquisition was triggered by the EKG, so that one image was acquired per heart beat, myocardial perfusion became visible on the harmonic B-mode image. *Triggered harmonic* imaging is now a standard way of imaging microvascular perfusion in the myocardium: how does it work and can it be applied to the liver?

Each pulse of sound destroys the agent, so that only bubbles which can ‘wash in’ to the imaging region between frames is normally visible. As this interval might be only about 20 milliseconds in real time imaging modes, the blood needs to be moving fast, that is, to be flowing in a large vessel. By reducing the frame rate to one per second, blood is able to carry the bubbles further into the vascular tree, rendering tissue blood visible. In fact, it takes up to five seconds for bubbles to completely refill a myocardial bed that has been emptied of contrast agent.

In the liver, we can exploit the same phenomena by simply freezing the scanner and allowing the contrast agent to fill the entire liver microcirculation, a process which takes approximately 8 seconds. Harmonic imaging is then performed, the first frame of which destroys the agent, producing a “flash”. This frame is then reviewed from the scanner’s cine-loop memory. In many cases, the effect of the entire circulation of the liver being filled with microbubbles is that the attenuation increases to an extent that the first frame only shows a flash from the most superficial parts of the organ. However, the second frame encounters only destroyed bubbles, so stimulates bubbles which lie slightly deeper, the third frame deeper still, and so on. The result is that one sees a flash which descends in real time through the liver dropping like a “veil” from top to bottom. By looking at the intensity of this veil as it passes through a lesion, one can ascertain whether the number of bubbles in the lesion is the same, less, or greater than that of the surrounding liver parenchyma—or whether the veil’s echo in the lesion is greater, less, or equal to that of

surrounding tissue. This examination of the relative echo intensity of the transient harmonics emitted from the agent at the microvascular level forms the basis of what we call *interval delay imaging*.

This then is the basis of a new liver imaging examination. We image at low MI continuously in order to visualise the geometry of resolvable vessels, which are clearly visible around metastases and penetrating into hepatomas. At higher MI we perform interval delay imaging, which reveals the relative number of bubbles in the microscopic vessels that lie below the resolution limit of the B-mode image. This manifests itself in a “veil”, whose intensity reflects the total vascular volume in the organ.

The Future

A useful way of combining the low MI, real-time “survey” mode with interval delay “destruction” mode, has yet to be devised. Other challenges, such as devising a way of scanning with a very dark tissue background in the low MI images without getting “lost”, remain open. One new approach which will be discussed is to overlay a low MI multipulse nonlinear image on a fundamental image of the liver, much as is done in colour Doppler, but with the MI of the fundamental tissue image sufficiently low that it does not disrupt the bubbles and reduce the effectiveness of the contrast image. This would allow realtime, conventional scanning of the liver with a bright, contrast specific overlay that could be optimised for bubble sensitivity. Multipulse methods such as pulse inversion Doppler may have a role to play here.

ULTRASOUND CONTRAST AGENTS: IMAGING THE VASCULAR PHASE

Stephanie R. Wilson, Peter N. Burns, Korosh Khalili
University of Toronto

Preamble

Imaging of the vascularity of a liver lesion contributes tremendous discriminatory information to allow for specific diagnoses. Historically conventional color Doppler and spectral waveforms allowed detection of arterial vascularity in some hypervascular masses, most notably focal nodular hyperplasia and hepatocellular carcinoma. Other masses, without such profuse arterial vascular supply, were often difficult or impossible to evaluate with conventional Doppler techniques. The introduction of microbubble contrast agents to clinical practice several years ago showed that Doppler signals were enhanced with the addition of contrast agents. This, however, did not materialize into the expected improvement in terms of diagnosis of focal liver masses. The advantage of the enhanced Doppler signals was frequently offset by many additional problems which prevented good interpretation of the Doppler signals. Tremendous color blooming and significant motion artifact, in particular, often led to images with little or no diagnostic information.

Improvements in imaging technology have been paramount in the evolution of this technique such that we can now see with exquisite detail the vascularity in many liver tumors. Harmonic imaging and, more recently, pulse inversion imaging represent the first two major technical advantages that have greatly facilitated our ability to use microbubble contrast agents for diagnostic purposes. These, however, are on a spectrum which continues to evolve.

Our *objectives in vascular imaging* are two fold, as follows:

Regarding the *lesional vessels*

to image in real-time the major and the small vessels within a liver tumor.

to assess their filling relative to the filling of the liver vasculature.

This is performed most optimally with *continuous low MI imaging* timed to coincide with the completion of a bolus injection.

Regarding the *microvasculature*

to estimate the relative vascular volume of a lesion as compared to the liver.

to image the changes in the filling of the vascular volume over time.

This is performed most optimally with variations of *an interval delay technique with high MI imaging*. Suspension of insonation allows the microbubbles to accumulate in the vascular volume. Reinsonation at high MI produces a bright flash in the liver and in the lesion as the accumulated bubbles disrupt. This flash is in proportion to the bubble distribution. These interval delays may be brief or prolonged and may be timed at the peak of arterial enhancement for an arterial phase, between 30 – 60 seconds for a portal

venous phase, or of even longer duration to image the late vascular phase. In all interval delay sequences, image review must be performed on the stored cine-loops. Frame one is essential as the bubble bursting is frequently brief and maximal on this frame.

General Imaging Choices

Choice of contrast agent – Perfluorocarbon contrast agents are preferred for vascular vessel imaging as they demonstrate a strong harmonic response with ultrasound insonation at low MI. Therefore, optimal continuous imaging may be performed without destruction of the agent. The air-containing contrast agent, Levovist, is however comparable to the perfluorocarbon agents if assessment of the microvascular volume is the motivation.

The intravenous – An angiocath is preferable, either a 20 or 22 gauge, with a simple hook up via a short tubing to a three-way stopcock. We attach a flush syringe, filled with normal saline, to the right-angle port and the contrast agent to the direct port. We have abandoned infusion bags as an inferior method for delivery of the contrast agent.

Administering the agent – we now prefer to give small boluses of contrast to allow for imaging in different planes and evaluation of more than one lesion with a single dose of the agent.

Choice of MI –

Low MI – To show the extent and the nature of vascularity in a hepatic lesion, low MI, nondestructive mode is the selection of choice. This affords two advantages. First we can look at the vessels continuously throughout the vascular phase to show the filling of the vessels relative to the filling of the liver vasculature. Also, the nondestructive mode allows for visualization of long lengths of the vessels facilitating assessment of their morphology. Currently, we select the lowest MI that provides sufficient visualization of the liver for identification of the lesion.

High MI – To produce bubble destruction, required for the determination of the volume of contrast agent within the lesional microvasculature, a high MI is the selection of choice. If a separate injection is to be used for this determination, maximal response will be directly dependent on both the MI and the volume of contrast agent. Alternately, following an interval delay, even with a low or medium MI selection, re-insonation will produce a bright flash which is a reflection of the accumulated microbubbles. This response will obviously be less than at a higher MI level.

Combination low and high MI – This is a highly desirable feature for vascular assessment, providing information about both the lesional vascularity and the vascular volume. Our motivation is to watch the wash-in phase of the contrast agent with low MI so as to preserve the bubble population. Then at the peak of arterial enhancement, a change to a high MI will burst the accumulated microbubbles. We document both the intensity of the flash and its distribution. The timing of the change to high MI is also facilitated by direct visualization providing the opportunity to image in the arterial and the portal venous phases of enhancement and even beyond. This is very difficult to do

manually and the equipment manufacturers are now trying to implement this by the simple push of a button.

Choice of Gain Setting

Continuous low MI imaging is performed with a relatively high gain as the low MI will diminish the ability to see the background liver on the real-time image. Interval delay techniques with a medium or high MI selection will result in a bright image. Therefore a reduction of gain is performed in anticipation of the result.

Image Storage – the large numbers of frames required for assessment of a contrast agent injection necessitates a computerized method of image storage. This is required on both the hard drive of the ultrasound machine as well as on a computerized archiving system. The ability to store cine loops is also mandatory, as review must be in real-time to allow for accurate interpretation. It is highly desirable for the image storage to accommodate loops which include a conversion from the low to the high MI imaging. Flexibility of cineloop length is also a benefit as long loops are required for continuous low MI imaging whereas a loop of 50 frames is more than adequate for many interval delay sequences.

Observations

Vascular Imaging

The number distribution and morphology of the lesional vessels all provide discriminatory information that helps to distinguish one lesion from another.

Interval Delay Flash Imaging

This is dependent on the volume of the accumulated microbubbles, the MI selection, and the choice of contrast agent. In all instances, the enhancement of a lesion is compared with the enhancement of the adjacent liver.

A greyscale veil – this is seen as positive enhancement first in the near, then in the mid, and ultimately in the far field as the accumulated bubbles burst. It is seen only with a high MI and only with the perfluorocarbon agents.

A single frame flash – this is seen as a bright enhancement of the image which lasts only for the first frame of reinsonation. It may be seen after an intentional interval delay with freezing of the ultrasound mechanism. However, more commonly it is observed spontaneously in two situations: as the transducer is moved during the scan over an area which was not previously insonated and with normal quiet patient breathing, which brings a lesion in and out of the ultrasound field. These flashes are seen with both perfluorocarbon and air based agents. The response is directly proportional to the volume of the contrast agent accumulated during the delay and the MI selection.

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IMAGING THE POSTVASCULAR PHASE

Martin Blomley

Department of Imaging

Imperial College School of Medicine, Hammersmith Hospital, London UK

Introduction

It is known that some microbubbles are selectively retained within the liver and spleen after completion of the vascular phase. The agents with well documented evidence of this are Levovist (Schering), Sonazoid (Nycomed Amersham), Sonavist (Schering), and BR14 (Bracco). The underlying mechanisms of the liver phase remain, however, incompletely understood, and there are probably some differences between different types of microbubbles.

The liver phases of both Sonavist and Sonazoid are known to involve intracellular (reticuloendothelial) uptake, and this has been proven with electron microscopy studies which have shown microbubbles within Kupffer cells after administration. An intracellular phase would also be consistent with the relatively long duration of the liver specific phase with these agents (hours or longer).

The mechanism of the liver phase of Levovist is still not understood. It is substantially shorter (<1 hour) than with these more robust agents, and it is tempting to hypothesize that, whilst some form of reticuloendothelial interaction occurs, the Levovist is not surviving for long enough to be completely engulfed.

Whilst a liver specific phase for the novel microbubble BR14 has been described, there is no information on the detailed kinetics or mechanism in the public domain.

Whatever the mechanism, it is clear that certain microbubbles enter a liver and spleen specific phase after completion of blood pool enhancement. They thus have kinetics analogous to SPIO agents in MR. Just as with these agents, they could be useful both in increasing sensitivity and specificity, as many benign liver lesions contain normal liver tissue (eg focal nodular hyperplasia, regenerating nodules) while malignant lesions do not.

From an imaging aspect, the key point is that the microbubbles are widely distributed and stationary (or near stationary) in the tissue parenchyma at this time. They cannot therefore be detected using conventional Doppler enhancement. Non-linear modes which can detect stationary microbubbles are needed, and a number of alternatives are now available. The best choice involves consideration of many factors, including the ultrasound equipment available, the clinical problem being addressed, and the microbubble being used. Currently, these modes can basically be divided into modes which detect resonance effects, such as second harmonic and pulse/phase inversion imaging, and modes which rely on microbubble disruption producing a “loss of correlation” of successive ultrasound pulses.

Resonance Based Imaging

Using second harmonic imaging, parenchymal grey scale enhancement can be seen. By scanning the liver of subjects who have received Levovist several minutes before, a study using the Toshiba second harmonic system has shown that the conspicuity of liver metastases can be increased [1]. The spatial resolution of second harmonic grey scale imaging is poor, however. The use of pulse or phase inversion imaging allows for much higher spatial resolution and sensitivity to microbubbles. By scanning several minutes after injection (2-5 minutes seems optimal) of Levovist, the conspicuity of metastases is greatly increased (Fig 1). Sweeps are performed through the liver parenchyma, scanning with relatively high acoustic powers and low frequency settings. It is very helpful to capture a cine loop and review it immediately afterwards. An interesting observation is that echogenic rims around lesions (“rolled edge effect”) has been described around many metastases and this may be a marker for a metastatic lesion. Two recent single centre studies (using the ATL Phillips pulse inversion software [2] and the Siemens phase inversion systems [3] respectively) have shown that the sensitivity of ultrasound to metastases can be increased.

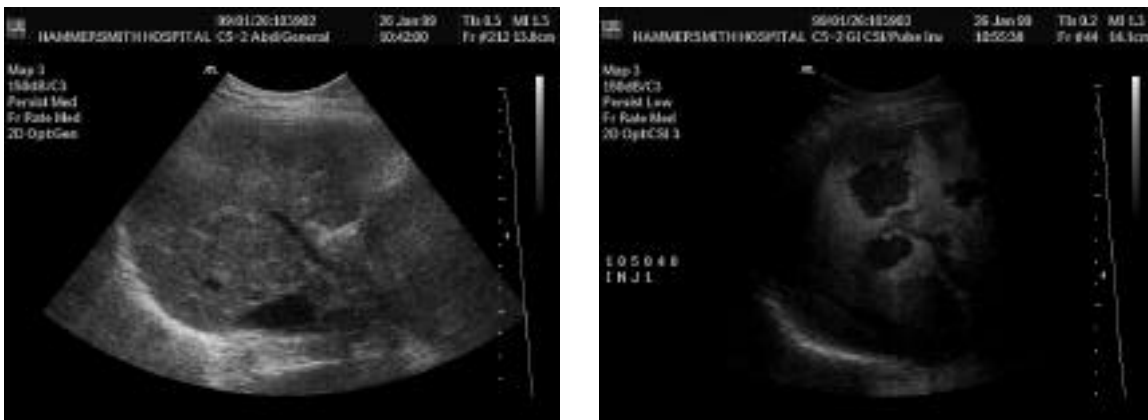


Fig 1. The left image shows a conventional view and the right a pulse inversion image acquired several minutes after Levovist injection in a subject with liver metastases. The lesions (seen as defects) are much better shown with the late phase pulse inversion study.

A multicentre study, supported by ATL Phillips, has proven the utility of this approach in a multicentre setting [4].

Air based agents, notably Levovist, seem to require relatively high acoustic powers to display good enhancement using this approach. Some non-air based microbubbles, notably Sonazoid, will produce good harmonic emissions even at lower acoustic powers. This means that liver phase imaging is possible with low MI settings, with minimal microbubble disruption. This means that sustained enhancement can be seen, greatly increasing the practicality of this mode of imaging. It might be possible to biopsy a lesion in real time for example.

Although highly appealing, these harmonic approaches have limitations. These include the fact that a conventional and microbubble specific image cannot be acquired simultaneously, that they cannot always be switched on and off rapidly and their dependence on particular ultrasound systems (The only commercial systems supporting pulse/phase inversion are the ATL HDI5000 and Siemens Elegra).

Loss of Correlation Methods

Another approach is to use microbubble disruption deliberately. One method is to scan in colour Doppler mode using high acoustic powers ($MI > 1$ and preferably at FDA maxima), and low to medium Doppler frequencies (about 2MHz.) With these settings, microbubbles are easily disrupted, especially less robust agents such as Levovist. The sudden disappearance of a reflector leads to a loss of correlation in successive ultrasound pulses, which is interpreted as a strong wide frequency Doppler signal by the ultrasound system. These are particularly well shown if “native” non-microbubble signals are suppressed using high pulse repetition frequencies settings and low to medium colour gain. Strong mosaic like signals can be seen from the normal liver and splenic parenchyma. This effect, which is at least theoretically platform independent (although marked differences in systems exist), has been dubbed somewhat inaccurately “stimulated acoustic emission”. [5]

This method has the advantages of providing simultaneous microbubble specific and conventional imaging (the colour Doppler data can be turned on and off a frozen image or captured cine loop) (Fig 2), the ability to “prescan” with low power settings to minimise microbubble disruption and extremely high sensitivity. It has major disadvantages however, including severe focal zone dependence, poorer spatial resolution than phase/pulse inversion and marked microbubble destruction [6]. Although the method has been shown to increase sensitivity in liver ultrasound [7], its main use will probably be in improving specificity. The ability to capture exactly registered images means that the distribution of a microbubble within and without a lesion can be carefully studied. A recent study has shown that a single acquisition 5 minutes after injection can improve specificity, as metastases and HCCs all showed as defects at this time while FNH and many haemangiomas show uptake. It may be that the presence of late phase enhancement is a strong marker of benignity [8].

The limitations of this approach have been addressed recently in a new LOC based approach, dubbed “Agent Detection Imaging” (ADI). This uses a LOC approach but provides an image with much less focal zone dependence and better space filling and spatial resolution. This can be used in a manner somewhat like pulse/phase inversion (with sweeps through the liver parenchyma) (Fig 3). Early experience suggests that this may be the optimal method for LOC imaging.

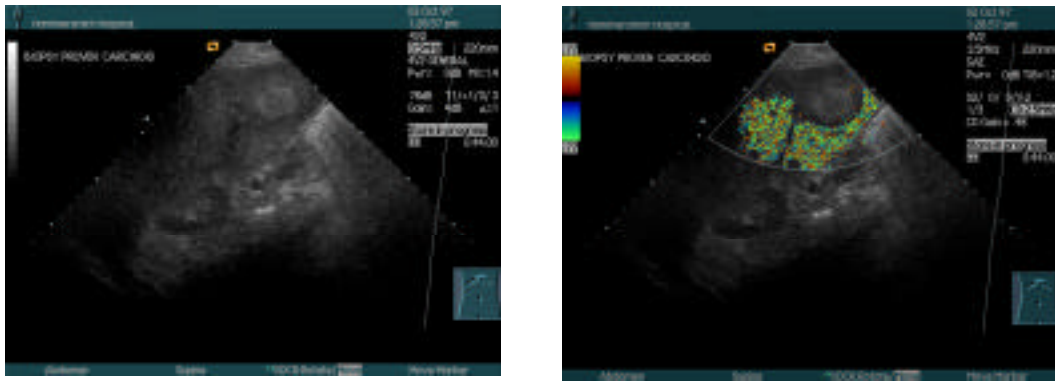


Fig 2. Conventional and “SAE” study of a subject with metastatic carcinoid. The metastases are shown as defects.

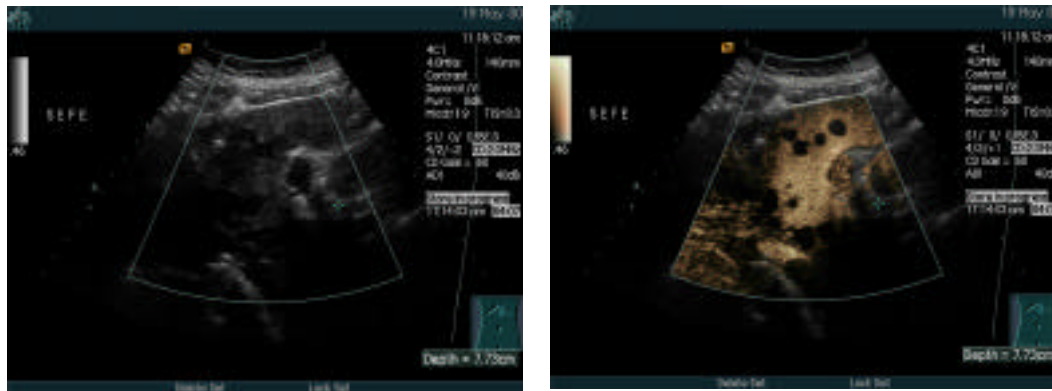


Fig 3. ADI study in a subject with metastatic melanoma

Current (somewhat simplified) impressions of the relative merits of different approaches:

| Technique | Improving sensitivity | Improving specificity |
|-----------------|-----------------------|-----------------------|
| “SAE” | ++ | +++ |
| ADI | +++ | ++++ |
| PI (high MI) | ++++ | ++ |
| PI (low MI) | | |
| Air-based agent | + | + |
| Non-air agent | +++ | ++ |
| Second harmonic | + | + |

Harmonic modes

| Advantages | Disadvantages |
|---|---|
| <ul style="list-style-type: none"> • Potentially less destructive • Good spatial resolution • Good space filling • May show “rolled edge”effect around metastases, a helpful aid in diagnosis | <ul style="list-style-type: none"> • Do not allow a conventional image to be acquired simultaneously • Hard to “prescan” • Platform dependent (for pulse/phase inversion*) |

*Currently available only on ATL and Siemens systems.

LOC

| Advantages | Disadvantages |
|--|--|
| <ul style="list-style-type: none"> • Allows simultaneous capture of two images • Extremely sensitive • Allows low MI prescan • Platform independent* | <ul style="list-style-type: none"> • Highly destructive • Highly focal zone dependent* • Poorer spatial resolution* |

*Not true for ADI, which offers much better space filling and spatial resolution but is only available on the Acuson platform.

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LIVER METASTASES, BUBBLES VERSUS CT: RESULTS OF A MULTICENTRE TRIAL

Thomas Albrecht¹, Martin J.K. Blomley, Peter Burns, Stephanie Wilson, Edward Leen, Michel Claudon, Fabrizio Calliada, Jean-Michel Correas, Michel LaFortune, Rodolfo Campani, David O. Cosgrove, Christian W Hoffmann, Chris Harvey Frederic LeFevre, Dario Pallavicini

¹Department of Radiology and Nuclear Medicine, Universitätsklinikum Benjamin Franklin, Freie Universität Berlin, Germany

Abstract

Background: Although non-invasive and inexpensive, ultrasonography remains second line to CT in the assessment of hepatic metastases. This study sought to determine whether assessment of hepatic metastases by ultrasonography is improved by the addition of pulse inversion imaging during the liver-specific phase of a microbubble contrast agent.

Methods: 128 patients were studied with conventional ultrasonography, pulse inversion harmonic contrast ultrasonography in the liver-specific late phase of Levovist and dual-phase spiral-CT. The conspicuity and number of metastases on the conventional sonogram and the contrast-enhanced pulse inversion sonogram were compared using CT as the reference. In a sub-group of 23 patients MR imaging, intraoperative ultrasonography or resection pathology were available as independent reference for comparison of pulse inversion ultrasonography and spiral-CT.

Results: Conspicuity of metastases was improved by contrast-enhanced pulse inversion in 87% by a mean of 10.8 dB ($p < 0.0001$). In 47 patients more metastases were seen than on conventional ultrasonography, in 3 of which the conventional scan was normal. Using CT as the reference, contrast-enhanced pulse inversion improved the sensitivity for the detection of individual metastases from 71% to 88% ($p < 0.0001$). On a patient basis sensitivity improved from 94% to 98% (NS) and specificity from 59% to 88% ($p < 0.01$). In the 23 patients with independent references, 7 patients showed more confirmed metastases on contrast-enhanced ultrasonography than on CT.

Conclusion: Contrast-enhanced pulse inversion ultrasonography markedly improved the sensitivity and specificity in detecting hepatic metastases. It revealed metastases that were not detected by spiral-CT in selected cases. It could complement or replace CT for assessment of hepatic metastases.

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LIVER IMAGING: TO BREAK OR NOT TO BREAK THE MICROBUBBLES?

Carlo Del Favero, **Alberto Martegani**, Luca Aiani, Daniela Bokor *
Como, Italy, * Bracco

Introduction

Until now the vast majority of the studies of the Focal Liver Lesions (FLLs) with contrast-enhanced ultrasound (CE-US) were based on bubble-destruction methods during insonation.

This approach allowed the direct visualisation of liver parenchyma and FLL microcirculation, and according to recent published clinical experiences, not fully sustained by biological evidences, the distribution of the ultrasound contrast agent used in those experiences in the liver's reticulo-endothelial system.

According to our experience this approach (i.e. bubble destruction) has the following limitations:

1. The inhomogeneity of liver enhancement, related to focus, at different depth
2. A completely different US scan method with "moving" or "flash" techniques for the visualisation of the liver microcirculation, with the drawback of reduced significant echo images
3. The difficulty, or in some cases the impossibility, to perform US scans during the liver arterial phase, fundamental for the correct characterisation of the FLLs, and moreover, due to the speed of the arterial phase duration, the difficulty to obtain an adequate liver coverage
4. The poor reproducibility of the method across patients with different acoustic windows, especially for in depth and trans-costal scans
5. The study of the spleen (around 7% of patients bearing GI tumours have spleen mets) is very difficult, if not impossible, because an accurate equipment setting is needed due to the particular acoustic window, this means massive bubble destruction without significant results.
6. Last but not least the reduced time available for the whole liver examination during all the phases (arterial, portal and late) and the complexity of the whole procedure

Even if recently some interesting results have been published^{1,2,3} is not surprising that the radiology world has not been impressed by these results, probably because of the above mentioned reasons, also considering the very good results and the potential new application of the well known and established imaging modalities such as MRI and CT.

