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