Based on what we have mentioned before, the ideal CE-US examination of the liver should have the following characteristics:

1. Imaging the Ultrasound Contrast Agent (USCA) without breaking the bubbles
2. Allowing a true real time imaging

3. Allowing a panoramic US examination of the liver (with the same limitations of conventional B-mode technique)
4. Allowing the visualisation of the macrovasculature and microvasculature during the same examination
5. Allowing perfusion studies and reperfusion studies after planned bubble destruction
6. Allowing the visualisation of the entire liver haemodynamics (arterial, portal and late phases) in the same US examination i.e. the only limitation should be the USCA half-life. In practice the SCA signal should be detected at least up to three minutes after injection.

In other word, the ideal CE-US examination of the FLLs should allow their detection, characterisation and vascular activity.

The experience we report is coming from a clinical trial with SonoVue[®], still on going, aimed to assess the accuracy of CEUS, with a technique preserving the USCA in the detection and characterisation of FLLs in comparison with pre and post contrast enhanced MRI as reference method.

Materials and Methods

The contrast agent used is BR-1 (SonoVue[®] Bracco S.p.A. – Milan, Italy), an aqueous suspension of SF₆ (Sulphur Hexafluoride) microbubbles with a phospholipidic shell. The mean bubble diameter of SonoVue[®] is 2.5 μ, which allows transpulmonary and sinusoidal passage, with a bubble concentration of 10⁸ – 5·10⁸ bubble/ml. SonoVue[®] does not diffuse into the extravascular compartment but remain within the blood vessels until the gas dissolves and is eliminated in the expired air.

The bubbles of SonoVue[™] Demonstrated

- to be robust enough to be proof against the mechanical effect of US waves and consequently to be detected during all the liver phases (arterial, portal and late)
- to have non-linear properties (harmonics)
- to have a wide-band harmonic response allowing superficial and in depth detection of the harmonic signal

We used, in our study three different SonoVue[®] doses, 2.4, 4.8 and 7.2 ml in rapid bolus injection followed by a saline flush (3 – 5 ml).

In our settings we observed a resonance frequency of BR-1 between 1.5 and 3 MHz, with a second harmonic between 3 and 6 MHz.

A wider range of resonance frequencies cannot be excluded and, in our opinion, is related to the US equipment used; this phenomenon could have important practical aspects.

The US equipment used is an Acuson Sequoia 512 (Acuson, Mountain View CA, USA) equipped with Harmonic Imaging (HI) and Coherent Contrast Imaging (CCI[®]) systems. CCI[®] is a particular scan algorithm aimed to allow the signal cancellation from the stationary tissues and the detection of the non-linear signal coming only from the USCA.

The cancellation of stationary tissues signal is obtained by the transmission of two inverted pulses on adjacent lines.

It has to be clarified that the results we obtained depend not only from the CCI but also from the method we identified as the best technical choice.

In fact using CCI, the fundamental signal (and the tissue harmonic signal) in the US images is still relevant when Mechanical Index (MI) higher than 0.3 are used and that the fundamental signal is added, and not totally subtracted, to the signal from SonoVue.

We observed that a true subtraction effect can be obtained only with lower MI (0.1 – 0.2), and we called this technique “Very Low Mechanical Index” (VLMI).

This technique is based on signal amplitude subtraction (and not only frequency subtraction), and it is possible thanks to the combination of two different component: the harmonic signal coming from SonoVue and the dynamic threshold of low amplitude signals which suppress the low amplitude signals returning to the transducer.

We already enrolled 84 patients with suspected FLL on baseline US examination. Only 21 patients underwent CE-US with VLMI technique. In this subset of patients a bolus of 2.4 ml of SonoVue was administered and only when the enhancement obtained was not satisfactory (especially in the far field) we increased the dose to 4.8 or 7.2 ml (about 33% of the patients). The experimental protocol allowed also additional SonoVue bolus

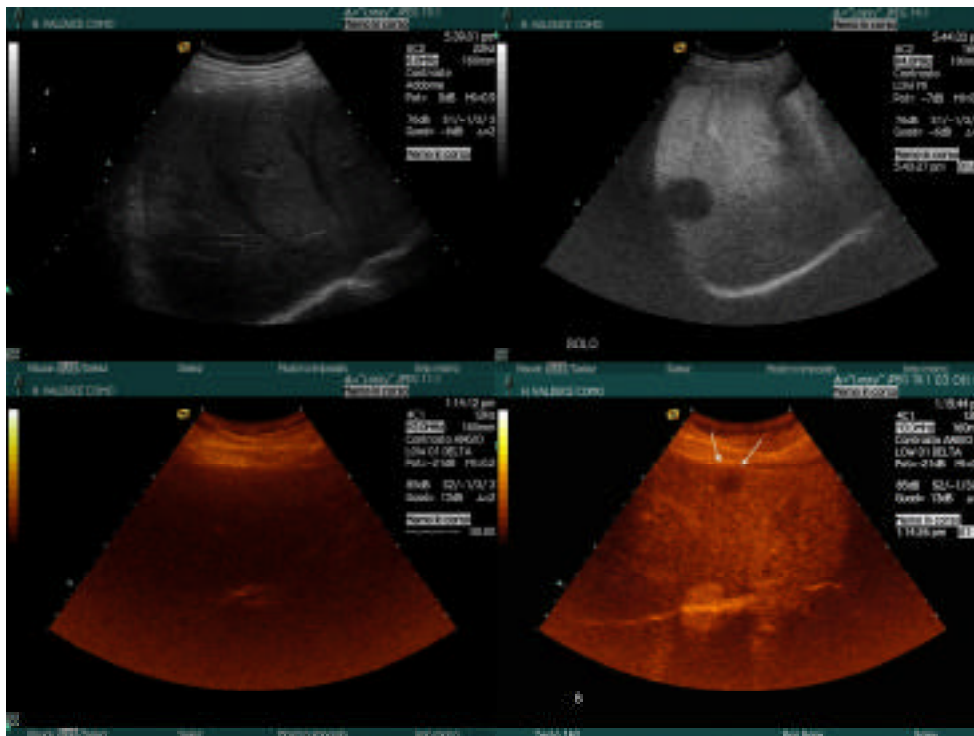


Fig 1a – Baseline US of the right lobe of the liver with CCI™. Metastatic FLL. **1b** – After SonoVue™ injection the fundamental tissue signal is still evident. **1c**–Baseline US with VLMI (Very Low Mechanical Index). This approach allows the visualisation of anatomical structures such as diaphragm, large vessels and all the interfaces with high difference in acoustic impedance. The signal coming from the parenchyma is almost absent, but is very evident the signal coming from the thermal noise. The image quality is poor. **1d**- After SonoVue™ injection

injections to study the suspected primitive tumour organs (pancreas, breast and nodes) and to study the haemodynamics of particular FLL.

The scanning technique was free and the scanning delay was to be evaluated directly on the screen on the basis of the contrast appearance in the arteries of the hepatic hilum. We scanned the right and left lobe of the liver alternatively; to preserve the synchronism of vascular phenomena, with a moving technique and, thanks to the high frame rate of the VLMI technique (between 12 and 27 Frame/sec. depending on line density), the scans can be obtained very rapidly.

All the significant images obtained were digitally stored during scans.

MR examinations were performed using a 1.5 Tesla machine (GE Signa Horizon) within 3 days after CE-US study. The following protocol was used: GE T1w, FSE T2w pre contrast and GE T1w post contrast scans in arterial, portal and late phase were acquired.

Results

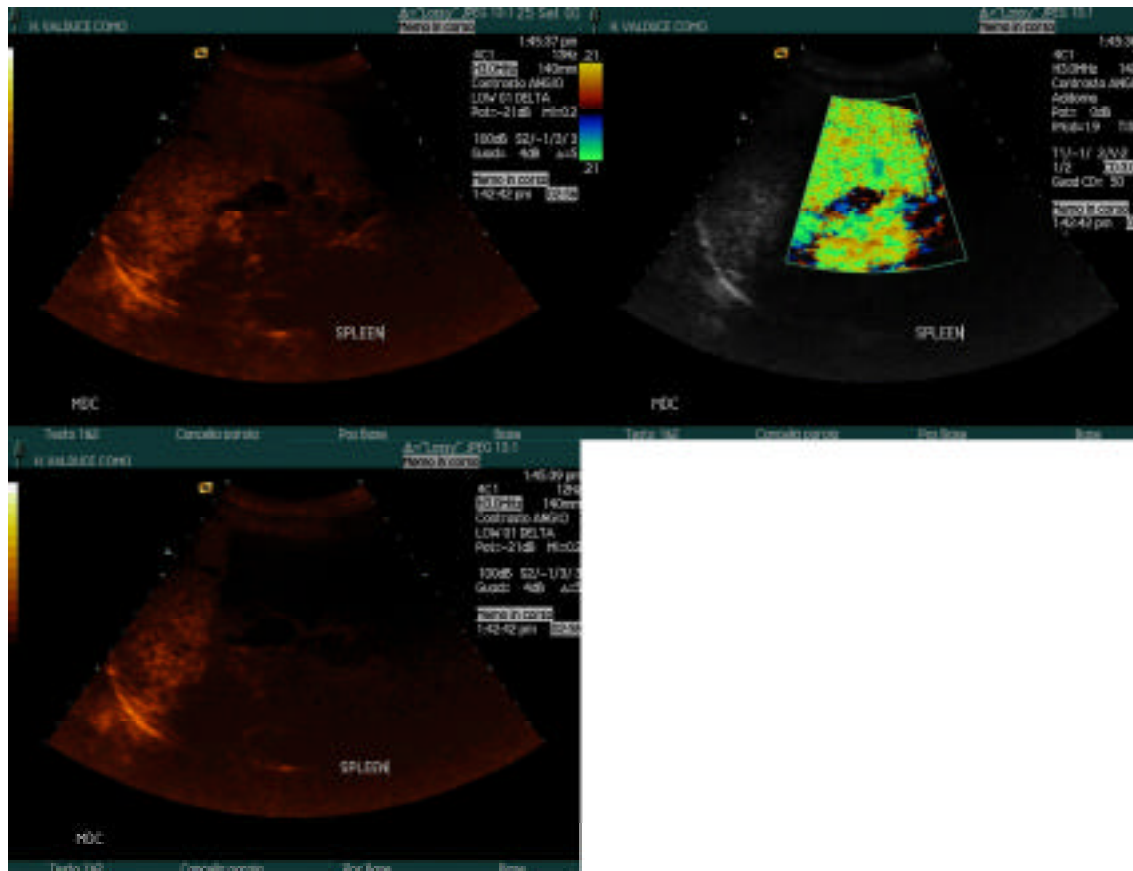


Fig 2a- Baseline US images with VLMI of the spleen. The parenchyma is enhanced due to SonoVue™ effect. **2b-** US images of the spleen parenchyma. Massive bubble destruction after SonoVue™ injection due to high power colour Doppler box (Stimulated Acoustic Emission) **2c** – US images of the spleen with VLMI obtained immediately after bubble destruction. Part of the spleen parenchyma is almost without signal detectable and the rest is still enhanced (no bubble destruction).

The harmonic image obtained on pre-contrast with VLMI technique allowed the visualisation of the high amplitude signals only, such as diaphragm, large vessels and all the interfaces with high difference in acoustic impedance. The signal coming from the parenchyma was almost absent, but was very evident the signal coming from the thermal noise.

On pre contrast images, in fact, the receiver amplitude threshold is automatically set at a relatively low level and this setting is not able to filter the thermal noise artefact. Using this VLMI technique the USCA is detected by the system as an independent signal generator with amplitude, thanks to the second harmonic signal, much higher than the parenchyma signal. The final effect is a dramatic increase in signal amplitude.

The post-contrast images obtained with VLMI reflected the described dynamic situation, with a substantial disappearance of the artefacts caused by the thermal noise, and with good visualisation of BR-1 distribution within liver parenchyma. The resolution of this VLMI technique was proportional to the BR-1 concentration within the micro and macro circulation and especially to the insonation frequency; moreover the penetration of the US beam seemed to be comparable with a much higher MI (around 5-fold increase).

Furthermore the enhancement obtained was scarcely dependent on beam focus and is homogeneous both in the near than in the far field.

Last but not least, and this is the most interesting aspect from a physiopathological point of view, no bubble destruction was seen and this allowed the continuous scanning of the liver, with a truly real time technique. The scanning technique can be a moving technique or stationary in a particular region of interest, allowing the visualisation of the liver macro and microcirculation only, thanks to the blood-pool characteristics of SonoVue . The VLMI approach with SonoVue allowed, without artefacts, the visualisation of the major liver vessels (arteries, portal branches, sovrahepatic veins) and the visualisation of the FLLs vascularisation with a sensitivity and spatial resolution much higher than Color or Power Doppler. Moreover the FLLs microcirculation and the hepatic sinusoid too, was easily detected without any bubble breakage such as “flash echo” techniques or intermittent imaging.

The appearance of arterial enhancement depends on the patient’s haemodynamics characteristics and on the FLL characteristics, but thanks to the fact that it was possible to visualise directly the USCA arrival in the hepatic artery, this allowed a “customisation” the start of each single examination. Generally speaking the arterial enhancement appeared between 12 and 25 seconds after USCA injection, with a little delay in cirrhotic patients. The portal enhancement started surprisingly early and is seen between 20 and 30 seconds after SonoVue injection.

It was possible to see the hepatic sinusoidal enhancement even earlier than expected and inhomogeneously, probably due to the arterial feeding. The hepatic sinusoidal system appears to be inhomogeneously enhanced after 40 – 60 seconds after SonoVue

administration with a plateau that lasts for 1 – 3 minutes depending on the administered dose.

SonoVue is a blood-pool agent and for this reason is not possible to talk about a “post-vascular” phase, also because a contrast effect in heart chambers is seen even after the contrast is disappeared from liver parenchyma.

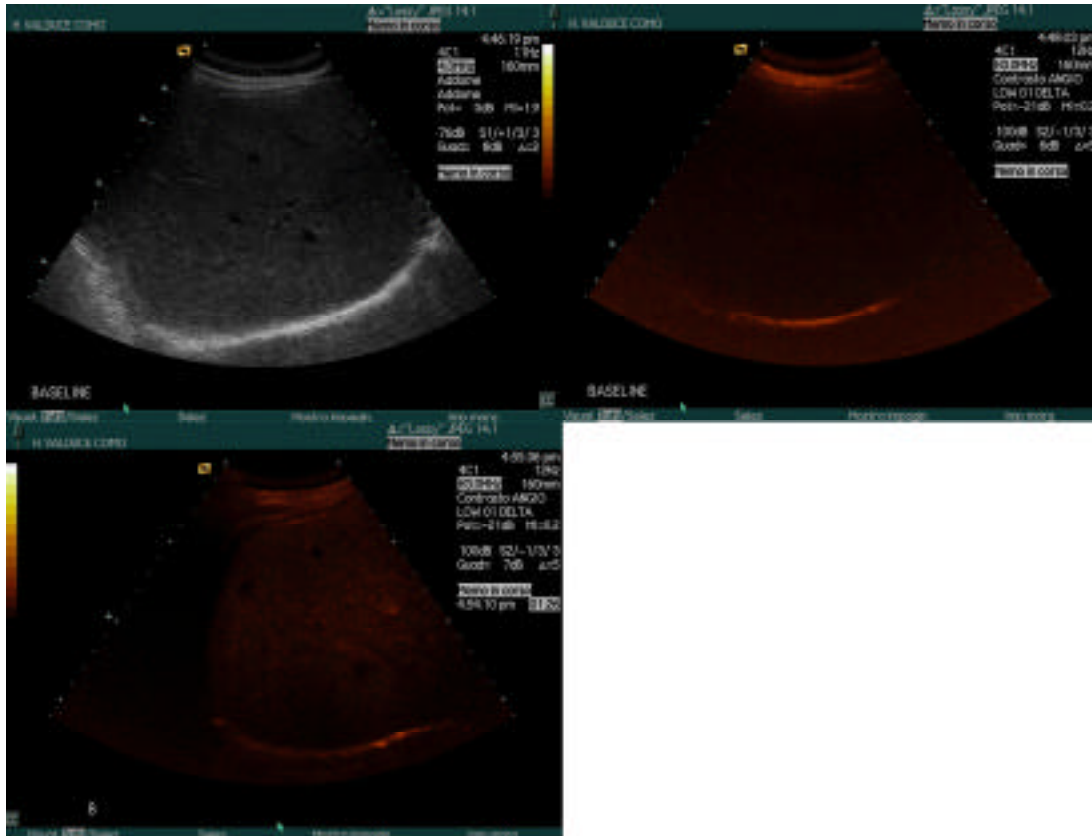


Fig 3a – Standard B-Mode baseline US scan of the right lobe of the liver. Detection of FLLs is difficult. **3b** – Baseline US images with VLMI: no visualisation of liver parenchyma or FLLs. **3c** – VLMI US images obtained during portal phase after SonoVue™ injection. FLLs can be easily detected.

The table below summarises the different vascular phases of the dynamic imaging with SonoVue .

| Vascular Phase | Arterial Phase | Early Portal Phase | Sinusoidal Phase |
|----------------|---|---|--|
| Imaging timing | 10- and 25 sec. after SonoVue injection | between 30 and 60 sec. after SonoVue injection; | Between 60 sec and 3 min. after SonoVue injection. |

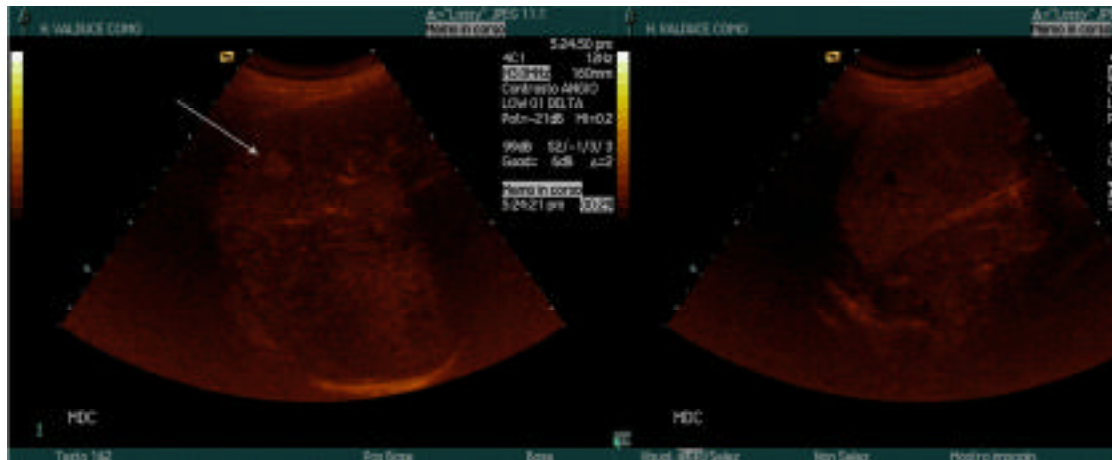


Fig 4a VLMI US images obtained during arterial phase (29 sec. after SonoVue™ injection): the FLL enhances homogeneously (Hypervascular met). 4b VLMI US images obtained during portal phase (179 sec. after SonoVue™ injection) The lesion appears hypoechoic vs. surrounding parenchyma.

We have also performed arterial reperfusion studies with bubble destruction using a flash of Colour Doppler with high MI. This technique must be done from 60' to 3 min. after SonoVue injection to obtain a visible reperfusion effect in the portal phase. This technique must be done within 3 min. after SonoVue injection to obtain a visible reperfusion effect, but if it's performed during portal phase the lesion conspicuity is less evident because the USCA reaches equilibrium between hepatic artery and portal vein.

Conclusions

The study is ongoing and is not possible to anticipate the results of the comparison between CE-US and MRI; but is possible for us to show some cases obtained with VLMI and to compare them with our previous images obtained with a bubble destruction technique.

The rationale is to support the theoretical basis of the VLMI and to demonstrate the potentiality of this new approach in the detection and characterisation of FLLs. What in our opinion is most interesting is that with VLMI technique coupled SonoVue can open a new “window” in the study of the microcirculation and neoangiogenesis of tumours.

¹ A. Martegani et al.: Contrast-enhanced US Pulse Inversion Harmonic grey scale imaging in diagnosis of liver masses (72 cases) Scientific Exhibit –RSNA 1999-Chicago

² Harvey et al.: Pulse Inversion Mode Imaging of liver specific microbubbles. Improved detection of subcentimeters metastases. Lancet Vol. 355• March 4, 2000

³ Wilson S. et al: Harmonic Hepatic Ultrasound with microbubbles contrast agent. Initial experience showing improved characterisation of hemangiomas, hepatocellular carcinoma and metastasis. Radiology 2000 215 155-161

FOCAL NODULAR HYPERPLASIA: CONFIRMATORY DIAGNOSIS WITH LEVOVIST?

Marcus Dill-Macky, Korosh Khalili, Peter N. Burns, Stephanie R. Wilson
University of Toronto, Toronto Canada

Introduction

Focal nodular hyperplasia (FNH) is the second most common benign liver mass after hemangioma. These masses are believed to be developmental hyperplastic lesions related to an area of congenital vascular malformation, likely a pre-existing arterial spiderlike malformation. Hormonal influences may be factors, as focal nodular hyperplasia is more common in women than in men, particularly in the child bearing years. Like hemangioma, FNH is invariably an incidentally detected liver mass in an asymptomatic patient.

FNH is typically well circumscribed and most often a solitary mass that has a central scar. The majority of lesions are less than 5 cm in diameter. Although usually single, cases have been reported with multiple FNH. Microscopically, lesions include normal hepatocytes, Kupffer cells, biliary ducts and the components of the portal triads, although no normal portal venous structures are found. As a hyperplastic rather than a neoplastic lesion, there is proliferation of normal, non-neoplastic hepatocytes that are abnormally arranged. Bile ducts and thick-walled arterial vessels are prominent particularly in the central fibrous scar. The excellent blood supply makes hemorrhage, necrosis and calcification rare. These lesions often produce a contour abnormality to the surface of the liver or may displace the normal blood vessels within the parenchyma.

On sonography, FNH is often a subtle liver mass which is difficult to differentiate in echogenicity from the adjacent liver parenchyma. Considering the similarities in histology of FNH to normal liver, this is not a surprising fact and has led to descriptions of FNH on all imaging as a “stealth lesion” which may be extremely subtle or hide altogether. Subtle contour abnormalities and displacement of vascular structures should immediately raise the possibility of FNH. The central scar is seen on gray scale sonograms as a hypoechoic linear or stellate area within the central portion of the mass. On rare, occasion, the scar may appear hyperechoic.

Doppler features of FNH are highly suggestive in that well-developed peripheral and central blood vessels are commonly seen. Pathologic studies describe an anomalous arterial blood vessel in FNH larger than expected for that locale in the liver. Our experience suggests that this feeding vessel is usually quite obvious on color Doppler imaging although other vascular masses may appear to have unusually large feeding vessels as well. The blood vessels can be seen to course within the central scar with either a linear or stellate configuration. Doppler interrogation usually shows predominantly arterial signals centrally.

Differential diagnosis of FNH includes other hypervascular neoplasms especially those with a central scar. Most frequently problematic is the differentiation from hepatic adenoma, hepatocellular carcinoma, and fibrolamellar carcinoma.

Confirmatory imaging includes sulphur colloid scintigraphy, MR and CT scan. On CT and MR scans, these lesions and their scars have recognized appearances on unenhanced and enhanced images characteristically showing a hypervascular mass in the arterial phase. Nonetheless, there is lack of confirmation of this diagnosis in many patients. Therefore, *sulfur colloid scan* is invaluable in patients with suspect FNH as 50% of lesions will take up sulphur colloid similar to the adjacent normal liver and a further 10% of lesions will be hot. This test has a very high specificity for the diagnosis of FNH and only 40% of patients with FNH will lack confirmation of their diagnosis after performing a sulfur colloid scan.

In the situation that conventional imaging does not confirm the presence of FNH, biopsy may be undertaken. Cytologic aspiration is not adequate as normal hepatocytes may be found in normal liver, adenoma, and FNH. Core biopsy is required to show the disorganized pattern characteristic of this pathology. Because FNH rarely leads to clinical problems and does not undergo malignant transformation, conservative management is recommended.

Role of Microbubble Contrast Agents in the Evaluation of FNH

As FNH is a known hypervascular liver lesion, it is ideal for evaluation with microbubble contrast agents and pulse inversion imaging. In virtually all of the patients that we have studied with this diagnosis we have observed consistent and unique features allowing for a firm confirmation of the diagnosis. We have routinely imaged patients in three phases as follows:

The vascular phase – consisting of continuous imaging during and immediately following a bolus injection of contrast agent. This component of the exam may be performed with either a perfluorocarbon agent or with Levovist. A reasonably high MI is required for Levovist vascular imaging. Perfluorocarbon agents allow for imaging with a lower MI which will show greater sensitivity for the detection of smaller intralesional vessels. Lesional vascularity is typically quite profuse and often shows a characteristic stellate or spokedwheel configuration. Selection of a scanning plane which affords visualization of the porta hepatis and the lesion will also frequently show a tortuous and large feeding artery, an observation which is neither sensitive or specific for this diagnosis.

At approximately the peak of arterial enhancement during the vascular phase, we then perform an *interval delay technique* with suspension of the acoustic output while the transducer is maintained over the lesion. Reinsonation, following an 8 to 10 second delay, will disrupt the bubbles accumulated during the delay producing a bright flash with enhancement of the normal liver. FNH is typically enhancing at least equal to and frequently in excess of the normal adjacent liver reflecting its profuse vascular supply. The central scar is most often nonenhancing on the interval delay flash.

Postvascular delayed imaging is dependent on the liver specific action of Levovist. Performed about 4 minutes following a separate injection of contrast agent, the liver is scanned in a continuous sweep to include the FNH. A high MI technique will disrupt all of the microbubbles persistent in the field following clearance of the agent from the vascular pool. The normal liver will enhance and FNH also uniformly enhances with this technique. The scar does not enhance. This postvascular phase enhancement of FNH is unique to this pathology. We hypothesize that the similarity of histologic characteristics of FNH to normal liver accounts for its participation in the liver-specific phase of enhancement. We believe that inclusion of this phase of imaging should become the definitive test to confirm the presence of FNH.

Conclusion

Our experience with microbubble contrast agents and pulse inversion imaging suggests that this highly vascular mass lends itself optimally to this imaging technique. Furthermore, we believe that Levovist should be the agent of choice when FNH is suspect, as the postvascular liver specific phase provides a definitive confirmation for this diagnosis. In our experience FNH is uniformly enhancing on the postvascular scans, often showing as well a central and nonenhancing scar. As FNH is the only tumor on which we have shown this enhancement, we believe that the specificity for Levovist enhanced postvascular delayed scans far exceeds the recognized specificity of sulfur colloid liver spleen scans

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HEMANGIOMA - A CHALLENGE FOR MICROBUBBLES AND ULTRASOUND IMAGING TECHNIQUES

Stephanie R. Wilson, Korosh Khalili, Marcus Dill-Macky, Peter N. Burns
University of Toronto

Introduction

Cavernous hemangiomas are the most common benign tumors of the liver, occurring in approximately 4% of the population. They occur in all age groups but are more common in adults. The vast majority of hemangiomas are small, asymptomatic, and discovered incidentally. Large lesions may occur and very rarely produce symptoms of acute abdominal pain caused by hemorrhage or thrombosis within the tumor. Once hemangiomas are identified in the adult population, they uncommonly change in size although their morphology may change over time. Isolated reports also document that hemangiomas may grow and that previous assumptions suggesting they never change in size are in error.

On histology, hemangiomas consist of multiple vascular channels that are lined by a single layer of endothelium, separated and supported by fibrous septae. The vascular spaces may contain thrombi.

On sonography, hemangiomas are recognized to have varied appearances. The *classic morphology* is that of a small, well-defined, homogeneous and hyperechoic mass. Inconsistently seen, increased through transmission has been correlated with hypervascularity on angiography. *Atypical features* of hemangioma on gray scale ultrasound are also now familiar and include: a nonhomogeneous central area containing hypoechoic portions which may appear uniformly granular or lacelike in character; an echogenic border, either a thin rim or a thick rind; and a tendency to scalloping of the margin. Larger lesions tend to be heterogeneous with central hypoechoic foci corresponding to fibrous collagen scars or thrombosis. In a fatty infiltrated liver, a hemangioma may appear as a hypoechoic mass within the echogenic parenchyma. *Doppler features of hemangiomas* reflect the very slow blood flow of these lesions. Therefore, conventional color and duplex Doppler rarely demonstrate signals within these lesions. Occasional lesions, however, may show quite marked vascularity which always raises the possibility of more significant pathology.

Confirmatory Imaging

Cavernous hemangiomas are commonly observed on abdominal sonograms performed for any reason and confirmation of all visualized lesions has proven to be costly and unnecessary. Therefore, it is considered acceptable practice to manage some patients conservatively without confirmation of their diagnosis. When a hyperechoic lesion, typical of a cavernous hemangioma, is incidentally discovered in a patient with no risk for either metastases from the gastrointestinal tract or for hepatocellular carcinoma, no further examination is usually necessary. There are significant lesions, however, which may mimic the morphology of a hemangioma on ultrasound and produce a single or

multiple masses of uniform increased echogenicity. Metastases from a gastrointestinal primary and neuroendocrine tumors, in particular, are recognized as mimickers of hemangioma. In other situations, hemangiomas may have an appearance which is nonspecific on sonography, not allowing a reliable prediction as to the nature of the mass. In these situations or others, where confirmation of the diagnosis is required, the following may be performed:

Technetium-99m labelled *red blood cell scintigraphy* using single photon emission CT (SPECT) has achieved a positive predictive value and specificity of almost 100% in evaluating hemangiomas. The classic appearance is decreased activity on early dynamic images with increased activity on delayed blood pool scanning.

Computed tomography and magnetic resonance imaging both have characteristic appearances for the diagnosis of hemangioma including peripheral enhancing nodules in the arterial phase, progressive centripetal enhancement, and persistent homogeneous enhancement on contrast enhanced scans. MRI, in particular, is more accurate than SPECT in diagnosing hemangiomas less than 2 cm and those adjacent to the heart and major intrahepatic vessels.

Role of Microbubble Contrast Agents in the Evaluation of Hemangioma

Hemangiomas are not characterized by profuse arterial flow. Their characteristic slow flow and slow fill in provides a challenge for satisfactory imaging on sonography with microbubble contrast agents. Our early evaluations of hemangiomas were performed with perfluorocarbon agents and conventional Doppler and harmonic imaging. Both imaging techniques failed to show significant vascularity within most hemangiomas when scanning was performed during or immediately after a bolus injection. The addition of pulse inversion imaging provided much improved sensitivity for the detection of the signal from the microbubble agents and allowed us for the first time to reliably detect the blood flow within a hemangioma. Further technical advances which have allowed for continuous low MI imaging have further improved this situation such that we now routinely see the early arterial filling of many hemangiomas.

The well recognized features of hemangioma described on CT and MR scan, peripheral nodular enhancement with centripetal fill in of the lesion, require an interval delay technique with a special modification to allow for the detection of slowly accumulating microbubbles within the circulation of the lesion. Our current technique for imaging of hemangiomas is as follows:

The vascular phase – consisting of continuous imaging during and immediately following a bolus injection of contrast agent. This component of the exam may be performed with either a perfluorocarbon agent or with Levovist. A reasonably high MI is required for Levovist vascular imaging. Perfluorocarbon agents allow for imaging with a lower MI with subsequent greater sensitivity for the detection of smaller intralesional vessels. The vascularity in the majority of hemangiomas is typically marginal, often appearing in pools or globules, in an asymmetric distribution, around the lesion edge. Some movement of the transducer may help in the detection of this marginal vascularity

as the globules may be few in number or located in only a single location. In the minority of lesions, particularly those which are very small, there may be rapid and diffuse arterial flow right to the center of a lesion.

Recognizing that the flow to the center of a hemangioma is usually very slow, we have attempted to improve our technical capability in terms of performing lower and lower MI studies which should allow for real time evaluation of the slow flow through the circulation of the hemangioma. To date, we still have not successfully shown this flow in realtime. In spite of this situation, we have modified our interval delay technique to allow for the demonstration of microbubble accumulation throughout the lesion over time. At approximately the peak of arterial enhancement during the vascular phase, we perform an *interval delay technique* with suspension of the acoustic output while the transducer is maintained over the lesion. Reinsonation, following an 8 to 10 second delay, will disrupt the bubbles accumulated during the delay producing a bright flash with enhancement of the normal liver. The response of the hemangiomas is highly variable in the arterial phase although most frequently we see a pattern of peripheral nodular enhancement which is highly analogous to the familiar appearance recognized in the arterial phase on CT scan. This nodular enhancement is usually of at least an equal intensity to the adjacent liver but is more often more intense. To show the centripetal progression of this peripheral nodular enhancement, we then perform increasing interval delays to allow a longer time for the accumulation of the microbubbles throughout the lesion. Typically, we do delays of 10 seconds, 30 seconds, 60 seconds and 120 seconds from the peak of arterial enhancement. With each reinsonation, the accumulated microbubbles will disrupt often enhancing more and more of the lesion, extending towards the lesion center. Characteristically, the entire lesion will not enhance. Sizeable regions of the mass may remain hypoechoic and unenhanced. These same areas which do not enhance on the interval delay sonograms typically do not fill in on dynamic CT scan.

Postvascular delayed imaging is dependent on the liver specific action of Levovist. Performed about 4 minutes following a separate injection of contrast agent, the liver is scanned in a continuous sweep to include the FNH. A high MI technique will disrupt all of the microbubbles persistent in the field following clearance of the agent from the vascular pool. The normal liver will enhance. Hemangiomas will not accumulate the contrast agent in the postvascular phase and do not enhance.

Both the vascular and the interval delay sequences for hemangioma are highly suggestive of this diagnosis. *Differential diagnosis* includes metastatic disease where marginal flow with marginal enhancement following interval delay is also recognized. The pattern of the marginal flow in a metastasis is usually different not appearing in such an asymmetric and globular distribution. Furthermore, metastases do not show centripetal progression of the enhancement towards the lesion center on interval delay.

Conclusion

Hemangiomas show characteristic vascular and interval delay appearances which should allow for their noninvasive confirmation without the necessity of referral for either MRI or CT scan. A meticulous technique is required for their confirmation and should

include modification of the interval delay sequences if the arterial phase interval delay image shows a pattern suggestive of peripheral nodular enhancement.

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HEPATOCELLULAR CARCINOMAS

Edward Leen

Royal Infirmary, University of Glasgow, Scotland UK

Introduction

HCC is the most common primary malignancy of the liver in the world with an annual incidence of approximately 0.2-0.8% in the industrialised world and 5.5-20% in high risk areas such as the sub-Saharan Africa, Southeast Asia, Japan, Greece and Italy. It is extremely lethal with over 90% overall mortality and a mean survival of only 6 months (median survival without treatment has been reported to be as low as 1.6 months). The disease is advanced at the time of initial presentation. It afflicts males more frequently than female with a ratio of 2.5 to 1 in the industrialised world and 5 to 1 in the high incidence areas. In the western world, it usually arises in previously damaged livers, most commonly in patients with alcoholic cirrhosis, hepatitis B & C or haemochromatosis. It is solitary in 50% of cases and is multi-focal or diffuse in the remaining cases. In contrast in the Far East, it may arise de novo or through steps of progressive atypia from adenomatoid hyperplasia to early and then advanced HCC.

Fibrolamellar HCC is an uncommon type of HCC with slow growth and better prognosis; it usually occurs in younger female patients, without any underlying cirrhosis or risk factors.

Unenhanced Sonographic Appearance

HCCs may be hypo- (26%) or hyper-echoic (13%) or of mixed echogenicity (61%) depending on the size of the tumour, the fat content, degree of differentiation, scarring or necrosis. They may either have irregular margins with/without the halo sign or have well defined smooth echogenic rim corresponding to presence of pseudo-capsule; the pseudo-capsule is not uncommonly seen especially in early or well differentiated tumours. Sometimes calcifications may be present. A characteristic feature of HCC is presence of tumour extension into biliary tree, portal (25-40%) or hepatic veins (16%). Venous invasion is commonly seen in large advanced tumours.

Fibrolamellar HCCs are generally large solitary partially or completely encapsulated tumours with heterogeneous echogenicity. An echogenic central scar is a feature, which may have radiating appearance. Central stellate / trabecular calcifications are more frequent in this type of HCCs.

On colour or power Doppler ultrasound, HCCs are commonly seen as hyper-vascular lesions and it is believed that the vascularity is dependent upon the degree of differentiation. Enlarged feeding tortuous vessels are demonstrated around and infiltrating the lesions giving rise to a "basket-like" appearance. Tumour thrombus can be clearly depicted and differentiation between tumour venous invasion from blood clot may sometimes be made on colour/power or spectral Doppler modes.

Spectral Doppler ultrasound waveforms of the feeding tumoral vessels is variable but usually demonstrate high resistive / pulsatility indices, high systolic velocities or presence of arterio-venous tumour shunting. Arterio-venous shunts are characterised by the high arterial diastolic flow, pulsatile venous flow as well as retrograde portal venous flow.

Contrast Enhanced Sonographic Findings: Vascular Phases

Contrast agents used for liver imaging can be broadly categorised into blood pool agents such as Optison (Mallinckrodt), Sonovue (Bracco) and Definity (Dupont) or agents with delayed liver specific phase such as Levovist, Sonovist (Schering AG) and Sonazoid (Nycomed Amersham). In clinical phase trials, all these agents have been shown to improve the demonstration of the HCC tumoral hyper-vascularity / vascular morphology on colour/power Doppler modes, during the vascular phase of the agent i.e. during the first 2-3 minutes following contrast administration.

Overt HCCs derive their blood supply predominantly from the hepatic arterial blood supply. In contrast, only 25% of the total liver blood flow to the normal liver parenchyma is derived from hepatic artery, with the remaining 75% via the portal venous blood flow. Therefore the arterial phase (15-30 seconds of the contrast agent administration is the optimum period to assess HCCs' vascular morphology over that of the surrounding normal liver parenchyma. On colour/power Doppler modes, chaotic peri-tumoral and intra-lesional vessels can be clearly depicted. Enhancement of the tortuous feeding tumour vessels is particularly early (10-15 seconds) in the presence of arterio-venous shunts. Necrotic areas are seen as an/hypo-echoic flow voids. Spectral Doppler waveforms from the tumour vessels can be easily obtained.

On these colour/power Doppler modes, the capillary enhancement (20-35 seconds after start of contrast injection) of the tumour or normal liver parenchyma is not be seen during continuous scanning. However with intermittent / flash / interval delay imaging, enough contrast is allowed to perfuse the capillaries in the plane of the scan which can then be demonstrated in the first frame as a result of stimulated acoustic emission or loss of correlation at high MI.

During the portal venous phase (30-90 seconds after start of contrast injection), the contrast enhancement in the tumour vessels is gradually diminished relative to that of the surrounding normal liver parenchymal vessels which is the at its peak.

Pulse Inversion Harmonic Imaging: Vascular Phases.

Similar findings are demonstrated during these three vascular phases described above, except in gray scale with improved resolution. Ideally scanning should be performed at low mechanical index to minimise microbubbles destruction. Vascular lakes, dense tumour stain, coarse neo-vascularity are seen in well-differentiated HCCs. Characteristic tortuous / corkscrew like vessels, with vascular encasement and fine neo-vascularity are demonstrated in anaplastic HCC. These easily stand out during the whole of the vascular phase (including portal phase, because of the prolonged tail end of the bolus injection of contrast). Hyper-vascular HCCs light up in a background of dark liver in the arterial phase and during the portal phase they may be as bright or still brighter than the

surrounding liver parenchyma. Continuous scanning at very low MI enables the demonstration of the differential capillary perfusion between HCC and normal liver with some agents.

On intermittent / time interval delay imaging, the hyper-vascularity of HCCs are frequently demonstrated as the lesions appear brighter than or as bright as the normal liver during the arterial and portal phases respectively. The lesions always participate in the veil of microbubbles destruction at high MI.

Late Phase Imaging: Liver Specific Agents

Late phase imaging can be performed with any of the liver specific agents from 4-5 minutes after the start of the contrast injection. It is believed that these liver specific agents are taken up by the Kupffer cells of the reticulo-endothelial system of normal liver and tumours such as HCCs which are devoid of Kupffer cells therefore do not show any contrast enhancement in the late phase. Indeed in our own experience, over 90% of the HCCs will show no appreciable contrast (Levovist and Sonazoid) uptake. On pulse inversion harmonic imaging these HCCs appear hypo-echoic in a background of bright liver. A hyper-echoic rim enhancement replacing the halo sign is often noted. On fundamental colour or power Doppler modes at high mechanical index, HCCs appears as colour free areas. The fundamental modes are very sensitive in depicting presence of contrast and are more commonly associated with artefacts, which may be confusing and limiting in assessing true degree of contrast uptake. There are some limitations in using Levovist in the late phase imaging:

- (a) Contrast uptake in the liver parenchyma may be non-uniform in presence of cirrhosis.
- (b) Vascular phase imaging is best kept separate from late phase imaging, therefore requiring 2 separate contrast injections.
- (c) Single sweep technique is difficult to adopt in routine practice, which may require repeated contrast administration and further prolonging the examination.

In contrast, recent studies suggest that Sonazoid has none of these disadvantages.

HILAR BILIARY OBSTRUCTION: THE UTILITY OF LEVOVIST DELAYED SONOGRAPHY

Stephanie R. Wilson, Korosh Khalili
University of Toronto

Hilar Cholangiocarcinoma (Klatskin Tumor)

Cholangiocarcinoma is a malignant hepatic tumor of the biliary epithelium and represents less than 1% of all newly diagnosed cancers in North America. Most of these tumors are adenocarcinomas, some of which produce mucin. They may be ductal or peripheral depending on their site of origin. Tumors that arise at the convergence of the right and left hepatic ducts are known as hilar cholangiocarcinomas (Klatskin tumors) and account for approximately 25 % of the total. These tumors may be nodular, infiltrating, or papillary. Predisposing conditions for the development of cholangiocarcinoma include primary sclerosing cholangitis, choledochal cyst, and pyogenic cholangitis. Tumor complicating PSC is particularly challenging as ductal dilatation and thickening of the biliary ducts are common to both.

Klatskin tumors are frequently advanced at the time of diagnosis and complete resection may be impossible. The most important observations that may contraindicate surgical resection of hilar cholangiocarcinoma are extensive and bilateral spread through the intrahepatic ducts, involvement of the portal vein, vascular involvement on one side of the liver with extensive contralateral bile duct involvement, and hepatic or peritoneal metastasis.

As jaundice is the most common presenting symptom in ductal cholangiocarcinoma, most affected patients initially undergo ultrasonography. Although early reports denounce the ability of ultrasound in terms of evaluating the cause and the stage of malignant biliary obstruction, more recent reports suggest that ultrasound can compete very favorably with other cross sectional modalities. On sonography, hepatic lobar atrophy with marked biliary dilatation and crowding of bile ducts is classic for the diagnosis of Klatskin tumor. This observation on any cross sectional imaging modality suggests a hilar tumor with dominant involvement of the duct that supplies the atrophic segment. However, the isoechoic nature of a Klatskin tumor, and its propensity to grow in an infiltrative, periductal pattern makes its detection and the determination of its extent quite difficult. Failure of union of the dilated segmental ducts is the strongest inference of this pathology. In this situation, the location of the tumor is inferred based on the level of ductal obstruction and irregularity of the dilated ductal margins, while the actual borders of the tumor are not visualized. Therefore, CT and MR scan are frequently performed to confirm ultrasound findings and to stage disease.

Delayed Phase Levovist Imaging

Levovist, an air based microbubble ultrasound contrast agent, has been shown to persist in the normal hepatic parenchyma after a brief vascular phase. This liver specific post vascular phase of enhancement has been utilized for improved visualization of hepatic

malignancies. Several studies have shown increased conspicuity of individual lesions allowing for detection of more and smaller lesions as compared to baseline. We have applied the principles of postvascular imaging with Levovist to investigate hilar biliary obstruction with equally promising result – showing consistent delineation of the tumor mass and improving the prediction as to the extent of the disease. The addition of Levovist enhanced postvascular scans has tremendously improved our performance for both the detection and the staging of Klatskin tumor. Identification of the infrequent patient who has hilar biliary obstruction on the basis of benign disease, most commonly primary sclerosing cholangitis, has also been facilitated on the Levovist enhanced scans.

Ultrasound Technique – Postvascular Levovist

All scans are performed as continuous high MI sweeps through the porta hepatis. Considerable reduction of the gain setting is required for the enhanced sweeps in anticipation of the increased brightness of the image on the basis of the liver enhancement. Sagittal and subcostal oblique views are standard with an additional view to focus on the pathology if necessary. Baseline and contrast enhanced sweeps are performed with an identical transducer placement and angulation to reproduce as closely as possible the baseline image on the contrast enhanced views. All sweeps are stored as a cine loop to allow for a frame by frame analysis. A separate injection is required for each enhanced sweep. Following the examination, single frames at the same location are viewed together to facilitate the interpretation of the contrast enhanced scan.

We mix Levovist in a concentration of 300 mg/ml. Following an intravenous injection of a bolus of 4.5 ml, a 4.5 minute delay is observed. Maintaining the exact same scanning plane as used for the baseline is requisite if the images are to be compared. Alternately, some of the currently available equipment allows for the removal of the contrast agent component of the image leaving only the fundamental greyscale background. This greatly facilitates the interpretation. A direct comparison of the baseline with the contrast enhanced images is required on a frame by frame basis to avoid over calling tumor. The portal vein, in particular, may still show some circulating microbubbles and can be mistaken for tumor by the unaware.

The tumor which is within the ducts and the tumor extent into the liver, in particular, are more conspicuous as the non enhancing tumor will appear virtually black against the enhanced liver background. These scans show masses which are often not seen on the baseline and invariably show a greater extent of the tumor.

In patients with benign biliary obstruction, the findings are variable. A benign stricture may show nothing on the scan at the site of the obstruction. Conversely, in patients with primary sclerosing cholangitis, we have seen extensive ductal thickening which may often involve all of the intrahepatic ducts. The differentiation of this appearance from that of cholangiocarcinoma may be difficult although lack of liver involvement, smooth and continuous involvement of many ducts, and the absence of any vascular invasion are all supportive of a benign etiology.

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STRATEGIES TO DIFFERENTIATE FOCAL LIVER LESIONS

HP Weskott

Dept of Internal Medicine

Klinikum Hannover, Siloah Krankenhaus, Hannover, Germany

In a clinical workup, we gain diagnostic value using contrast agents primarily with patients who have tumor diseases. The most important aspects to be evaluated are: viable tissue (infarction, tumor necrosis after ablation therapy, tumor detection (Late Phase Imaging, Albrecht,1) and tumor characterization (early and late phase imaging, intermittent or flash imaging, etc).

To date, the only contrast agent which is approved for radiology in most European and Asian countries is SH U 508A (Levovist). Optison is approved for cardiac indications and can only be used off label with the patient's consent. An increasing number of contrast agents with different acoustic properties are still waiting for approval. Thus, the ultrasound manufacturers are providing new contrast imaging modalities.

There are presently a number of imaging modalities that can be used in conjunction with contrast agents (CA): Color Doppler Flow Imaging modes (fundamental /2nd harmonic Color Doppler and Power Doppler Imaging modes); and more recently the introduction of Non-Doppler based techniques (Pulse or Phase Inversion Imaging and Coded Harmonic Imaging Modes).

With all of these modalities, two goals can be achieved:

- (1) Blood flow enhancement (better visualization of vessels)
- (2) Blood pool imaging by exploiting SAE

While SAE is mainly used for tumor detection in the late phase, vessel imaging contributes to tumor characterization. Since SAE is dependent upon the magnitude of the MI number (should exceed 1.0), there seems to be a trade off between vessel and blood pool imaging. In CFI Modes, only the harmonic settings have the capability to decrease the amount of SAE in vessel imaging. Color blooming, and thus overestimation of vessel size is still a problem.

Contrast enhanced US increases the diagnostic confidence of an US examination. All technical data aside, it is most important to know what characterizes a tumor when looking at its architecture, the vessels, and the entire blood pool of the tumor in comparison to normal liver tissue.

From a practical aspect, it would be best to have a contrast agent that ensures a strong enhancement for vessel imaging as well as for SAE. From a technical aspect, an imaging modality is needed that produces few artifacts, and has a high spatial, time and contrast resolution. Non Doppler based gray scale imaging techniques are about to achieve this goal by using lower MI numbers (in our studies between 0.6 and 0.8). When the MI

number is even lower, SAE is not sufficient enough to be diagnostically useful in focal liver disease.

In comparison to other imaging modalities ultrasound has many advantages in tumor characterization:

- CA volume: A smaller CA volume is needed in US when compared to the volume of contrast media used for CT (1% -Optison - to 10% -Levovist). Therefore, the time resolution for changes in CA concentration in the early arterial phase is higher.
- In contrast to CT protocols, the time interval between the administration of the bolus and scan begin can be chosen freely.
- US is fast in creating numerous images in one scan plane, so that one can follow the change in CA concentration very closely.
- Single, small vessels can be imaged – with images similar to angiography (Non Doppler techniques like Coded Harmonic Angio)

The most important clinical question to be answered is whether a benign lesion can be differentiated from a malignant one.

Before this question can be answered, certain diagnostic criteria based on histopathological, pathophysiological and angiographic findings need to be defined for each tumor.

The examination technique should consider these criteria and merge them together with the information CA enhanced US can give us. This should be the background of any examination strategy based on the use of contrast agents.

In order to obtain as much information as possible during one contrast examination, we follow a specific protocol introduced in Fig. 1. It takes into account that all phases (arterial, portal-venous and postvascular) contribute in characterizing the tissue.

The arterial arrival of the first portion of CA should be watched very closely (continuous scanning). If arteries are detected, continuous scanning is stopped, and the next period of 30secs to 60secs is replaced by intermittent scanning with time intervals between 1-4secs. In the cases where no single vessels or no major enhancement is detected, the time interval between two frames can be longer. If the clinical question has been answered, the last part of the exam, and/or a second bolus can be used for tumor detection in other liver areas or 3-D imaging of the tumors vessels. The protocol can always be adapted relative to the findings during the exam. In highly vascularized tumors, the intermittent scanning can be performed much earlier. In tumors with a low arterial supply, the intermittent phase can be performed later than the protocol suggests.

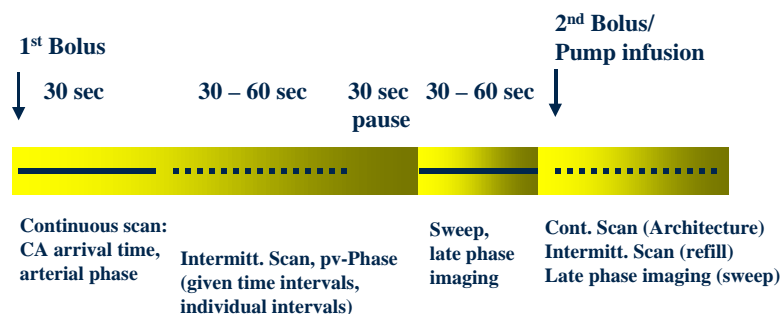


Fig. 1 protocol for us contrast examination of focal liver lesions with ca levovist.

The most common benign lesions of the liver are cysts, calcifications, hemangiomas and focal nodular hyperplasias (FNH).

Along with vessel malformation which can already be seen with CDI, certain tumors have a cystic appearance, and show no flow information in CDI. In these cases, the use of CA can definitely help in confirming the diagnosis.

With ultrasound, 70% of hepatic hemangiomas have a typical appearance, and 30% have an atypical echogenicity and echo-pattern. In comparison to other imaging modalities, US is very sensitive in detecting especially small hemangiomas below 2cm. If a typical hemangioma is detected by chance in a healthy subject, a further diagnostic work up is not needed (Leifer, 2). All suspected hemangiomas in patients with a history of malignant disease have to be proven with MRI or Nuclear Medicine methods.

A non-enhanced color flow examination is not helpful in hemangiomas. Perkins (3) reported that neither CDI nor PDI can add any diagnostic value in cavernous hemangiomas.

There is no typical vessel architecture in hemangiomas that can be detected by contrast enhanced US; with the exception of some large veins in larger hemangiomas around the periphery of the lesion (“cotton wool appearance”, Johnson,4).

A continuous flow of CA from the periphery to the center during late phase is a characteristic sign for a hemangioma in contrast enhanced US (Weskott,5).

In order not to misinterpret atypical hemangiomas, US contrast studies should therefore consider these unique vascular situations for a further diagnostic work up. All areas in a hemangioma show a marked enhancement during late phase except the areas which are thrombosed.

So far there is no explanation why some hemangiomas have an echo-poor appearance. These hemangiomas are often difficult to interpret. An arterial filling, or quick filling, and the proof of arterio-portal shunts (Naganuma,6) can raise doubts about the

characterization of the hemangioma. Therefore, Wachsberg (7) recommends other imaging modalities before puncturing the lesion.

Our laboratory examined 11 echo-poor hemangiomas ranging from 8mm to 3.2cm in size; all of these hemangiomas showed an early CA filling during the first 60 secs, and all were refilled during a time interval of up to 5 secs. This finding may explain the hypoechoic echotexture. It remains unclear whether arterial supply or arterio-portal shunts reported by Naganuma are responsible for the echo-poor appearance of hemangiomas.

In all tumors, it is therefore absolutely essential to watch CA during all phases. Late phase enhancement is highly suggestive, if not a definitive diagnosis of an hemangioma.

The typical vascular architecture of an FNH is a feeding artery and the so called “spoke and wheel” sign. The RI of the corkscrew-like supplying artery is quite low ($0,51 \pm 0,08$ compared to liver artery: $0,65 \pm 0,06$) of the same patient (Uggowitz, 8). The specificity of imaging techniques regarding FNH is limited. In only 71% of the cases could blood tests, patient history, different imaging modalities and liver biopsy differentiate between FNH and liver adenoma (De Carlis, 9).

With regards to CA kinetics, a quick CA filling of the tumor is seen, and a central scar is spared from enhancement. During the first 15 to 20secs, the FNH is completely filled; while the liver parenchyma shows no enhancement at this time. In the intermittent scanning mode, this behavior can be reproduced. At late phase, an enhancement may still be there (when using Levovist), since FNH consists Kupfer cells. Small FNH below 2-3cm may not show a typical vascular architecture. In this cases, an early and complete CA filling during arterial phase can be seen, but a late phase enhancement may be missed. In this situation, an overlap with malignant lesions may cause a differential diagnostic problem.

Malignant lesions are in 70-80% supplied by arteries and liver parenchyma in only 20-30%. Contrast agents have been mostly if not completely washed out at the postvascular phase (this being the principle of tumor detection). Depending on the arterial blood volume that is supplying the tumor, early enhancement may largely differ. Refill images during the portal-venous phase predominantly shows enhancement in the area of neovascularization in the periphery of the tumor. Some hyperperfused metastases may be difficult to differentiate from primary liver cancer.

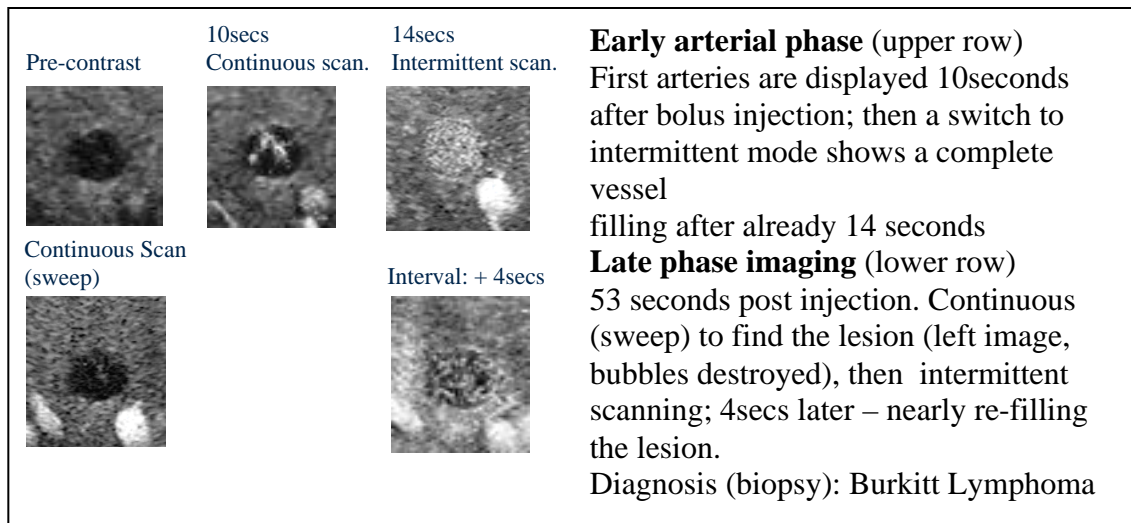


Fig. 2 contrast study in 35 year old male with a non Hodgkin lymphoma. Very early arrival time, and a dense pattern of tiny vessels seen with intermittent scanning may be typical for liver lesions in NHL.

With gray scale techniques like Coded Harmonic Angio contrast enhanced 3-D vessel imaging -with only minor SAE- may add clinical value in demonstrating the local vascular situation (Fig. 3).

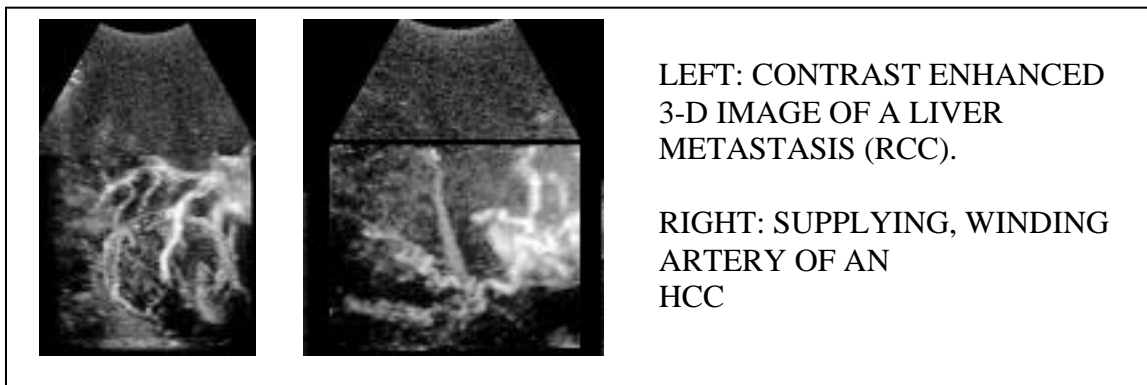


Fig. 3 3-D images demonstrate the vasculature of different tumors with only little SAE

It should be mentioned that US-CA studies are limited like all other imaging modalities and clinical tests. There are overlaps between different tumor entities, regarding their vascular architecture or CA kinetics. This will be a source of doubt in differentiating tumors, no matter how sophisticated examination protocols are and how well trained the examiner may be.

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ORGAN IMAGING WITH CONTRAST: THE CHALLENGES.

David O. Cosgrove

Hammersmith Hospital, London, UK.

Changes in Tissue Vascularity and Flow

Most non-invasive imaging confines itself to large vessels but there are two main areas where demonstrating tissue flow are clinically important: states of reduced flow in ischaemic or infarcted tissue and, states of increased flow or vascularity in hypervascular congested tissue or those where vascularization is abnormal, the most important of which is in malignant tumors.

Documenting and if possible quantifying reduction in flow is especially important in cardiology where ischaemic disease is a major concern.¹ As in most other vascular diagnoses, angiography remains the reference standard but is, of course, invasive and carries some risk and so cannot be repeated frequently. Nuclear medicine techniques have long been established methods for cardiac ischaemia but are expensive and so an ultrasound alternative would be attractive.

In the general imaging field reduced flow is importantly associated with pathology in the central nervous system, in ischaemic bowel and peripheral vessel disease and in torsion of the testis and ovary. In most of these situations, Doppler offers some useful information, becoming a standard in testicular torsion for example, but in others, notably cerebrovascular disease and in the limbs, only the larger supply vessels can usually be examined this way. In reductions in tissue flow a major limitation is the usable sensitivity of Doppler systems: no signal does not mean no flow, merely indicating that any flow present is undetectable. This limits confidence in the diagnosis of ischaemic disorders.

Increased flow may be easier to demonstrate and is important in inflammation² as well as in the physiological changes of the menstrual cycle and in pregnancy. Even in these instances, however, the limits of Doppler in detecting signals from small vessels impairs diagnostic reliability. Neovascularisation is a special and important case where newly formed vessels are the key to pathology.³⁻⁷ Most important in malignancy, physiological neovascularisation also occurs in the corpus luteum, in healing and in growth. The characteristics of healthy and malignant neovascularisation seem to differ in several important respects that can be summed up by saying that malignant neovasculature has chaotic properties, both structural and functional, whereas both the normal system and the new vessels formed in the physiological processes are orderly. Malignant new vessels, which are stimulated to grow from normal stromal vessels by the angiogenesis factors that the tumor secretes, are more abundant than normal and have a disorderly patterns with an irregular course and branching arrangements as well as forming loops and shunts (the former being vessel branches that rejoin the parent vessel, the latter being vessels that join an artery directly to a vein without traversing a capillary bed). Despite their abundance, they perform poorly and so the tumor tissue is often underperfused such that ischaemic regions and necrosis occur while at the same time other tumor regions may be oversupplied with blood. In addition to these

anatomical disturbances, tumor vessels often exhibit functional deficiencies which may arise from their chaotic anatomy, shunts, for example causing rapid transit of blood through the lesion. There may be blind-ending vessels in which blood and contrast agents can pool. Deficiencies in the muscle layer of these vessels deprives the arteriolar sphincters of their ability to control flow and this lack of vasomotor tone leads to unregulated perfusion which allows flow to continue throughout the cardiac cycle. On the other hand, the endothelium is abnormally permeable and this allows large molecules to leak. One result of this is an increase in interstitial pressure that may in turn compress small supply vessels and lead to regional underperfusion.⁸

Overall, therefore, the vascularity of tumors is complex both in their architecture and function, and is inefficient. Whether these abnormalities are unique to malignancies is not entirely clear since in the new vessels that form in some chronic granulomatous diseases may share some of these characteristics. However, benign tumors do not seem to have the chaotic malignant patterns: not only are they more sparsely vascularised but their vessels, which usually are simply encased as the tumor grows, retain the simple architecture of normal vessels as well as their vasomotor control.

In imaging terms, malignant vessels are seen as tortuous, irregular vessels with disordered branching (trifurcations, for example, that are rare in normal vessels as often found in cancers) that penetrate the mass at an angle that is almost perpendicular to their surface. Loops and shunts may be detected and many of these features are more clearly demonstrated in three dimensional displays.^{9,10}

Why Do We Want to Visualize the Flow in Organ Tissues?

Visualizing tissue flow is most important in both ischaemic conditions and in tumors to improve diagnostic accuracy, to quantify changes and to aid with decisions on management and in providing a prognosis. In ischaemic heart disease perhaps the most important is assessing the severity of ischaemia and its response to stress but evaluation of the viability of underperfused muscle is critical to predicting the success of reperfusion interventions (coronary artery bypass or angioplasty). Not surprisingly, investigation of angina has become one of the commonest procedures world wide.

Visualizing the neovasculature associated with malignancies is important for differential diagnosis (benign versus malignant) and for assessing their invasive potential; for example, it has been shown that the vascular density of breast carcinomas is closely related to the likelihood of spread to regional lymph nodes⁷ and, in the prostate, relates to the Gleason grading which indicates the tumor's invasive potential.¹¹ Tumor neovasculature has proved to be very valuable in assessing the adequacy of interstitial therapy in the liver where small amounts of remaining viable tumor may be difficult to detect on B-mode ultrasound. In many centers the ablation procedure is monitored with Doppler. Tumor vascularity can be used to distinguish between scarring and recurrent tumor in the breast and elsewhere: in essence any vascular region in a scar must be biopsied.¹² Tumor vascularity is also used as a guide to response to chemotherapy where it may predict volume changes and allow earlier adjustment of regimen.¹³ This application is likely to increase as anti-angiogenesis drugs are introduced.¹⁴

How Can they be Visualized with Ultrasound?

Demonstrating flow in the microvasculature supplying soft tissue is a major challenge for ultrasound. The flow velocity in capillaries is only about 1 μ per second, which is slower than the motion of the tissue itself under cardiovascular and local muscular forces, including the pulsation of nearby arteries. If, in addition, the transducer is swept across the tissue, probe-induced movements compound the problem. Conventional B-mode and Doppler ultrasound are unable to visualize this circulation and conventional microbubble approaches seem unlikely to be able to disentangle such slow flow from this clutter artifact, but it may be possible to demonstrate the presence of microbubbles even in very small vessels by detecting unique microbubble signatures, for example harmonics on gray scale or transient destruction signals using color Doppler. This task may be aided by the very large volume of blood in capillaries compared to the larger vessels, despite their very slow flow.

Using the harmonic mode in experimental animals, flow has been detected within interlobar arteries in the kidney that are less than 40 μ in diameter.¹⁵ In fundamental mode, detection will probably be limited to small arteries and veins of perhaps twice this diameter. Nonetheless, detection of flow in such vessels pushes the frontiers well beyond that which is achievable without microbubbles. Newer non-linear modes such as dual and single pulse phase inversion for gray scale, and power pulse inversion for Doppler. Some of these methods are able to detect bubbles that are not moving (because they are trapped in the microcirculation or have been phagocytosed by the reticulo-endothelial system for example in the liver or spleen and this has proved useful in distinguishing the normal function of these organs as compared to the lack of uptake in malignancies and other focal diseases.¹⁶ This area is likely to expand as microbubbles targeted to specific tissues are designed.

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BREAST

David O. Cosgrove

Hammersmith Hospital, London, UK

With almost 60000 new cases per year world wide, breast cancer is the commonest malignancy in women world-wide and the single commonest cause of death in the 40-50 age group: two out of every 1000 women aged 50 will have breast cancer diagnosed every year.¹ This underlies the importance of breast screening programs for early detection. While mammography is of proven value for screening and the most widely used imaging method, ultrasound has taken its place in characterizing lesions and even, to a limited extent, in screening.^{2,3}

The importance of tumor angiogenesis and neovascularity as indicators for tumor growth, local invasion and distant spread was first shown in breast cancer, and is now widely recognized in many malignancies.^{4,5} Thus, the assessment of vascularity – and neovascularity in particular – should be helpful in the differential diagnosis and grading of tumors as well as in guiding therapy, especially since vessel invasion is a major route of spread and because the blood supply of tumors is being specifically targeted with some forms of treatment.⁶

For these reasons, tumor vascularity has been studied intensively using a variety of techniques. Special stains for endothelium and angiogenesis factors have made tumor vessel microscopy a specialized field in histopathology, while contrast-enhanced CT and MRI have largely replaced conventional X-ray angiography for characterization of tumors in vivo. In breast lesions MRI has become the most sensitive technique for the detection of multicentric and multifocal breast malignancy.⁷ The pathophysiologic basis for different MRI enhancement patterns is abnormal vascular permeability rather than vessel density and architecture.⁸ Because echo-enhancing agents behave as predominantly intravascular contrast media, they may have a special role in improving Doppler ultrasound in the assessment of the morphology of tumor vascularity.⁹

Doppler Investigations

Doppler ultrasound has proved to be helpful in imaging many of the features of malignant neovascularisation and the differential diagnosis of breast diseases was the first application.^{10,11} Spectral continuous wave and pulsed Doppler systems revealed high velocity flow in tumor feeding vessels and indicated that this was a useful discriminating feature for breast masses. The use of color Doppler, which enables the display of flow signals over wide areas, opened further possibilities in the assessment of tumor vascularity.^{12,13,14}

Early promising semiquantitative approaches in the interpretation of color Doppler measurements were followed by more quantitative assessments to segment and analyze Doppler signals.¹⁵ Color Doppler pixel density can be calculated to give a “perfusion index” that can discriminate benign from malignant breast masses.¹⁶ This was taken

further by the development automated quantification software – now commercially available ('CQ System', Kinetic Imaging, Liverpool, UK. <<http://www.kineticimaging.com/cq.htm>>) – and expanding this to the intensity of Doppler signals using power Doppler. Many ultrasound systems now offer inbuilt quantitative software that avoids the non-linearity problems inherent in offline quantification.

In breast diseases, a number of early Doppler studies quantified the peak systolic velocity and the resistance index. Good sensitivity was achieved (88%) but with rather poor specificity (57%) and, in practice, the methods were very tedious.^{17, 18 19} Perhaps surprisingly, in view of the consensus from other malignancies, the RI was found to be higher in breast cancers than in benign masses.²⁰

Echo-Enhanced Doppler – Anatomical Studies

The Doppler sensitivity of state-of-the-art scanners has improved so dramatically that small vessels with low flow can be depicted and this capability has been furthered by the use of microbubble echo-enhancing ("contrast") agents⁹ for intravenous use in the breast.²¹ By boosting the Doppler signal intensity by several-fold (a 25dB increase is typical), a more complete display of the vascularity is achieved..

Small studies have indicated that this improves the accuracy of ultrasound in differentiating malignant from benign breast masses.²¹⁻²⁴ In addition, ways to display the three dimensional architecture of the vasculature have been developed: while it is not yet clear that this results in a more accurate diagnosis, the displays are spectacular and it seems intuitive that displaying such a complex vasculature in a volume mode will be more revealing than reliance on tomograms, since neovasculature is intrinsically three dimensional.²⁵

The breast has been one of the most intensely studied areas, mostly using Levovist but also using InfoSon²⁶ and EchoGen²¹. In the InfoSon study, 16 patients were entered and modest color Doppler enhancement was demonstrated. The cancers tended to show more vessels than the benign solid lesions and the enhancement improved diagnostic accuracy from 50% to 69%, though it was not felt to be capable of demonstrating the small vessels associated with neovascularisation

In the careful study from Freiburg²⁷, 22 patients with equivocal diagnoses (suspicion on B-mode but negative color Doppler findings) were selected for Levovist-enhanced Doppler studies from 200 women with breast masses. Only 4 of the 14 biopsy-proven carcinomas were suspicious clinically while 12 were suspicious on sonography. Diagnostic confidence increased from 3 to 9 (on a scale of 1-10) after enhancement with Levovist and, on average, 3.6 tumor arteries were detected before enhancement in the carcinomas, increasing to 8.4 after Levovist; 7 tumors were stage T1. In the single patient with *in situ* cancer, 8 vessels were seen in the affected quadrant, which showed only diffuse parenchymal changes on B-mode. Only enhanced Doppler showed the 2 malignant foci of a cancer that was multicentric. Before contrast agent injection, the average blood flow velocity was 13.5 cm/s and this increased to 16.9 cm/s

after injection; the Velocity Sum, calculated by adding the peak systolic flow velocity measurements of all vessels in each tumor, increased from 52 cm/s to 135 cm/s after Levovist.

The benign pathologies were one case of gynecomastia, 2 fibroadenomas, one case with non-proliferative benign breast change, one with proliferative disease without atypia and 3 with benign diseases showing atypical proliferation. All were considered suspicious both on mammography and ultrasound. Diagnostic confidence increased to 9 and the average number of tumor arteries increased from 4.5 to 4.9 after injection of Levovist. The average flow velocity was unchanged at 13.7 cm/s after a dose of 300 mg/mL, but the Velocity Sum increased from 84 cm/s to 92 cm/s.

The study reported by Kedar et al. included 36 cases, of which 18 were cancers, and attempted quantification, albeit rather crudely.²⁴ Using a blinded observer review of video tapes, the increased number and complexity of the vasculature was scored subjectively: the cancers scored higher in both, though there was overlap between the benign and malignant groups. An intriguing finding was the description of apparent interconnections between adjacent vessels that the authors tentatively called shunts: these were seen after enhancement in every breast cancer but not in any of the benign lesions. This preliminary result requires further evaluation before it can be recommended as a useful diagnostic criterion.

A computer-based quantitative system for estimating both the morphological and dynamic features has been developed and applied it to 18 breast lesions using EchoGen: the same trend emerged and this was also found in a series of 20 patients using Levovist and a similar computerized quantification system.²¹

One particular diagnostic problem, the differentiation between scar ring and recurrent tumor, has been reported to be achievable with contrast enhancement: demonstration of vascular signals in a scar nodule after healing (say, 6 months post surgery) is a suspicious feature and warrants a directed biopsy.^{19, 28}

Functional Studies

Since they can be used as tracers in a manner that is analogous to radio-isotopes, microbubbles offer a new ultrasound approach to determining haemodynamic function. By injecting the dose as a bolus, the transit of the microbubbles through the lesion (or any region and even potentially a volume of interest) can be measured.²⁹ Functional indices can be extracted from these wash-in, wash-out or transit intensity curves and they seem to reveal new, unique information; preliminary studies suggest that some may improve diagnostic accuracy. The indices can also be presented as true functional images, in which a useful feature is displayed as a color-coded overlay on the corresponding gray-scale scan. (Fig. 3) This approach offers the possibility of highlighting local hemodynamic differences, such as may be expected in malignancies with their heterogeneous circulations.

Kedar et al. attempted to perform a dynamic analysis by subjectively timing the delay from the bolus injection to the moment of peak enhancement and to the point of return of the Doppler signal strength to baseline in breast masses.²⁴ An earlier peak enhancement was typical of the carcinomas (45 sec compared to 60 sec in the fibroadenomas), though this difference was not statistically significant. The enhancement persisted for longer in the cancers (>5 min) than in the fibroadenomas (3 min). These hemodynamic alterations are what would be expected from a knowledge of the patterns of neovascularisation, with early peaking because of shunts and vessels with a low resistance to flow and delayed washout because of wide, tortuous vessels.

Monitoring Response to Therapy

Studies investigating the potential of color Doppler measurements to monitor the effects of adjuvant chemotherapy, show that response to therapy not only coincides with a reduction in vascularity, but that altered Doppler signals often antedate changes in tumor size.³⁰⁻³¹ No study investigating whether this also applies to contrast enhanced studies in the breast have yet been reported.

Assessment of Regional Lymph Nodes

Several studies have investigated Doppler signals in lymph nodes. Results are controversial and the use of contrast agents does not seem to give any diagnostic advantage.³²⁻³⁴ In the prostate, the difficulties with access that beset conventional ultrasound are unlikely to be overcome with contrast agents.

In animal studies, microbubbles have been shown to detect sentinel nodes in a similar way to lymphoscintigraphy.³⁵ In this “lympho-echography” approach, microbubbles are injected intradermally around a primary tumor site and signal enhancement is detected along the draining lymphatics and within the “sentinel” lymph node. The immediacy with which ultrasound can be performed and used to guide biopsy make it a more attractive modality than nuclear medicine. Some obvious limitations include the presumption that conventional bubbles might be too large to cross into the lymphatics and licensing restrictions to non-intravascular use in humans.

Three dimensional Doppler Angiography

Overall only a few studies using true 3D Doppler have been reported and only one is evaluating morphologic criteria in a quantitative way.³⁶

Conclusion

Thought the results in general have been too indecisive to warrant recommendation as a useful clinical tool, the consistent results reported in the difficult decision of whether a mass in the vicinity of a scar following excision of a breast cancer represents local recurrence (vascular) or scar nodularity (avascular) seems to be emerging as a truly valuable application. Contrast enhanced Doppler (and probably gray-scale using non-linear techniques) seems to be approximately as effective as contrast-enhanced MRI but is more readily available and is better suited to guided biopsy of suspicious regions.

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MICROBUBBLE-ENHANCED SONOGRAPHY OF PROSTATE CANCER

Ethan J. Halpern

Jefferson Prostate Diagnostic Center
Thomas Jefferson University, Philadelphia, PA

Widespread application of the blood test for prostate specific antigen (PSA) has resulted in a large number of men referred for biopsy of the prostate. A rough estimate of the number of biopsies performed each year may be obtained from the number of new cases of prostate cancer diagnosed each year. The number of new cases of prostate cancer in the United States for the year 2000 is estimated at 180,400.¹ Since approximately one-third of patients subjected to biopsy are found to have cancer, the number of prostate biopsies performed annually in the United States must be over 500,000.

There is currently no definitive imaging study to detect prostate cancer. When imaged with conventional sonography, cancer of the prostate is classically described as a hypoechoic lesion,² but can also appear echogenic or isoechoic.³ Color Doppler and power Doppler have been proposed to supplement conventional gray scale imaging and increase the overall sensitivity of ultrasound. The diagnostic accuracy of conventional gray scale and Doppler ultrasound, however, is limited.⁴ A retrospective study at Thomas Jefferson University demonstrated that increased color flow with Doppler ultrasound correlates with both tumor stage and grade, as well as with the risk of recurrence after treatment.⁵ In spite of this correlation between color Doppler and disease prognosis in subjects with cancer, our prospective imaging data demonstrate that conventional gray scale and Doppler imaging are minimally superior to random chance in the detection of prostate cancer.⁶

There is strong pathologic evidence to suggest that the vascular supply to malignant prostate tissue differs from the vascular anatomy of normal prostate tissue. Studies of microvessel density within the prostate demonstrate a clear association of increased microvessel density with the presence of cancer,⁷ with metastases,⁸ with the stage of disease^{9,10,11} and disease-specific survival.^{12,13} Quantitative assessment of microvascular density may actually provide important data to guide therapeutic decisions.¹⁴ Based upon clinical experience with computed tomography and magnetic resonance imaging in other parts of the body (ie. kidney, liver), one might expect contrast enhanced imaging to be useful for the detection of prostate cancer. Unfortunately, contrast enhanced computed tomography has not proven useful for the detection of prostate cancer. Recent reports have suggested that dynamic magnetic resonance imaging following gadolinium administration may demonstrate different enhancement patterns for benign and malignant prostate tissue.¹⁵ Nonetheless, although magnetic resonance imaging has been used extensively to stage known prostate cancer, its utility has not been demonstrated for detection of prostate cancer.¹⁶

The poor performance of standard imaging studies for the detection of prostate cancer may be related, in part, to the growth pattern of prostate cancer. Primary neoplasms in most organs tend to grow as solitary, round masses. Prostate cancer presents with multiple foci of tumor in 80% of cases. Furthermore, most of the lesions are irregularly shaped rather than round. Many prostate lesions are oblong, and quite narrow in the short axis. Furthermore, these lesions are imbedded in an organ that is often heterogeneous because of inflammation, previous infection, or due to the process of benign prostatic hyperplasia.

Ultrasound contrast agents may be used to enhance visualization of the microvasculature associated with prostate cancer. These agents are gas-filled microbubbles that can increase the echogenicity of the intravascular space. Recently developed ultrasound contrast agents have intravascular residence times of several minutes, pass through the pulmonary circulation, and may be used for parenchymal organ enhancement.^{17,18} Some agents demonstrate only Doppler enhancement in the prostate, while others show both gray scale and Doppler enhancement. Studies of one such agent, Echogen (Sonus Pharmaceuticals; Bothell, Washington), suggest that enhanced color flow with contrast may be associated with the presence of prostate cancer, though gray scale enhancement was not demonstrated.^{19,20} A dose response relationship has been demonstrated for Doppler enhancement of the prostate with BR1 (Bracco SpA; Milan, Italy).²¹ A study of men with biopsy proven prostate cancer demonstrated that power Doppler enhancement with the contrast agent Levovist (Shering; Germany) is reduced after androgen therapy.²² A more recent study using power Doppler imaging of Levovist (Shering; Germany) suggested that contrast-enhanced imaging provided increased accuracy for the diagnosis of prostate malignancy.²³ These preliminary studies suggest that contrast agents may be useful in ultrasound evaluation of prostate vascularity for the presence of cancer.

In order to enhance tumor neovessels within the prostate, contrast agents must traverse into the capillary bed. If microbubbles are destroyed before they reach the neovasculature, however, enhancement of tumor vessels will not be observed. Conventional ultrasound systems generally deliver power levels which are sufficient to destroy contrast microbubbles before they can reach the neocirculation. A potential solution to this problem is the use of transient response imaging, also known as intermittent ultrasound imaging. Intermittent imaging has been shown to increase the enhancement provided by ultrasound contrast agents.^{24,25,26} The observed degree of enhancement with intermittent imaging is dependent upon flow rate, acoustic power output and the frequency of insonation.²⁷ With continuous gray scale ultrasound imaging, contrast agent within the imaging plane may be destroyed during the acquisition of each frame of the ultrasound image. Since a typical gray scale ultrasound image is refreshed at 30 frames per second, the available contrast agent for each new image frame is that amount which enters the imaging plane in 1/30th of a second. In this short time between frames, contrast may enter larger vessels, but will not generally reach the microcirculation. With intermittent imaging, the ultrasound beam is turned off for longer periods between each image frame. More contrast material may enter the imaging plane during this interscan period. Furthermore, the contrast material will have time to traverse further into the capillary bed. Thus, intermittent imaging demonstrates a quantitative

increase in contrast enhancement, as well as a qualitative difference in the enhancement pattern that may be related to contrast in smaller vessels. Adjusting the interscan delay may control the depth of penetration of contrast material into the capillary bed. Larger interscan delays will produce greater enhancement, and may permit more selective imaging of contrast within the distal portions of the capillary bed and within neovessels.

We recently reported phase II results for the intravenously injected ultrasound contrast agent Imagent (Alliance Pharmaceutical Corp.; San Diego, CA).²⁸ Our initial experience with 26 subjects suggested that intermittent imaging can provide enhanced visualization of the neovascularity associated with cancer of the prostate. Two cases in this series demonstrated enhancement of tumor foci that were invisible to conventional gray scale and Doppler imaging.

Harmonic imaging represents yet another recent advance in ultrasound imaging of contrast agents.^{29,30,31,32,33,34} When contrast agents are imaged with ultrasound, the reverberations created by microbubbles may resonate at frequencies which are different than the frequency of insonation. Various harmonics of the insonating frequency are produced by the contrast agent and can be imaged. Since ordinary tissue predominantly reflects ultrasound at the frequency of insonation, most harmonic signals will come from contrast material. Thus, harmonic imaging may be used to improve the imaging of contrast material, and reduce the background signal from surrounding structures.

We are presently investigating the combination of intermittent imaging with wideband harmonic imaging. Our initial experience on 60 subjects studied with Definity™ (DuPont Pharmaceuticals; Billerica, MA) suggests that the combination of intermittent imaging and wideband harmonic imaging results in improved signal-to-noise ratio for visualization of prostatic vasculature.³⁵ Sensitivity and specificity for detection of prostate cancer were computed with pathologic interpretation of the biopsy core specimens as the reference standard. For baseline imaging without contrast, sensitivity was 37.8% with a specificity of 82.7%. Contrast-enhanced imaging provided a sensitivity of 64.9% with a specificity of 79.6%. The difference in sensitivity at baseline and during contrast infusion was highly significant ($p = 0.004$), while the difference in specificity was not significant ($p = 0.239$). Of the 20 patients with cancer detected on biopsy, 11 were identified on baseline imaging (sensitivity = 55%) while 15 were identified with contrast-enhanced sonography (sensitivity = 75%). The maximum Gleason scores of those subjects whose cancers were detected with contrast-enhanced sonography included 1 subject with a Gleason score of 8, 7 subjects with a Gleason score of 7, 5 subjects with a Gleason score of 6 and 2 subjects with microfoci. Each of the 5 subjects whose PC was not detected by contrast-enhanced imaging had only a single positive biopsy core. Two of these subjects had microfoci, and 3 had foci of Gleason score 6, comprising 10%, 40% or 80% of the biopsy core.

Since microvascular density is correlated with Gleason score, one might expect that a larger number of high grade tumors would be detected with contrast-enhanced imaging. In fact, confidence ratings for the presence of malignancy during contrast infusion did demonstrate a significant correlation with Gleason score ($p = 0.04$). A higher percentage

of high grade lesions were detected during contrast infusion. Spearman's rho was computed as 0.15 for correlation between baseline confidence ratings and Gleason score ($p = 0.36$). Spearman's rho was computed as 0.33 for correlation between confidence ratings during contrast infusion and Gleason score ($p = 0.049$).

Although the detection of prostate cancer with contrast-enhanced sonography is improved relative to conventional imaging, contrast-enhanced imaging does not detect all cancers. Furthermore, many of the lesions that are detected are quite subtle. Future research efforts must concentrate on improvement of the signal-to-background ratio for detection of malignant lesions. In order to reduce microbubble destruction, the energy of the transmit pulse should be adjusted to the lowest possible level that still provides adequate image quality. When a specific region of the prostate is in question, improved enhancement may be obtained with bolus administration of contrast. Nonetheless, new imaging techniques will be necessary to reduce bubble destruction and improve contrast enhancement. Newer bubble agents that resonate at higher imaging frequencies may provide better signal since the prostate is generally evaluated at 6-7Mhz. Alternatively, harmonic imaging at lower frequencies or with subharmonics may be useful with current contrast agents.^{36,37} Time-intensity curves may provide an objective measure to demonstrate the more rapid enhancement of tumor relative to normal parenchyma. Three-dimensional presentation and other post-processing image enhancements may increase the conspicuity of cancers. In the final analysis, a simplified protocol will be needed with clear enhancement of malignant foci if contrast-enhanced sonography is to be generally applicable in screening for prostate cancer.

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CONTRAST ENHANCED TRANSCRANIAL DOPPLER

Roland Brassard

Montreal Neurological Institute and Hospital
McGill University

Transcranial doppler is gaining popularity and acceptance in a variety of clinical uses. The technique itself is relatively simple. It can be done using any standard US equipment on patients with a variety of neurological disorders. The most important physical limitation is crossing the bone barrier. Because of the thickness of the cranial vault, up to 30% of examinations are incomplete or impossible. The use of contrast increases the sensitivity and allows a complete or satisfactory exam in several of these cases and broadens the indications for TCD.

Indications For TCD

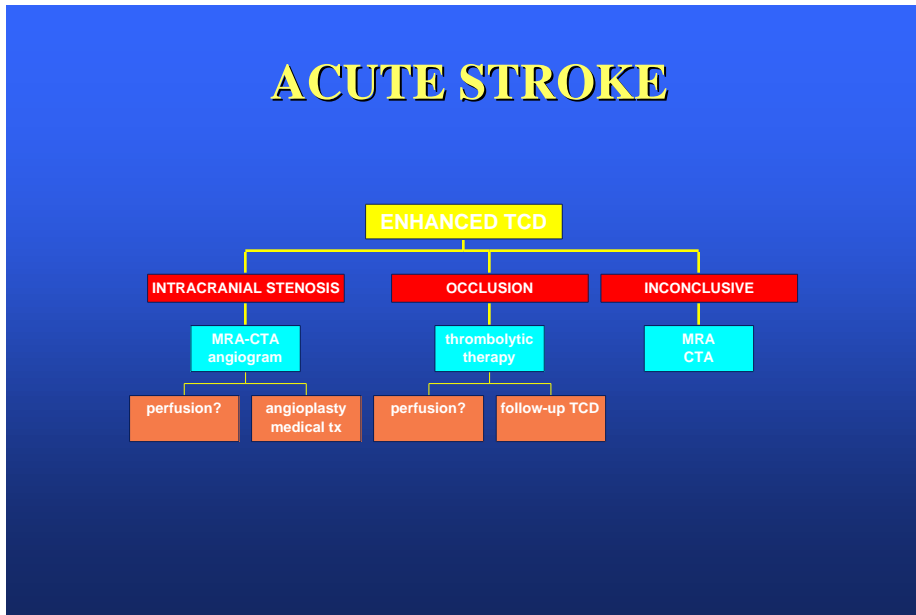
- Intracranial stenosis
- Assessment of collateral circulation
- Vasospasm
- Brain death
- Detection of emboli
- Intracranial aneurysms
- Arterio-venous malformations
- Tumor vascularity.

In cases of ischemic disease, TCD should become a natural extension to the cervical examination, especially when the cause of symptoms is not found on the standard neck exam or in the medical history. Detection of intracranial pathology, stenosis or occlusion, as well as evaluation of collateral flow pathways will orient further investigation and therapy. Contrast enhanced TCD allows better definition of intracranial vascular anatomy for direct assessment of flow dynamics. Flow velocities, direction and waveform can be better studied in each vessel segment. Evaluation of parenchymal perfusion also becomes possible.

Intracranial stenoses although not very common are probably underdetected because we don't look for them. TCD is a simple method of identifying a stenosis and assess its severity. It is also a most simple method to assess the results of treatment either endovascular or medical.

TCD, in addition to the elective evaluation of ischemic disease, can be a useful tool in acute stroke.

ACUTE STROKE



For the other indications, contrast will increase diagnostic accuracy.

Assessment and follow-up of vasospasm is routine in most cases of sub-arachnoid hemorrhage. TCD is the first line exam for this indication relying on flow velocity. TCD in conjunction with clinical findings helps to titrate medical therapy of vasospasm and to decide on the need for angiographic study and endovascular treatment.

TCD, in cases of suspected brain death, can give the necessary information and help guide decision on treatment.

Although not useful in detection of aneurysms, TCD can identify aneurysms and assess adjacent arteries, intra-aneurysmal flow and be used in follow-up. Contrast is essential in these cases. Many aneurysms, because of the turbulent and low intra-aneurysmal flow, will only be seen with contrast enhancement. Visualization depends on size (as small as 3 mm can be seen) location, flow and window.

Evaluation of AVM again requires use of contrast. Identification of feeding arteries, nidus and draining veins can be achieved , pre or intra-operative. Follow-up after treatment of flow velocity and resistivity index of the feeding arteries is a useful addition to standard imaging.

Similarly, evaluation of intracranial tumors for vascularity, shunting and surrounding vessels can be done pre and intra-operative.

The contrast we have been using is Levovist in a concentration of 300 to 400 mg/ml. The reconstituted volume is injected IV in repeated small boluses of 1 to 2 ml. Slow infusion can also be done, but this requires a pump. The simple technique of multiple small

boluses gives enhancement for approximately 15 to 20 minutes, enough to complete a full examination.

TCD is a simple non invasive method of imaging that is probably underutilized. With the addition of contrast, the potential of the technique is very interesting and exciting.

KIDNEY AND RENAL ARTERIES

JM Correas, O Hélénon, M Cherkaoui, D Chauveau, MN, Peraldi, A Méjean, JF Moreau

*Departments of Radiology, Nephrology, Renal Transplantation and Urology,
Necker Hospital, Paris*

Color Doppler ultrasonography (CD US) tends to become the modality of choice in the screening of renal diseases, and particularly renal vascular disorders, because of a significant improvement of the equipment and image quality during the past ten years. However, CD US remains a rather long and difficult operator-dependent examination with some anatomical and technical limitations including ultrasound beam attenuation with depth, abdominal gases and motion artifacts.

The recent introduction of efficient and safe ultrasound contrast agents (USCA) based on stabilized microbubbles is participating to the development of renal US. The latest generations are stable enough to cross the pulmonary capillary bed and reach the renal arteries after peripheral intravenous injection. USCAs increase the Doppler signal intensity in all modes and may also increase the renal parenchyma echogenicity. With an increased understanding of the microbubble behavior within the ultrasound field, specific image sequences such as harmonic imaging and pulsed inversion Doppler technique have been developed to improve USCAs efficacy and reduce artifacts.

A – The ultrasound contrast agents

The concept of contrast enhancement has extended to ultrasound imaging during the last decade. The microbubble persistence has been dramatically improved using two different approaches: external bubble encapsulation or stabilization and selection of low solubility gases. Microbubbles are stabilized using sugar matrix (such as galactose) or albumin microspheres, lipids or polymers. Low solubility gases also participates in the persistence improvement. Most of recent USCAs combine several of these techniques to achieve prolonged enhancement of the Doppler signals and sometimes of B-mode imaging.

The properties of recent USCAs are getting close to that of an ideal USCA, such as being injectable intravenously, non toxic and capable of passing through the pulmonary capillary bed after a peripheral intravenous injection. With prolonged duration and infusion protocols, the contrast enhancement lasts during at least 10 to 15 minutes, reaching the duration required for the study of both kidneys and renal vascular bed. They enhance the Doppler signal intensity (including continuous Doppler, spectral Doppler, color and power Doppler imaging), and sometimes the B-mode echostructure using specific imaging sequences such as harmonic imaging, intermittent harmonic imaging and pulsed inversion Doppler technique.

Artifacts and optimization of contrast-enhanced examinations

At the peak enhancement, some oversaturation and color blooming of the color signal are detected on conventional Doppler. Such artifacts can be limited by reducing the color gain, increasing the color Doppler wall filter and the pulse repetition frequency, resulting in a decreased sensitivity of the color Doppler system. An increase in the color Doppler resolution as well as a reduction in the color Doppler persistence may be also useful for

this purpose. Slow bolus infusion is likely to reduce these artifacts because of a decrease in the peak signal intensity. Moreover, the constant delivery of the USCA provides more uniform contrast enhancement and the Doppler settings do not require constant adaptation. Second harmonic imaging is a recently developed modality that considerably reduces color and spectral artifacts. Such modality appears to be more appropriate in improving the image quality than limiting the sensitivity of the Doppler system.

The systolic peak velocity might increase up to 50 % with the Doppler signal intensity increase. This artifact may be limited by reducing the Doppler gain and by infusing the USCA. It is not a real problem as the diagnosis of significant RAS also requires the presence of flow disturbances, but the operator needs to be careful in grading the lesion.

New imaging modalities, such as harmonic imaging, intermittent harmonic imaging and pulse inversion Doppler technique. These techniques rely on the non-linear behavior of the microbubbles within the ultrasound field. However, they require further studies in humans to evaluate precisely their potentials for kidney disease screening, but our preliminary data shows promising future in opacifying the renal cortex

B – Normal CD US renal vascularity

Normal color Doppler encoding of the RA includes a homogeneous color appearance when using an appropriate PRF from the main RA to the arcuate branches into the deep cortex. The diagnostic criteria of RA stenosis rely on spectral analysis of the main renal vessels and the interlobar arteries (at least 3 measurements). The normal Doppler spectrum of proximal RA encompasses continuous forward flow in diastole due to the low resistance of the renal vascular bed. The laminar flow results in Doppler spectrum well-defined spectral borders and dark systolic “window”. At the level of the intrarenal segmental or interlobar arteries, the normal Doppler tracings includes a marked systolic-diastolic modulation, a rapid rise in systolic velocity up to at least 20cm/sec, and an inconstant early systolic compliance peak (ESP). The normal Doppler waveform parameters are an acceleration time of early systole less than 0.07 sec, a systolic acceleration over 3 m/s² and a resistive index (RI) in between 0.56 and 0.70.

C – Pedicular vascular disorders

1 – Renal artery stenosis

The diagnostic strategy in screening RA stenosis in patients at risk for renovascular hypertension (RVH) is equivocal. Numerous modalities including intravenous pyelography, captopril nuclear renography, spiral computed tomography, angiography and magnetic resonance imaging have been proposed as screening tests. Unfortunately, none of them fulfill the ideal criteria, which are being a non-invasive and cheap modality with a high sensitivity and a high negative predictive value. Doppler US offers proper advantages: it is a cheap, easy to perform and widely available non-invasive technique. However, it remains operator-dependent and requires a perfect knowledge of the anatomy and its variants, as well as the diagnostic criteria and the limitations of the technique. A screening strategy based on Doppler US providing that experienced operators are available appears to be cost-effective while it decreases dramatically the number of diagnostic angiography and increases the number of patients with RVH that can benefit from an appropriate revascularization procedure. It also improves the management of

RVH in providing additional criteria that help in patient selection for revascularization and optimizes the angioplasty itself, which can be performed as a single-step diagnostic and therapeutic procedure. Therefore, CD US examination encompasses some major challenges such as to improve the diagnostic performance of the technique and increase the number of skilled operators. For these purposes, USCAs represent a promising additional tool that might increase significantly the number of contributive Doppler US examinations.

- **RAS diagnosis**

- The diagnosis of non-hemodynamically significant RA stenosis has become possible with the assessment of the RA walls using an anterior abdominal window. Morphological findings of the RA walls include focal narrowing, thickening and calcification of the arterial wall that indicates atherosclerotic lesion and typical beady appearance in case of fibromuscular dysplasia. However, the pulse Doppler waveforms taken from the immediate post-stenotic segment remain normal. Power Doppler mode underlines the RA wall due to an improved arterial lumen filling with color flow signals and therefore facilitates the assessment of RA wall lesions.

- Diagnostic criteria of a hemodynamically significant ($\geq 50\%$) stenosis include flow acceleration at the site of the stenosis with high systolic peak velocity above 150 cm/sec, post-stenotic flow disturbance resulting in spectral broadening and reversed flow. The limitations in studying extrarenal arteries prompted to evaluate downstream hemodynamic repercussions from the distal intrarenal arterial bed in order to indirectly diagnose RA stenoses. Numerous parameters that have been evaluated are still much controversial except in cases of critical stenoses ($> 80\%$). These parameters obtained from the interlobar arteries include a decrease in RI with an increased side difference ($RI > 10\%$), a prolonged acceleration time (>70 msec) with loss of the early systolic peak (ESP) and a decreased acceleration (<3 m/sec²). However, the performance of the study of the distal vascular bed remains insufficient. Tight stenoses may also exhibit severe downstream arterial flow repercussions responsible for a tardus-parvus pattern with a smoother and smaller envelope of the Doppler trace. This pattern is easily recognized in comparison with the normal opposite side. In tight stenoses developed in a RA branch or an accessory supernumerary artery, the tardus-parvus pattern can be demonstrated within a portion of the kidney. Unfortunately, downstream repercussions are missing in about 20% of tight stenoses because of a well-developed collateral blood supply.

At present, the most reliable diagnostic criteria remain those obtained from the stenotic site including flow acceleration and post-stenotic turbulence. Since they require a technically successful Doppler examination with adequate interrogation of extrarenal arteries, the value of Doppler US is mainly influenced by the rate of technical failure which can reach 20% when including multiple RA, even in experienced hands. USCAs improve the detection of main and supernumerary RAs following a peripheral intravenous injection and thus reduce the frequency of technical failures particularly in the juxta-ostial and middle segments. At the peak enhancement, color and spectral artifacts can be found on conventional Doppler modes. They can be reduced by limiting the sensitivity of the color Doppler system. A better approach consists in specific image

sequences that can take fully advantage of the non-linear behavior of the microbubbles within the ultrasound field.

USCAs also improve the placement of the pulsed Doppler sample window onto the lumen as well as the angle of the insonation. The increase in the signal to noise ratio is critical as the major RAS criteria rely on the spectral analysis of the extrarenal RA.

In renal transplants, the entire pedicle can be assessed successfully in almost all cases. A careful study of the complete vascular bed including intrasinus and extrarenal arteries is required especially in case of multiple arteries anastomosed separately or to a common trunk prior to the iliac artery. RA stenoses are detected through color encoding alteration due to turbulence and aliasing phenomenon. A peri-vascular artifact surrounding the stenotic segment can be detected. Spectral analysis reveals acceleration with a systolic peak velocity over 1.5 to 1.9 m/sec and flow turbulence with typical spectral broadening and reversed flow. Downstream repercussions occur in tight stenoses (> 75 %). The assessment of the intrasinus vascular bed with color Doppler may also reveal RAS involving segmental branches. The hemodynamic disturbances are detected at the stenotic site within the sinus and the downstream repercussions can be detected with a segmental distribution. USCAs have a little interest since a complete examination of the entire vascular bed is usually successful, except in the early days following surgery. USCAs may help in demonstrating a hypoperfused territory due to a RA branch severe stenosis.

2 – Renal artery occlusion

The diagnosis of RA occlusion requires both direct visualization of the mute RA and severe intrarenal velocimetric abnormalities (tardus-parvus pattern or absent Doppler signal). The visibility of a mute RA or an arterial stump on color Doppler image is the only way to differentiate RA occlusion from tight RA stenoses associated with severe downstream repercussions. USCAs increase the diagnostic confidence in demonstrating a mute RA. The lack of visibility of any RA even after the enhancement of the blood pool is a strong argument for RA occlusion. USCAs also help in differentiating sub-occlusion from complete occlusion of the RA. When the kidney is atrophic and deep, the color and spectral Doppler signal enhancement from the interlobar arteries increases the detection of the downstream repercussions, which is a key finding for the diagnosis.

3 – RA aneurysm

Aneurysms usually arise from a renal arterial branch or distally from the main RA. CD US demonstrates an echo-lucent mass filled with color Doppler signal on the course of the RA. Spectral analysis shows a turbulent flow with a reverse component within the aneurysm but without the so-called “to-and-fro feature”. False negative results are due to aneurysmal thrombosis and peripheral calcifications, or to the inaccessibility of the involved arterial segment. However, the B-mode findings can suggest the correct diagnosis especially when curved calcifications are present. USCAs might be useful in detecting blood flow within a calcified or a partially thrombosed RA aneurysm. False positives result from arterial loops and post-stenotic dilatation.

4 – Renal vein thrombosis

The diagnosis of RV thrombosis relies on the detection of an echogenic thrombus within a mute or partially flowing RV at CD US. CD US should evaluate the extent of the thrombus within the RV and the inferior vena cava. Two situations must be distinguished: fibrino-cruoric thrombosis and tumoral thrombosis. Fibrino-cruoric thrombosis of the RV can be found in nephropathies with severe nephrotic syndrome or in renal allografts. At the early stage, spectral analysis shows normal intrarenal venous signal and a slight increase in RI values from the renal arteries. The lack of intrarenal venous flow disturbances and the limited changes in the RI in native kidneys reflect the quick development of collateral venous supply. Conversely, the thrombosis of the renal transplant vein is associated with the absence of intrarenal venous flow and high RI with plateau-like retrograde diastolic flow due to the lack of collateral venous supply. CD US of native renal veins is difficult due to the attenuation of the US beam related to the depth, the low flow and the bad insonation angle. In hypoperfused renal transplants due to non-specific increase in arterial resistance, it is difficult to rule out renal vein thrombosis. Moreover, the detection of partial thrombosis is a challenge despite the use of Doppler modalities with higher sensitivity such as power Doppler. However, CD US is a very promising technique as it provides a noninvasive assessment of the renal veins in patients with impaired renal function. The use of USCAs makes the RV assessment much easier as microbubbles increase the RV Doppler signal intensity due to their capacity to cross the renal microvasculature. The CD settings of should avoid excessive blooming that might obscure partial RV thrombosis. USCAs facilitate the diagnosis of RV patency and thrombosis in case of technical failure. They enhance the detection of collateral venous blood supply on the pericapsular circle and within the perirenal fat in case of RV thrombosis in native kidneys. USCAs are also useful in the follow-up of RV thrombosis during treatment or in case of suspicion of recurrence.

When the renal tumor extends to the venous system, it is critical to evaluate the upper limit of the thrombus, as it will change the surgical procedure. The CD study of the permeability of the RV including the distal segment and the inferior vena cava might be difficult. USCAs enhance the Doppler signal intensity of these veins and thus improve the detection of thrombosis. The detection of renofugal arterial flow within the RV thrombus is typical in case of tumor extension.

D – Intrarenal occlusive and non occlusive disorders

They include occlusive disorders such as renal infarction and intrarenal non-occlusive disorders such as renal tumors, acute pyelonephritis, iatrogenic false aneurysms and arteriovenous fistulas.

1 – Renal infarction

CD US is a valuable technique in the detection of renal allograft cortical necrosis. However, it seems less accurate for the diagnosis of small perfusion defects in native and transplanted kidneys because of anatomical limitations, movement artifacts and use of lower frequency. Despite the introduction of power Doppler and broadband high-frequency transducers that improves the cortical vascularity assessment, the technical limitations and/or the small size of the infarct may prevent CD US from visualizing perfusion defects resulting in false negative studies, particularly in native kidneys. Large segmental infarcts show a complete loss of Doppler signal on color, power and pulsed

Doppler modes typically within a hypoechoic area of cortex early after infarction. The presence of hemorrhage within the lesion can be responsible for a hyperechoic appearance.

USCAs improve the detection of the cortical vasculature and fill the distal cortical vessels with color Doppler signals up to the capsule. The renal infarcts are seen as color flow defects with sharp edges. However, the appropriate settings should be used to avoid excessive blooming that may obscure small infarcts. Harmonic imaging with the most recent USCAs might have a better sensitivity and specificity. Specific images sequences such as intermittent harmonic imaging, transient scattering or pulse inversion Doppler techniques might provide direct visualization of the renal perfusion on B-mode, with a superior resolution when compared to that of conventional color Doppler imaging. In the very next future, they should improve the diagnosis of small cortical infarcts.

In renal transplants, the deepest portions of the cortex typically show a decrease in the CD signal intensity that may mimic ischemia or infarction. This pattern might be increased in case of medical complications inducing a non-specific hypoperfusion and particularly in severe acute tubular necrosis, severe acute rejection, cortical necrosis and RV thrombosis. It is critical to differentiate hypoperfusion territories from infarction, as prognosis and treatment differ. USCAs improve the detection and delineation of the infarct. They can distinguish severe hypoperfusion from infarction. Adequate settings are required to avoid missing small peripheral lesions due to the blooming artifacts, and the appropriate direction will certainly be the use of new imaging sequences, as they provide superior resolution and less artifacts. USCAs can play a key role in this case because they do not have any renal toxicity, and the examination can be repeated to allow a close follow-up of the complication.

2 – Renal tumors and pseudo-tumors

US is a major modality for the detection of renal masses. The reduction in renal tumor size at the time of the discovery of the lesion is mainly due to the increasing number of renal masses incidental diagnosis with US. B-mode imaging plays a key role as a solid hypoechoic mass is assumed to be a renal cancer. An anechoic mass with no detectable wall and increased transmitted echoes distal to the mass is a typical benign cyst. However, small masses (< 1 - 3 cm in diameter) are difficult to detect and characterize with conventional B-mode US. Some improvements are expected with harmonic B-mode tissue imaging which does not require the administration of an USCA. The Doppler value for detecting and characterizing renal masses is very limited, as benign tumors exhibit the same color patterns and spectral waveforms than malignant lesions.

USCAs increase the detection of the intrarenal vascularity from the interlobar up to the interlobular arteries and veins using color and power Doppler modes. The detection of small masses is improved as the normal renal vascular architecture is altered. At the peak enhancement, small masses appear as rounded-shape cortical defects underlined with the massive blood flow enhancement of the normal surrounding vessels. There is no difference in between typical and atypical cysts or malignant lesions. The same effect can be achieved with B-mode perfusion image sequences such as broadband imaging or transient scattering harmonic imaging. Renal masses enhance little compared to the normal renal cortex and are detected as an hypoechoic round defect among the hyperechoic

enhanced normal cortex. Later, the microbubbles reach the tumor vascularity and increase its detection. Although not much useful in differentiating benign to malignant lesions, the presence of blood vessels in complex renal cysts might change their classification and management toward more aggressive surgical procedures. Contrast enhanced US participates to the staging of the venous extension (see renal vein thrombosis).

USCAs might be useful in differentiating between tumors and pseudo-tumors as hypertrophic columns of Bertin. In pseudo-tumor, internal and peripheral vasculature exhibits smooth and homogeneous branching.

3 – Acute pyelonephritis

The diagnosis of acute pyelonephritis (APN) is based on clinical findings (acute cystitis symptoms, back pain and fever) and laboratory investigations (urine infection). At the acute phase, imaging is used only to rule out immediate complication, with plain abdominal X-ray film and renal ultrasonography (US) to diagnose obstructive APN and renal or perinephric abscesses. When the clinical features are atypical or if a complication is detected, contrast-enhanced computer tomography is performed and is the gold standard imaging. However, it is an expensive modality that requires administration of an iodinated contrast media and exposition to radiation. B-mode and power Doppler imaging might be useful in children to detect hypoechoic hypoperfused areas. Unfortunately, these findings are inconstantly detected in adults, even with severe APN. US appears to be of low sensitivity in our adult experience. However, it has great potentials as an easy to access and cheap imaging modality, which do not require the administration of a potentially toxic contrast media and exposure to radiation. USCAs might increase the detection of perfusion defects in the renal cortex. The Doppler settings should be appropriate to increase the vascularity ratio between normal and ischemic areas. These defects will be easily differentiated from small infarcts, as some perfusion is likely to be detected. This differential diagnosis is critical particularly in renal transplants. However, the use of USCA might be difficult and the sensitivity of the technique reduced due to the blooming artifact. A large improvement is expected with new US imaging sequences such as harmonic imaging, intermittent imaging and pulse inversion Doppler techniques.

4 – RA false-aneurysm

RA false-aneurysm is an iatrogenic complication of surgical and percutaneous renal procedures. The flowing cavity arises from the injured artery without venous drainage. US shows a hypoechoic round shaped mass located within the renal parenchyma that is filled with color signal on CD image. Spectral analysis obtained at the level of the communicating channel demonstrates typical finding known as the to-and-fro sign which reflects both systolic feeding arterial flow and diastolic draining arterial flow. In our experience, USCAs have the potential to improve the detection of the feeding vessel and the flowing cavity in challenging cases. They might also demonstrate active bleeding rising from the false aneurysm.

5 – Arteriovenous fistulas

CD US is the only non-invasive modality that allows the detection of post-biopsy arteriovenous fistulas. They produce local tissue vibrations due to flow turbulence responsible for a peri-vascular artifact on CD imaging. Typically, spectral analysis shows accelerated and highly turbulent flow at the site of the arteriovenous shunt. The flow velocity in the feeding artery is increased with a marked decrease in RI values (0.30-0.40). The draining vein exhibits an increased flow velocity with systolic-diastolic modulation. In long-standing post-biopsy fistula, pseudo-aneurysmal draining vein can be detected with large peri-vascular artifact extending into renal hilum.

Congenital arteriovenous malformation is a rare pathologic condition which can also generate a peri-vascular artifact at the site of the arteriovenous shunt. Such typical findings suggest the potential of CD US as a noninvasive diagnostic tool for the detection of AV malformations responsible for chronic hematuria with negative excretory pyelography or CT. Angiography is still mandatory, especially when color Doppler suggests the presence of an AV malformation, to make the final diagnosis and treat the lesion with percutaneous transcatheter embolization.

USCAs might be useful in native kidneys to study low flow arteriovenous fistulas and malformations as well as to confirm the complete occlusion of the shunt after embolization. In renal transplants, USCAs value is reduced in this indication because flow disturbance is easily detected in all cases due to its intensity and to the superficial location of the fistula.

D – Conclusion

CD US appears to be an effective imaging modality in the diagnosis of renal diseases. USCAs can improve the diagnostic capabilities of renal Doppler US in case of technical failure, despite an increase in the duration of the examination. USCAs will increase the role of CD US as the primary modality in the screening of patients at high risk of renovascular hypertension. Their impact might be critical in patients with impaired renal function that preclude the use of iodinated contrast agents. USCAs are likely to reduce the operator-dependency of ultrasonography, but a specific training will be required in addition to the renal CD US training. USCAs are well tolerated and do not have any renal toxicity. Specific settings will be implemented in the US units to facilitate the use of USCAs, increase their efficacy and reduce the artifacts. New imaging sequences such as harmonic imaging, intermittent imaging and pulse inversion Doppler technique are likely to provide perfusion imaging.

APPLICATIONS OF US CONTRAST IN RENAL IMAGING

Michelle L. Robbin

University of Alabama, USA

Introduction

Ultrasound contrast agents (UCAs) have significant promise in expanding the role of ultrasound in Radiology. Potential renal applications of ultrasound contrast agents (UCA's) will be discussed, emphasizing solid and cystic renal mass evaluation.

Solid Masses

The incidental detection of a solid renal mass is becoming increasingly common at cross-sectional imaging. More than 30,000 new cases of renal cell carcinoma are diagnosed, and more than 12,000 people die of renal cell carcinoma each year in the United States. Renal cell carcinoma represents approximately 2% of all new cancer diagnoses each year (1). Early detection and surgical removal remains the best treatment for the disease, although chemotherapy and radiation therapy has been widely investigated.

At ultrasound, a solid hypoechoic or isoechoic renal mass is assumed to be a renal cell carcinoma until proven otherwise (2). Although benign lesions such as oncocytoma can be similar in appearance to renal cell carcinoma, the incidence of oncocytoma is much lower (3% vs. 90 – 95% of malignant renal neoplasms) (3). As there is no reliable way to differentiate renal cell carcinoma from oncocytoma with imaging, nephrectomy is usually performed.

Interestingly, even though solid renal masses may have little or no detectable flow at ultrasound, (4-6), they will usually demonstrate significant contrast enhancement at CT or MRI (7). If the renal mass is hyperechoic at ultrasound, a thin-section non-contrast CT can assess for the presence of intratumoral fat that is diagnostic of an angiomyolipoma (8). If definitive intra-tumoral fat is not found, a CT or MRI with contrast needs to be performed, as a small echogenic renal cell carcinoma can mimic an angiomyolipoma at sonography (9-12).

Detection of the small, less than 3 cm renal tumor is difficult on sonography unless it is contour deforming (13). When a renal mass is found, a second renal tumor must be excluded, as they can be bilateral in 2% of patients (14). It is not uncommon to perform a careful sonographic search for a renal lesion only to discover a non-contour deforming mass on a subsequent CT or MRI. Unless there is a marked difference in echogenicity or blood flow, small renal masses have been very difficult to detect with conventional ultrasound. In a recent study, seventy-nine of 205 small renal lesions were not seen with US, but seen with CT (15). UCAs may enhance the depiction of renal intra-tumoral vessels, and thereby increase the detection rate of these small masses.

A detection rate for synchronous tumors similar to that of CT or MRI may obviate the need for additional studies in the patient with a known renal tumor. If diagnostic confidence in renal vein visualization is high, further radiographic evaluation to determine renal vein patency and presence of (tumor) thrombus may not be necessary. Currently, many institutions now perform CT angiography and multiplanar CT reconstructions for preoperative evaluations of small renal masses, rather than angiography (16). Although a CT may still be needed as a baseline for postoperative follow-up, patient diagnosis and management may be clear after the first imaging study (ultrasound) in a higher proportion of cases.

Pseudo-Masses

Persistent fetal lobularity and/or a prominent column of Bertin can be difficult to differentiate from a focal renal mass. Disarray of the renal tubules may cause a slightly different echotexture from the adjacent renal parenchyma. The use of UCAs may allow the differentiation of prominent normal tissue from renal masses, without expensive ancillary studies. Definitive identification of normal from abnormal would also lessen patient and physician anxiety due to diagnostic uncertainty until a subsequent imaging test is performed.

After the injection of an UCA, a normal kidney will show a brightly echogenic cortex and relatively hypoechoic pyramids on gray scale contrast specific imaging, similar to the nephrogenic phase of contrast at CT or MRI. To differentiate a mass from a pseudo-mass, the pattern and spacing of pyramids within the cortex can be examined for alterations in contour and mass effect. Color and power Doppler can be used to examine the vascular pattern for uniformly branching vessels and the absence of tumor vascularity. With greater experience with this technique, it is likely that further CT evaluation of pseudo-masses will be unnecessary in the majority of cases.

Cysts

Simple renal cysts are benign by definition. Cysts with only one or two thin septations at ultrasound (Bosniak 2) usually appear simple at CT because of the lack of vascularity of the septations (13). Bosniak type 3 lesions usually have more or thicker septations, or peripheral nodularity. Contrast CT may show enhancement of the septations consistent with renal cell carcinoma, even though no detectable flow is found at ultrasound.

Considerable experience needs to be gained with analysis of cystic lesions with ultrasound contrast. However, investigation of the vascularity of the septations with ultrasound contrast in a non-simple renal cyst (Bosniak type 2 or 3) may help identify those complex cysts at higher risk for malignancy (17). In the future, a Bosniak 2 or 3 renal cyst work-up with ultrasound contrast enhancement may be as follows: 1. If no enhancement is seen on color or power Doppler in combination with an ultrasound contrast agent, either no follow-up or sonographic follow-up is performed. 2. If enhancement of septations is seen, consider surgical removal (18).

It is important to realize that vascular enhancement of renal septations at US is machine and technique dependent. Thus, an objective method of quantifying the degree of

sonographic enhancement of a septation (such as a scanner based videodensitometry technique), similar to the CT Hounsfield unit would be useful. Significant experience with large numbers of complex cysts needs to be obtained before clinical management of these lesions will change, however.

Renal Artery Stenosis

Renal artery stenosis (RAS) is a potentially curable cause of hypertension. The invasive nature of the angiogram and possible deleterious effect of the iodinated contrast media limits the actual number of examinations performed to rule out RAS to those patients with uncontrollable or sudden onset of hypertension. Although many tests have been investigated for the accurate diagnosis of RAS, none is completely satisfactory. Controversy exists in the ultrasound literature as to the accuracy, sensitivity and specificity of the sonographic analysis of intrarenal arteries and main renal arteries (19-21). Up to 20% of the population have accessory renal arteries, further increasing the difficulty of renal artery stenosis detection.

Sonographic contrast enhancement has been shown to increase the percentage of diagnostic examinations in main renal artery analysis (22). Ultrasound contrast agents can enable a more rapid and complete visualization of intra and extra-renal arterial anatomy using color flow imaging. One of the acknowledged difficulties precluding widespread use of ultrasound in the detection of RAS is the technically demanding nature of the examination. UCAs may make the examination technically easier, allowing some equalization of success among laboratories.

Renal Transplants

Current scanning techniques usually adequately image renal transplants. Spectral analysis of intrarenal vessels provides a limited amount of information regarding the transplant. Abnormal elevations of the resistive index are not specific for rejection, and can be found in pyelonephritis and cyclosporine toxicity, among others (23-25).

Visualization of the main renal artery and vein to their anastomosis with the iliac vessels is important when assessing for renal artery stenosis or renal vein thrombosis (26). UCAs may be useful in the occasional obese patient with deep vessels.

There has been some suggestion that the number of branch vessels may prove a more sensitive indicator of rejection (27, 28). However, fine small cortical vessel detail can only be obtained in thinner patients using current technology. UCAs may improve our ability to reliably assess smaller transplant vessels, so this hypothesis can be further evaluated. It also may provide a means to assess renal perfusion, potentially a very useful tool in renal transplant evaluation (29).

Conclusion

The emerging field of contrast ultrasound has many potentially important and exciting radiologic applications. New gray scale contrast imaging techniques may substantially increase the detection rate and characterization of kidney masses. Contrast ultrasound use may increase diagnostic certainty, decrease the number of non-diagnostic ultrasound

examinations, and decrease those exams requiring another imaging study for diagnostic proof. However, further investigation is needed in large numbers of patients to determine diagnostic utility and cost-effectiveness with respect to CT, MRI, angiography and fundamental ultrasound.

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CONTRAST AGENTS IN RENAL DISEASES

HP Weskott

Dept of Internal Medicine

Klinikum Hannover, Siloah Krankenhaus, Hannover Germany

In contrast to liver or spleen examinations, the length of the diagnostic window after bolus injection of Levovist (3 x 1.33g or 2 x 1.25g) or Optison (1 – 3 x 1ml) is quite short. In order to image blood pool a sweep should be performed, and continuous scanning of the ROI is recommended to demonstrate vessel architecture. The best time interval to perform a sweep is 20 to 30 seconds after administration of the agent. In order to expand the diagnostic window, we use a continuous pump infusion of Levovist (300mg/ml, 1 – 2ml/min). With pump infusion, sweeps can be performed every 30secs. This procedure provides sufficient time to examine both kidneys in two scan planes.

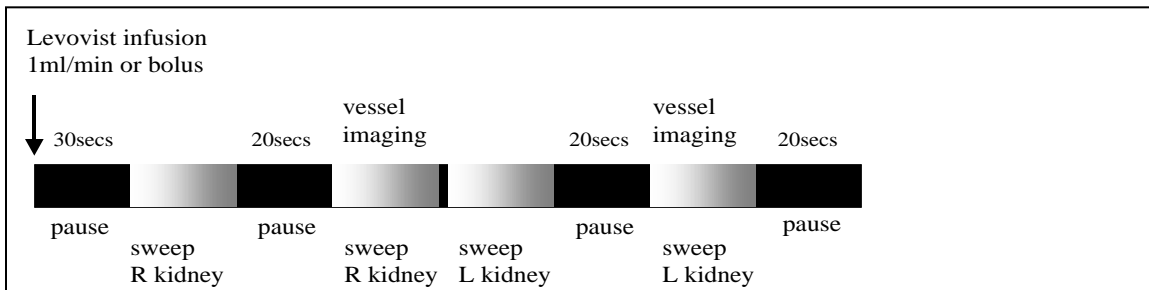


Fig. 1 Scheme for contrast infusion or bolus injection of levovist. Depending on the clinical question one may start to image blood pool or vasculature. Due to the relatively short enhancement time after bolus administration, multiple boluses every 2 minutes are recommended.

In renal imaging the five main goals are: Detection of renal tumors, its characterization, tumor staging, evaluation of vascular status and renal function.

RCC: 2-3% of all malignancies in adults are renal cell carcinomas, RCC (Landis, 1). 85% - 90% of all solid renal tumors are malignant (Russo, 2). Today, more than 3/4 RCC are discovered incidentally in asymptomatic patients.

Although CT is superior to US in detecting small RCC below <25mm (Jamis-Dow, 3), US-CA will -in contrast to liver lesions- not play a major role for tumor detection.

CT and US do comparable well in characterizing solid renal tumors that range between 10mm and 30mm (80% and 82% respectively, Jamis-Dow, 3). The high number of correctly characterized cases does not surprise, as the percentage of malignomas is high (see above). As there is no typical vascular architecture in RCC, the benefit of CA enhanced US is to image blood pool and thus the volume of viable tumor tissue. Therefore the role of CA in renal tumor US imaging is limited.

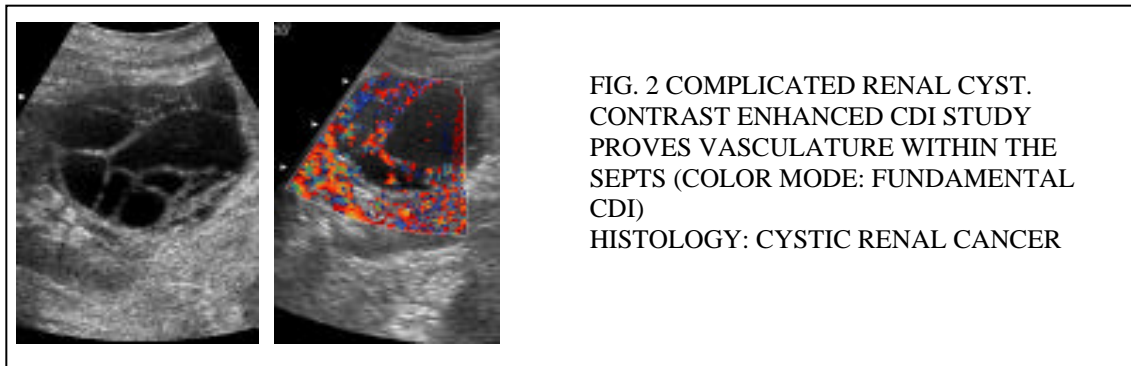
In most studies dealing with renal tumors we used CDI. In recent month color flow mapping modes have often been replaced by Non Doppler gray scale techniques (Coded

Harmonic Angio) in order to avoid the well known artifacts of color flow mapping modes in displaying renal vessels.

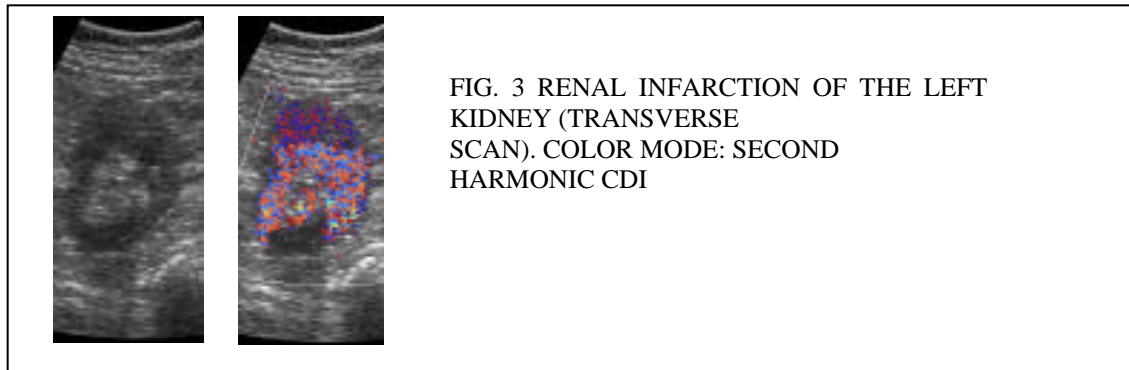
AML: Due to the great variance in vasculature and due to the fact, that AMLs may have intratumoral vessels that are also characteristic for tumor vessels in RCC, US CA can only be of limited value. With the detection of only very few (or even no tumor vessels) a small echogenic renal lesion is more likely a AML than an echogenic RCC. We made these observations in 18/21 pts. with AML <2cm. In 10/10 echogenic RCC <3cm, the tumor was nearly completely covered with SAE. As CT can demonstrate even small amounts of intratumoral fat, it is the method of choice to prove AML (Bosniak, 4).

Renal Cysts are by far the most frequent renal masses. In order to characterize complicated renal cysts, Bosniak described different categories by their gray scale appearance ranging from I – IV, with III and IV being the categories with the highest chance of malignancy (Bosniak, 4-7).

Contrast enhanced color flow Doppler modes are most sensitive in detecting vessels in thickened septa or the wall of complicated cysts. This is a sign of viability and thus may be a sign of malignancy (Fig. 2). We examined over 50 pts. with complicated cysts (mainly category III) with CA Levovist and referred pts. with a proof of vasculature to the surgeon. 2/3 had cystic renal cancer. We found that CA enhanced US examinations are at least as sensitive as CT or MR (Weskott, 8).



US CA is most useful in the characterization of complicated cysts, detecting tumor venous thrombus, and improved imaging of renal artery (-ies), to prove of renal infarction and focal renal diseases like pyelonephritis or abscess.



New gray scale, Non Doppler imaging modalities (like Coded Harmonic Angio, CHA) allow a higher spatial and temporal resolution of blood flow. Future will show whether US-CA will be of clinical relevance in diagnosing renal artery stenosis or may contribute in the evaluation of renal function.

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CONTRAST ENHANCED RENAL ULTRASOUND

Richard G. Barr

North Eastern Ohio Universities College of Medicine, USA

Renal masses are frequently encountered in daily radiology practice. The management of these lesions often requires the use of multiple imaging modalities. The use of contrast-enhanced ultrasound can be of significant clinical utility in diagnosis and appropriate management of renal masses. Contrast enhanced ultrasound has the advantages of ultrasound and the evaluation of enhancement patterns as used in Computer Tomography in diagnosis of renal masses. The Bosniak classification system used in Computer Tomography for cystic renal masses can be modified to be used with contrast enhanced renal ultrasound. When contrast agents are approved for routine use, contrast can be administered when a renal mass needs further characterization eliminating the need for an additional imaging modality.

Definity Enhanced Ultrasound of Indeterminate Renal Masses

Methods: Thirty five patients with indeterminate renal masses on an unenhanced ultrasound, CT or MRI scan were enrolled in a two-center open-label trial. Definity was administered both by bolus (10mL/KG) and infusion (1.3ml diluted in 50cc given at a rate of 4 to 10 ml/min) methods. One bolus followed by infusion followed by subsequent bolus injection was performed. The examination was performed with harmonic and contrast specific non-linear models such as phase inverted technology scanning. Interval time delay imaging techniques with high mechanical index (MI) were also utilized. Comparison was made to unenhanced ultrasound as well as to the comparative CT and/or MRI.

Results: The duration of contrast enhancement was sufficient for examination completion in all patients. Definity provided an increased confidence in diagnosis in 57% of cases. Post contrast diagnosis was changed compared to the unenhanced exam in 10% of patients. The second bolus was found most helpful in 86% of cases. With the bolus technique low MI phase inverted scanning and interval delay imaging was the most beneficial. With infusion low MI scanning and Power Doppler were found most useful. Additional diagnostic information was obtained in all patients after the administration of Definity, including facilitated vascular assessment (86%), improved conspicuity (57%), improved lesion characterization (52%), and improved lesion delineation (67%). The diagnostic information gained with Definity-enhanced ultrasound could have altered patient management in 67% of patients, including no additional follow-up (52%) and recommendation of needle biopsy/surgery (14%).

Conclusions: The use of bolus injection for contrast enhanced ultrasound was found more useful than infusion. Low MI continuous scanning with inverted phase technology and interval delay imaging were found to be the most useful techniques. Definity-enhanced ultrasound in patients with indeterminate renal masses significantly improved

the diagnostic capabilities over unenhanced ultrasound in a majority of patients. It could have altered patient management in a large portion of the population studied.

Characteristics Enhancement Patterns of Renal Lesions

Column of Bertin (Pseudomasses): Pseudomasses have the same enhancement pattern as normal renal tissue on all imaging sequences, including varying interval delays. Characteristic triangular renal pyramids are seen within the mass at regular intervals. The Arborization pattern of vessels seen best at low MI continuous scanning is not distorted.

Angiomyolipoma: Angiomyolipomas are seen as echogenic well circumscribed masses on non-contrast enhanced imaging. The lesions do not enhance significantly and are often Isoechoic to normal renal parenchyma with contrast enhancement. Peripheral blood vessels are often seen around the lesion. Central blood flow is rarely seen.

Complicated Cysts: In our experience benign cystic renal masses do not have blood flow associated with the mass. With contrast enhanced ultrasound we feel confident that blood flow is not missed. With interval delay imaging the lesions are more conspicuous since they do not enhance. Low MI continuous scanning is helpful in evaluating for blood flow in small nodules or septations within the mass.

Renal Cell Carcinomas: Renal cell carcinomas have significant blood flow within the mass as well as having increased enhancement on interval delay imaging with short (1s) interval delays. With longer interval delays the mass is often less enhanced than the surrounding normal renal parenchyma.

Echogenic Renal Cell Carcinomas: Echogenic Renal Cell Carcinomas often present as diagnostic problems as they can mimic an Angiomyolipoma. In our limited experience differentiating features from an Angiomyolipoma include significant blood flow in the central portion of the mass as well as more enhancement on short (1s) interval delays. Further evaluation is necessary to determine the accuracy of these characteristics.

Comparison of Image Characteristic

| Mass | Echogenicity precontrast | Enhancement Post contrast | Interval Delay | Blood Vessels |
|------------------|---|---------------------------|----------------|----------------|
| Simple Cyst | Anechoic | None | None | None |
| Complex Cyst | Anechoic to hypoechoic with internal echoes | None | None | None |
| Angiomyolipoma | Hyperechoic | Slight | Slight | Peripheral |
| Column of Bertin | Isoechoic | Same as Kidney | Same as Kidney | Same as Kidney |

Comparison of Image Characteristic

| Mass | Echogenicity precontrast | Enhancement Post contrast | Interval Delay | Blood Vessels |
|----------------------|---------------------------|---------------------------|----------------|---------------|
| Renal Cell Carcinoma | Hypoechoic to Hyperechoic | Marked | Marked | Yes |
| Metastatic Disease | Variable | | | Yes |
| Lymphoma | Variable | | | Yes |
| Infection | Hypoechoic | | | |

NON VASCULAR IMAGING: FALLOPIAN TUBES - HYSTEOSALPINGO-CONTRAST-SONOGRAPHY (HYCOSY)

Annette Schmider, Wolfgang Henrich
Ultrasound Division of the Department of Ob/Gyn
Charité, Campus Virchow Clinic, Humboldt University Berlin, Germany

Introduction

Infertility is defined as the inability to conceive after attempting to become pregnant for one year. Infertility is a significant health problem affecting 10 to 15% of couples in industrialised countries. It has definite physiological, psychological and social implications. The more women delay starting a family, the more the number of couples affected by infertility, or better subfertility is likely to increase. As a result, a higher proportion of couples will require assisted reproduction techniques. There is, in fact, evidence that these procedures are being increasingly publicised as more couples request such services (Hanson, 1998, Trantham, 1996).

The single, most common reason in women for difficulties with conception is a tubal factor. This proportion of infertility attributable to a tubal anomaly is gradually increasing due to the increasing prevalence of salpingitis, the growing number of ectopic pregnancies and the fact that microsurgical and laparoscopic interventions are becoming more common (Hamilton, 1998, Buck, 1997).

Awareness of this as well as the trend for couples to seek fertility treatment at a more advanced age have resulted in wider acceptance that accurate diagnosis – including early assessment of Fallopian tubal patency – should be a priority.

Moreover, investigation of the tubal factor is an important part of the diagnostic work-up of infertility, since the prognosis and further diagnostic measures are very much dependent on tubal patency.

Consequently, there is a need for a rapid, reliable and well-tolerated technique that can be performed on an out-patient basis.

Hysterosalpingo-Contrast Sonography (HyCoSy) can be recommended as an initial screen in parallel with endocrinological and andrological investigations before expensive and complicated treatments are started. If tubal patency is diagnosed with HyCoSy, the patient can be spared more invasive examination procedures and can be allocated all the faster to a relevant treatment strategy. On the other hand, the discovery of abnormal findings at HyCoSy can be further investigated by more invasive methods in a more selected group.

HyCoSy with Echovist®-200 has significant advantages over more commonly used methods such as chromolaparoscopy ('lap and dye') and X-ray hysterosalpingography (HSG). These are as following:

- Real-time demonstration of tubal patency increases patient compliance with the procedure and provides positive patient reassurance
- Highly versatile with concurrent assessment of uterine cavity and ovarian morphology
- Avoids surgical and anaesthetic morbidity
- Avoids exposure of the reproductive tract to ionising radiation
- Simple, virtually non-invasive and rapid out-patient procedure
- Cost-effective and time-saving
- Requires no referral to Radiology, i.e. the examination can be performed in gynaecological surgeries

HyCoSy can nowadays be recommended as a first-line screening test in female subfertility investigations in patients with an unremarkable conventional ultrasound and no medical complaints and has replaced the HSG.

Mechanism of Echovist®-200

Echovist®-200 is an ultrasound contrast medium indicated for examinations of the female genital tract and, in particular, for the visualisation of the Fallopian tubes and investigations of their patency. The contrast effect of Echovist®-200 is based on microbubbles of air stabilised by galactose microparticles. Apart from a small proportion which dissolves in the ready-to-use suspension, the microparticles become stabilised in the carrier solution and remain in suspension. The air attached to and within the granules causes gas saturation of the liquid compartment. Excessive gas volumes tend to form tiny bubbles (microbubbles) predominantly at the solid surfaces of the microparticles.

For application in HyCoSy, the contrast medium is administered transcervically, preferably by use of an intrauterine balloon catheter.

When the microparticle-microbubble suspension is instilled into the uterine cavity for assessing tubal patency, the microparticles dissolve completely within a few seconds after warming up to body temperature. The remaining microbubbles are sufficiently stable to provide contrast for approximately ten minutes. The galactose solution and the small amount of air originating from the microbubbles are released into the abdominal cavity after passing through the tubes. They are absorbed predominantly by the peritoneum. The use of Echovist®-200 for HyCoSy allows organ boundaries to be delineated more accurately and flow phenomena to be observed directly on two-dimensional scans. The quality of ultrasound investigations can be increased by improving the signal-to-noise ratio. Additionally, flow detection can be observed in areas that did not show any signal without contrast application. By improving both the anatomical and, to some extent, the physiological information obtained, the use of ultrasound contrast agents improves the diagnostic value of ultrasound.

Clinical Experience

Up to date, over 30 clinical studies including phase III trials concerning safety as well as diagnostic accuracy have been performed.

Clinical experience with this method as well as clinical studies are now suggesting that HyCoSy is able to select those patients in whom lap and dye and hysteroscopy are most likely to reveal pathology (Holz, 1997, Hamilton, 1998).

The use of Echovist[®]-200 in the evaluation of tubal patency has been investigated in eight phase III/IIIb trials (involving 3,297 patients) designed to assess the HyCoSy technique with respect to efficacy, safety and tolerability.

The data show that HyCoSy provides reproducible findings in the evaluation of uterine abnormalities and tubal patency. Both uterine malformations and tubal patency in particular can be diagnosed with an accuracy similar to that of the standard procedures. HyCoSy allows visualisation of the Fallopian tubes and their course in B-mode sonography and permits evaluation of left/right tubal patency.

In 25 prospective comparative clinical studies as well as in phase III clinical studies (involving a total of 1,989 patients) the accuracy of HyCoSy showed a high overall concordance rate per tube with either lap and dye or HSG (range: 68% to 100%). This result is similar to the reported concordance of 55 - 84% between lap and dye and X-ray HSG (Matorras, 1998, Adelsi, 1995, Philipsen, 1981).

There is, however, a large variability among the individual studies regarding diagnostic accuracy which may be attributable to the fact that only small samples were analysed in some of the trials as well as to the increasing skill of the operator over time. A comparison of only those trials involving at least 50 patients shows that the concordance rate was considerably higher, varying only between 83% and 94%. There was no clear indication that the lower and higher figures correlate with either HSG or lap and dye when used as the reference method.

Typically, the sensitivity figures for tubal patency were slightly higher (well over 80%, often over 90%) than the specificity figures. Likewise, the positive predictive value in almost all studies and in all studies involving 50 patients or more was higher than 90%, while the negative predictive value characteristically ranged from 60% to 85%. Consequently, tubal patency can be diagnosed by HyCoSy with greater accuracy than tubal occlusion.

The importance of the operator's experience with regard to the clinical results was demonstrated by Hamilton (1998): In a clinical study involving 500 patients, the rate of correct interpretation of the sonographic results increased considerably with experience. While 7.4% of the total number of 905 tubes were not clearly interpretable, this number decreased to 4.8% after exclusion of the data from the first 100 women.

In a phase III multicentre clinical study analysing the consistency between HyCoSy and the two reference methods of L&D and HSG as well as that between the two reference techniques, concordance with HyCoSy was 83% and, therefore, greater than that between the two reference methods themselves (concordance of 76%). Similarly, all figures for

sensitivity, specificity, PPV and NPV were higher for HyCoSy as compared to HSG or L&D than for HSG as compared to L&D (Holz, 1997).

There is evidence suggesting that the combination of B-mode and Pulse Wave (PW) Doppler sonography may enhance the accuracy of HyCoSy, particularly because of improved visualisation of the distal tubal regions (Kleinkauf-Houcken, 1997).

The diagnostic accuracy of HyCoSy may be further improved by transvaginal tubal catheterisation (Bloechle, 1996). Such an intervention may particularly improve the negative predictive value of HyCoSy with Echovist[®]-200, which has been shown in all large studies to be inferior to the positive predictive value, probably largely because of tubal spasm of temporarily occluded tubes (Campbell, 1994, Hamilton, 1998).

There are several possible reasons for a *false negative* finding for tubal patency (i.e. the diagnosis of an occluded tube in the presence of an anatomically patent tube), including tubal spasm, differing resistance between the two tubes so that the tube with least resistance drains most of the contrast agent, and poor positioning of the catheter balloon leading to blockage of the interstitial segments of the tube.

Another explanation for the lack of concordance with a reference method might be that either spontaneous recanalisation or, for example, infection-induced occlusion of the tubes has occurred in the interval between the examinations.

Possible reasons for a *false positive* finding for tubal patency include misinterpretation of partial patency in the proximal segment of the tube after salpingostomy in the case of an ectopic pregnancy or the extremely rare case of a tubal fistula, a possible result of e.g. inflammatory small bowel disease. In addition, peritubal adhesions could affect tubal fertility even if patency is demonstrated.

Improved pregnancy rates have been reported as a consequence of HSG examinations (Cundiff, 1995, Rasmussen, 1991). Initial results indicate that pregnancy rates following HyCoSy are similar to those after HSG (Degenhardt, 1996, Gamble, 1998, Holz, 1994).

Also, information is now accumulating on the outcome of treatment strategies devised on the basis of the results of HyCoSy. In a study involving 500 consecutive women, Hamilton (1998) showed that there were no statistically significant differences in the clinical pregnancy rates per cycle at intra-uterine insemination between those women who were assessed by HyCoSy versus a matched cohort undergoing treatment simultaneously but which had been investigated by lap and dye test.

If performed as a first-line examination procedure - where HyCoSy is most appropriate - along with endocrinological and andrological investigations, the technique helps to direct the patients to optimised treatment at an early stage: If tubal patency is diagnosed with HyCoSy, the patient can then be spared invasive examination procedures and further steps in the diagnostic work-up and treatments such as hormonal stimulation therapy or insemination therapy can be initiated early on.

The discovery of abnormal findings on HyCoSy can then be followed either by the more invasive method of lap and dye, for example to investigate the ovum-intercepting mechanism and the ovaries or by preparing for therapeutic laparoscopy or microsurgery, the latter in specialised centres, based on an optimised estimate of the actual finding. Determination of tubal patency is essential prior to the start of treatment: Ovulation induction in anovulatory cycles, intrauterine insemination – a low-tech assisted conception technique utilised in unexplained infertility, abnormal mucous sperm test and moderate oligospermia - and microsurgery.

If tubal pathology is found, the degree of complexity of the surgical intervention which may be required can be accurately assessed. The HyCoSy examination indicates the degree of difficulty with which subsequent surgical laparoscopy might meet, enabling the best choice of clinical centre - i.e. surgical experience - to be made.

In general, however, experience in this special field of microsurgery is still available at only very few centres. Because of the greater success in assisted conception techniques such as IVF (In Vitro Fertilisation) and ICSI (Intracytoplasmic Sperm Injection), good microsurgical therapy holds hope of success in only a few, highly experienced centres. HyCoSy provides excellent information on uterine structure.

Another aspect of HyCoSy in patient management is its use to check on the results of surgical procedures such as microsurgery on the tubes, tube-conserving surgery following a tubal pregnancy, surgical hysteroscopic enucleation of myomas and sterilisation. As a consequence, HyCoSy helps to avoid unnecessary diagnostic and therapeutic procedures and to guide the patient to the most appropriate intervention, thereby saving the patient risks and valuable time and the health care system costs.

Comparison of methods

Laparoscopy

At present, diagnostic laparoscopy is arguably the best procedure for comprehensive assessment of the pelvis. It is, however, obvious that the uterine cavity cannot be assessed with this method unless combined with hysteroscopy. The advantages of lap and dye include highly reliable assessment of tubal patency assessment of pelvic pathologies such as endometriosis, adhesions and ovarian status and assessment of fimbrial function.

However, because of the following pronounced disadvantages, lap and dye should be reserved for a pre-selected group of patients (e.g. those with a suspected tubal factor) rather than being carried out on every subfertility patient (Jansen, 1997): invasive procedure requiring general anaesthesia associated surgical and anaesthetic morbidity (mortality rate 1:12,500) potential risk of injury to abdominal vessels and bowel post-operative pain requiring analgesia in up to 50% of patients recuperation period is longer than with alternative procedures may require hospitalisation costly in terms of medical resources required patients who need therapeutic laparoscopy are often subjected to second operation if not pre-screened.

HSG

Like HyCoSy, conventional HSG is an out-patient procedure that does not require general anaesthesia. Its diagnostic accuracy for tubal patency testing is comparable to that of HyCoSy. In contrast to HyCoSy, HSG provides static X-ray pictures, while HyCoSy is a real-time procedure with the option of video-taping for later reviewing.

The main disadvantage of HSG is the exposure of the female genital tract, the ovaries in particular, to radiation. In conjunction with the following additional limitations of HSG, it is obvious that this technique offers no benefits compared to HyCoSy while posing the following disadvantages to the patient:

- potential adverse reactions to iodinated contrast media
- requires referral to radiologist in radiology department
- patient-guided adaptation of procedure not possible
- patient separated from medical personal while being examined, therefore no psychological support of patient
- no real-time demonstration to the patient
- requires additional investigation to visualise the uterus, ovaries and other pelvic lesions

Consequently, HSG should be replaced by HyCoSy.

Inclusion criteria

In most cases, the couple should have been actively trying to achieve a pregnancy for at least 12 months before investigations are initiated, although this may be waived if the woman is more than 30 years old.

Ideally, HyCoSy is performed between the *eighth and thirteenth day* of the patient's menstrual cycle, since the cervical canal is physiologically dilated at this time and allows easier insertion of the intrauterine catheter. Moreover, a very early pregnancy can be ruled out in the first half of the cycle. Consequently, the procedure should be performed in the second half of the cycle only in exceptional circumstances. Once learned, the HyCoSy technique is relatively easy and quick to perform, taking only 10 to 15 minutes. After an acute infection of the cervix has been ruled out, the surface of the cervix should be disinfected before the examination catheter is introduced. The transcervical balloon catheter is inserted gently through the cervix using a pair of forceps, and the balloon is inflated with room air (1 - 2 mL depending on whether the patient is a primipara or multipara) to fix it in position. The pre-contrast scan may be suggestive of intrauterine pathology, which should be more specifically delineated after contrast instillation.

The position of the catheter or balloon is checked and the endometrium - to the extent that it can still be seen cranially in the region of the uterine body - assessed in the B image.

The amount of free fluid in the pouch of Douglas can be assessed in the sagittal plane – which is useful for comparison if any depots appear later.

With the currently available high-resolution ultrasound scanners, it is possible in the transverse projection to assess the region of the intramural tubal section - i.e., the examiner "notes" where the inner ostia of the two tubes are to be expected in the further course of the examination.

As Echovist[®]-200 passes along the Fallopian tube from the uterine fundus to almost always spill adjacent to the ovary, prior knowledge of the ovarian situation is essential to avoid unnecessary prolongation of the procedure for the patient and the use of larger volumes of Echovist[®]-200 than should be required. Potentially technically difficult Fallopian tube assessments can be predicted; they most often occur with an acutely retroverted or oblique uterine position, when the ovaries are situated very laterally or high in the pelvis or closely adjacent to the uterus, usually in the pouch of Douglas, or if multiple loops of overlying gaseous bowel are present.

The anticipated course of the tubes can be examined in the B image – in particular to rule out existing tubal pathology such as a hydrosalpinx.

The pre-contrast scan may also identify a cystic structure adjacent to the ovary in which case subsequent injection of Echovist[®]-200 should help to determine whether this represents a hydrosalpinx or a para-ovarian or fimbrial cyst.

Over and above this, imaging of the region of interest can be optimised before contrast instillation using the focus and zoom facilities of the ultrasound scanner.

The examination should be started with demonstration of the tubal ostia and intramural parts of the Fallopian tube, which are best assessed in the transverse projection. The suspension does not always enter both tubes at the same time because of different situations at the openings of the tubes, differing resistance in the sections of the tubes or a transient spasm. This should not, therefore, be taken as a sign of abnormality. Neither should it be considered an abnormality if the fluid does not immediately arrive at the section of the tube to be assessed. This may also be due to tubal spasm, so it is important to wait for at least 2 minutes before drawing any conclusions. When assessing the different segments of the tube, an attempt should first be made to demonstrate all segments by repositioning the transducer and following the presumed proximal to distal course of the tube. Not infrequently, the intramural and isthmic tubal segment can be demonstrated and then, because of the tortuosity of the tube, a distal ampullar segment or the fimbrial region. Unimpeded flow in the intramural section and in a far distal section makes uncomplicated patency of a section lying between them plausible as well. It must, however, be admitted that discreet "guitar string adhesions", i.e. extremely fine periovarian adhesions, may not be demonstrable and that this is where the HyCoSy method with Echovist[®]-200 finds its limits. If the ampullar section is patent, the Echovist[®]-200 suspension spills out over the ovary and can be visualised on the ultrasound scan. It may even be possible in some cases to visualise the infundibulum and fimbriae, although this is unusual and also unnecessary to establish tubal patency. The demonstration of an accumulation of Echovist[®]-200 around the ovary is one aspect of

assessment in the evaluation of tubal patency. At the same time, the non-accumulation of Echovist®-200 does not preclude tubal patency. On occurrence and intensification of pain, which is compatible with the picture of proximal unilateral or bilateral occlusion, the contrast medium should be removed from the uterine cavity again by means of re-aspilation. Applicable to all criteria is that the development of a hydrosalpinx must be ruled out.

Examination of the uterine cavity

The sonographic study of the uterine cavity should be commenced with a longitudinal projection. The normal endometrium then appears as a longitudinal oval of varying thickness depending on the particular stage in the menstrual cycle. The lining of the cavity can be scanned step by step by slightly altering the position of the scanner in the longitudinal and transverse direction. The balloon catheter in the uterine cavity appears as a ring-shaped, echogenic structure. Examination in a transverse projection allows demonstration of the shape of the uterine cavity and the bilateral intramural segments of the Fallopian tube. The uterine cavity is thus depicted in a typical triangular shape. The balloon catheter is situated in the lower part of the uterine cavity and is recognisable by its round shape and hypointense central zone. The uterine cavity fills first of all after injection of the contrast medium. If findings are normal, the run-off of the contrast medium via the intramural section into further distal tubal segments can be seen almost immediately. The current generation of high-resolution ultrasound scanners permits an assessment of the uterine cavity in about 80% of cases without the additional use of contrast material or the use of hydrosalpinx. Problems can arise in the differential diagnosis of polyps and myomas or - and this applies more to the postmenopausal patient - endometrial carcinoma. In addition, synechiae or adhesions in the region of the opening to the tubes cannot be demonstrated by plain ultrasound. Unless they are the result of an operation, deformities of the uterine cavity are due to congenital anomalies. However, some investigators believe that a prior injection of negative contrast (physiological saline) improves the examination of the endometrial cavity because it allows an exact assessment of the endometrium and exclusion of irregularities or anomalies. Only a narrow band of saline needs to be present in the endometrial cavity for assessment resulting in considerably less distension than is necessary for hysteroscopy. Some experts recommend the use of saline prior to Echovist®-200 only if abnormality of the uterine cavity was observed on the baseline scan. Laparoscopy and hysteroscopy are always advisable if a major uterine anomaly is found at HyCoSy.

Criteria demonstrating tubal patency

During the entire examination, it should be possible to see run-off of Echovist®-200 without the formation of a hydrosalpinx. The demonstration of the emergence of contrast medium and flow around the ipsilateral ovary should be visible. A 10-second flow profile (continuous anterograde flow) in the interstitial section of patent tubes is seen in the B-mode. A hydrosalpinx must be ruled out in addition. The reliability of this criterion is increased by positioning a pulsed-wave Doppler gate beyond the presumed interstitial section of the tube and demonstration of a 10-second flow profile in duplex mode. The flow velocity demonstrated should be proportional to the injection pressure.

Complementary to the B-mode scan and pulse-wave demonstration, the colour-coded Doppler and power Doppler mode also permits demonstration of the course of the tube.

Possible fluid depots in both adnexal regions and in the pouch of Douglas in particular should be assessed at the end of the examination. In extremely unfavourable examination conditions, the demonstration of large amounts of free fluid in the pouch of Douglas would be a sign that at least one tube is patent. In cases of intramural tubal occlusion, it is important to determine whether the occlusion is unilateral or bilateral. If both openings to the tubes are closed, the uterine cavity will become distended as more Echovist[®]-200 is injected, and this may cause pain. If the occlusion is unilateral, the uterine cavity may not become distended. In some cases, increasing the pressure in the uterine cavity might allow the Echovist[®]-200 suspension to open the blocked tube. An isthmic occlusion may appear as an abrupt interruption of the band-shaped course of the contrast media. Dilatation of the tubal lumen by the suspension is unlikely in this section of the tube since the pressure exerted by the fluid is not particularly high. The patient usually feels a dragging pain on the relevant side which can be used to support the diagnosis.

Ampullar occlusion usually causes fluid to collect in the tubal lumen resulting in a hydrosalpinx; although this is only demonstrated after a delay in most cases. A hydrosalpinx which can already be seen in the B image makes Echovist[®]-200 administration superfluous. Longitudinal scanning shows a cylindrical structure filling the ampulla and tapering towards the uterus. In the transverse projection, the suspension can be seen in the dilated lumen of the tube. The distension causes pain in the vast majority of patients, although the ampulla can accommodate 20 - 25 mL of fluid without causing pain in rare cases. If a hydrosalpinx is demonstrable even in the baseline scan and the examination is being performed to demonstrate the contralateral side, the contrast media will become visible only slowly. It is sonographically demonstrable until just before the sactosalpinx, and appears in the ampulla only after a delay as it becomes diluted by the fluid already present. The cylindrical shape of the sactosalpinx then becomes visible, although the echogenicity may still be reduced.

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DELIVERY OF THERAPY AND DRUGS WITH BUBBLES AND ULTRASOUND

Evan Unger

University of Arizona & ImaRx Pharmaceutical Corporation, USA

Microbubbles are now FDA approved for limited cardiology applications. Soon, effective microbubble contrast agents will be FDA approved for radiology applications. As these agents enter routine clinical radiology practice we will begin to see the full potential of ultrasound contrast agents in clinical practice. The enhanced ability to visualize flow and characterize tissues will likely make ultrasound contrast agents an important part of radiologic ultrasound practice. Therapeutic applications of microbubbles could ultimately prove to be even more important than the already appreciable diagnostic utility of these agents, however.

The potential of microbubbles as therapeutic entities dwarfs their diagnostic utility. Not only do microbubbles reflect sound, important for their role as ultrasound contrast agents, they also can act as efficient absorbers of ultrasound energy. One of the ways ultrasound contrast agents (microbubbles) can effectively absorb sonic energy is through cavitation. When cavitation occurs, the microbubbles may resonate or oscillate and expand and contract in concert with the sonic energy. When energy reaches a certain threshold the microbubbles collapse catastrophically, destroying or breaking up the microbubble³⁻⁴. Figure 1 (below) adapted from work by Holland and Apfel shows how microbubbles lower the threshold of energy for cavitation and how lower frequency ultrasound is generally more efficient at causing cavitation.

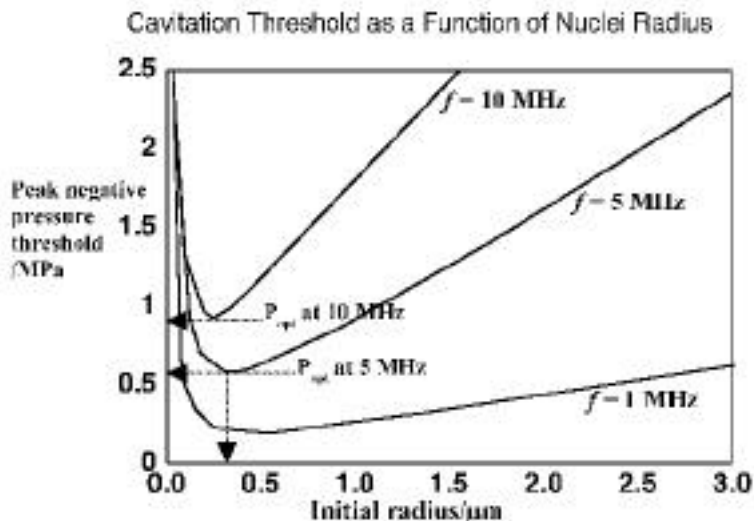
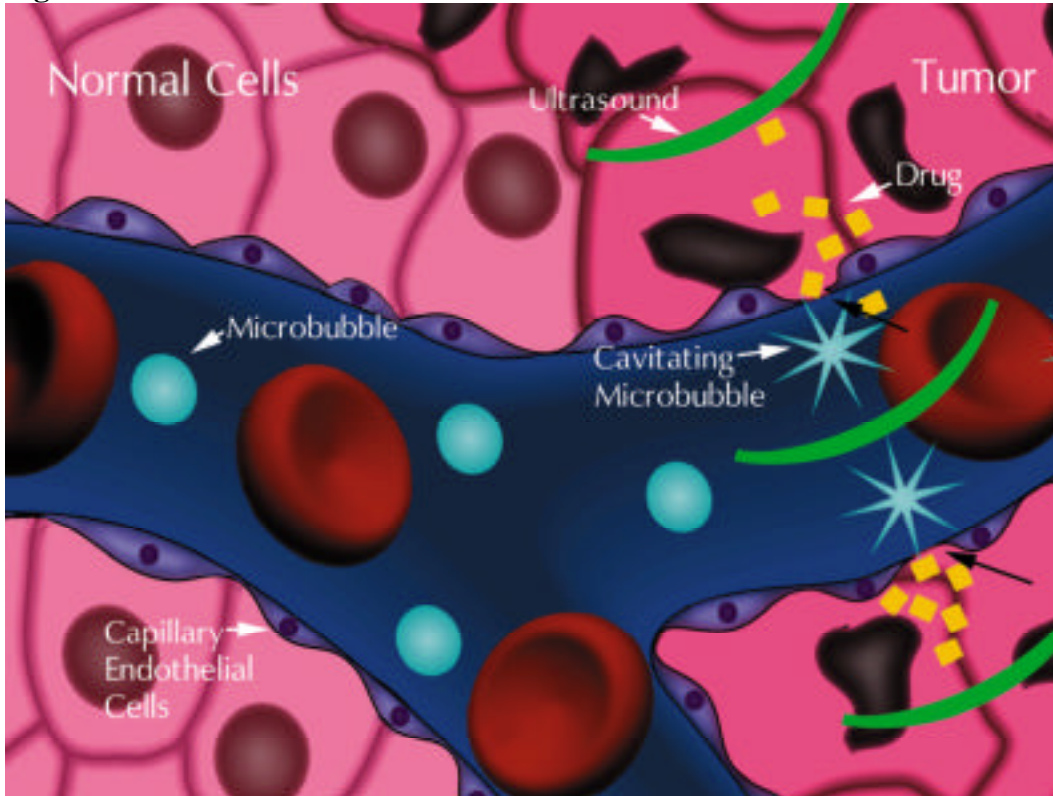


Figure 1

Microbubbles can be used as ultrasound sensitive carriers for site-specific drug delivery and gene therapy. Microbubbles may be co-delivered with drugs and ultrasound applied to the target tissue⁵. Experimental work from Sanjiv Kaul's group showed that microbubbles in concert with ultrasound increased capillary permeability and caused

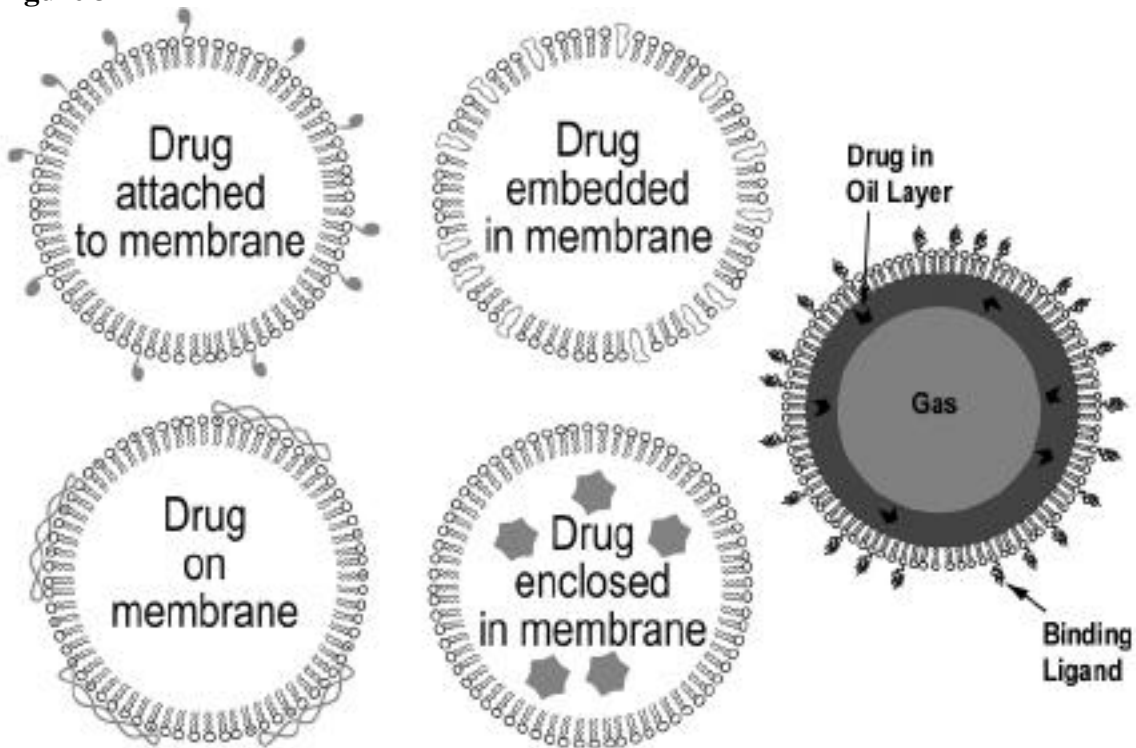
nanoparticles to be delivered into the interstitial tissues of the insonated region^{6,7}. Our own work has also shown that dye loaded into microbubbles is delivered locally to tissues through application of ultrasound⁸.

Figure 2



Drugs can be loaded into or onto microbubbles so that the ultrasound energy is concentrated just where the drug is spatially present. Microbubbles can be formulated to carry therapeutic agents in a number of ways. Hydrophilic compounds can be encased within lipid membranes or polymeric shells stabilizing microbubbles. To incorporate hydrophobic compounds, an oil layer, in which the compound has been suspended, is incorporated into the microbubbles creating acoustically active lipospheres (AALs). Genetic material can be incorporated into microbubbles that have negatively charged lipids in their membranes.

Figure 3



The ability of microbubbles to cavitate under the range of energy used in diagnostic ultrasound makes them an ideal delivery vehicle. The circulation of microbubbles can be followed via ultrasound and when the microbubbles are in the area of interest they will be destroyed, releasing their therapeutic payload to the surrounding tissue. In addition, the cavitation of microbubbles in the capillary beds will increase capillary permeability, allowing the released therapeutic agent better access to the interstitial tissue.

As a further extension of therapeutic delivery, microbubbles can be labeled with targeting ligands to direct the bubbles to cell specific receptors. Endothelial cell receptors and thrombosis can be targeted by specific ligands designed to interact with particular receptors. A number of receptors are expressed on endothelial cells including the receptor for Vascular Endothelial Receptor (VEG-f) and a variety of different integrins⁹. Some of these receptors are expressed or upregulated in particular diseases such as angiogenesis associated with cancer, inflammation, ischemia or atherosclerosis. By targeting microbubbles through the use of ligands binding to these specific receptors we may be able to image and diagnose specific diseases and also perform therapy with ultrasound and bioactive materials.

Our group is working on a targeted microbubble agent to image and treat thrombus^{10,11}. MRX-408 has a surface ligand comprising a peptide which binds to the GPIIB/IIIA receptors on activated platelets, allowing the microbubbles to bind to thrombus and deliver thrombolytic agents. Figure 4 is a schematic diagram of an MRX-408 microbubble bearing targeting ligands to thrombus.

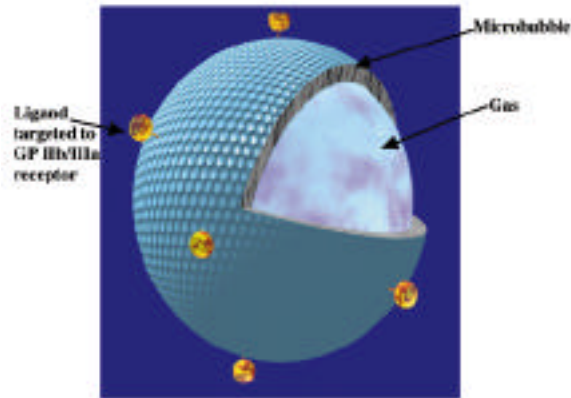


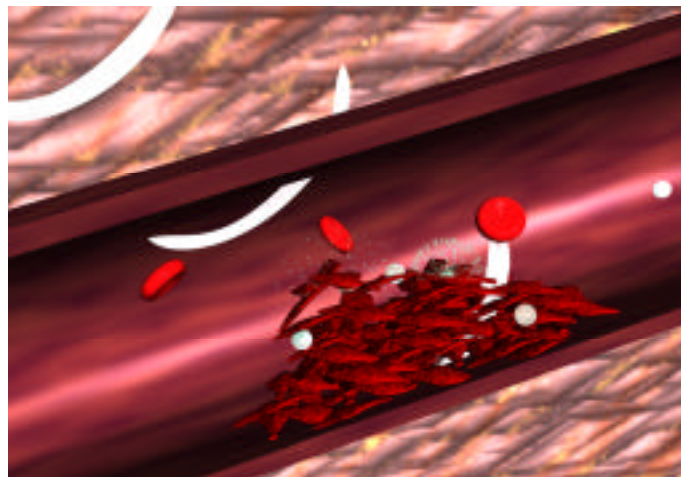
Figure 4

MRX-408

Vascular imaging with MRX-408 shows that the targeted microbubbles act like a "hot spot" agent to selectively enhance blood clots. Pre-clinical imaging has shown that this agent improves radiologists' confidence in detecting blood clots^{10,11}.

Experimental studies *in vitro* and in animals have shown that microbubbles and ultrasound are effective agents for thrombolysis^{12,13}. As shown in Figure 5, by concentrating the microbubbles on the clot through targeting ligands, the therapeutic effect is concentrated at the clot. Sonothrombolysis using clot-specific microbubbles could be very useful clinically. The technique may allow transcutaneous application of ultrasound (i.e. non-invasive) to effectively and rapidly lyse thrombolysis.

Figure 5



The most significant therapeutic applications of microbubbles could be in gene therapy. Gene delivery is difficult and has a number of hurdles to overcome¹⁴⁻²². Microbubbles and ultrasound could help to overcome the obstacles impeding the progress of gene therapy.

Genes are macromolecules that encode the sequences for production of proteins. In gene therapy, genes must be introduced into specific cells and the gene usually must be transcribed in the cell nucleus. After transcription of the gene an RNA transcript is

produced which is then translated into protein. A wide variety of different genes may potentially be used therapeutically to treat cancer²³, immune disease²⁴, cardiovascular disease²⁵ and congenital diseases. The development of successful clinical gene therapy has been delayed by difficulties in delivery genes to the right cells as well as in sending the genes to right cellular compartments. New and improved gene delivery systems are needed for successful gene therapy. In order for gene therapy to be successful, however, the genes must be delivered to the target cells and be expressed. Ultrasound might be used in conjunction with acoustically active carriers to overcome many of the problems with gene delivery.

Acoustically active carriers can be designed to encapsulate genes and other genetic material²⁶. These carriers can be administered intravenously and ultrasound applied to the target tissue. Localized gene delivery and transgene expression is attained in concert with acoustically active carriers and ultrasound²⁷.

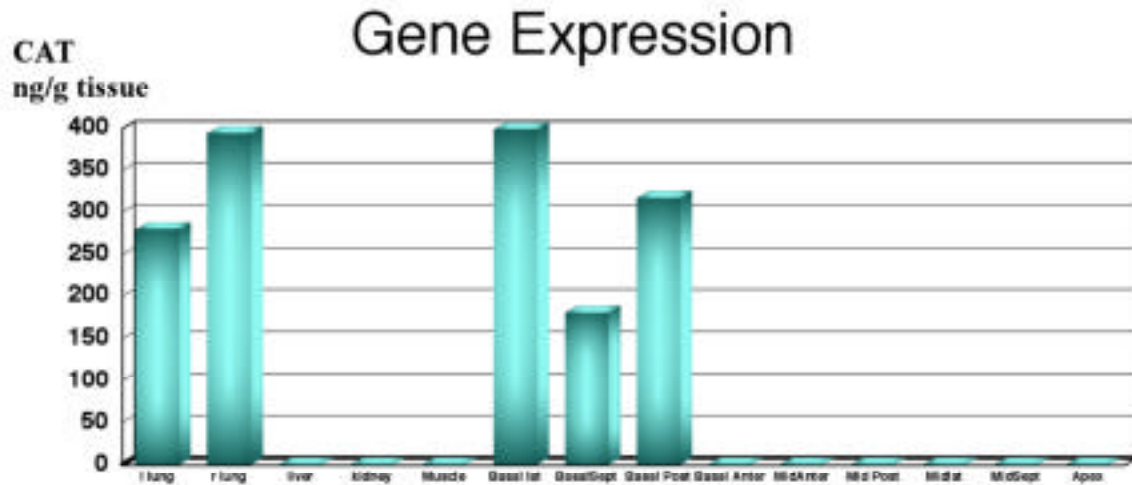
We have developed microbubbles and fluorocarbon emulsions to encapsulate genes²⁸⁻³⁰. Genetic materials are generally made of polymeric polyanions. These polyanions (negatively charged) can be electrostatically bound by positively charged structures. We have made formulations of microbubbles and emulsions bearing positive surface charge. These materials, under proper conditions, will bind 100% of DNA.

Microbubbles composed of cationic and neutral lipid are gently agitated by hand with aqueous suspension of DNA. The DNA coated microbubbles are then ready for IV injection.

Studies have been performed *in vitro* in cell culture and *in vivo* in mice, rats and dogs. Recent dog studies are illustrative of the potential of gene delivery with acoustically active carriers and ultrasound³¹⁻³². Cationic microbubbles binding the gene for chloramphenicol acetyl transferase are injected IV. Focussed ultrasound at 1.2 megahertz is applied transthoracically to the left ventricle at MI = 1.7. This results in delivery of the CAT gene to the left ventricular myocardium and high levels of gene expression within the insonated tissue. Fluorescent *In Situ Hybridization* (FISH) confirms distribution of gene expression within the insonated tissue.

Figure 6 shows the enhancement in expression of CAT gene in the heart following IV injection of microbubbles containing the CAT gene. The highest levels of gene expression were observed in the myocardium within the focal zones of ultrasound³³. Therapeutic genes such as VEG-f might be delivered this way, IV injection of microbubbles carrying genes, with ultrasound application to the target tissue, e.g. to the myocardium to treat ischemic disease.

Figure 6



Conclusions

Acoustically active carriers can be formulated as drug delivery and gene delivery vehicles. Ultrasound energy within the range proscribed for diagnostic ultrasound can be used to rupture these microbubbles and deliver therapeutic agents and genes locally to a tissue. High levels of local drug delivery and, in the case of gene delivery, gene expression are attained. Ultrasound and acoustically active carriers might represent a “magic bullet” for targeted gene delivery to regional tissue. Microbubble assisted sonothrombolysis may represent a whole new paradigm in treating vascular occlusions. Ultrasound enhanced gene delivery could be used to deliver genes to different tissues for treatment of vascular disease, cancer, immune and congenital disease. Work to date has primarily been with marker genes, which have made proof of concept and helped to develop this new technology. In the subsequent phase of development, more work will have to be done with therapeutic genes to assess the potential of this technology in animal disease models. Pending successful results in *in vivo* disease models, this new technology will need to be tested extensively in humans in clinical trials.

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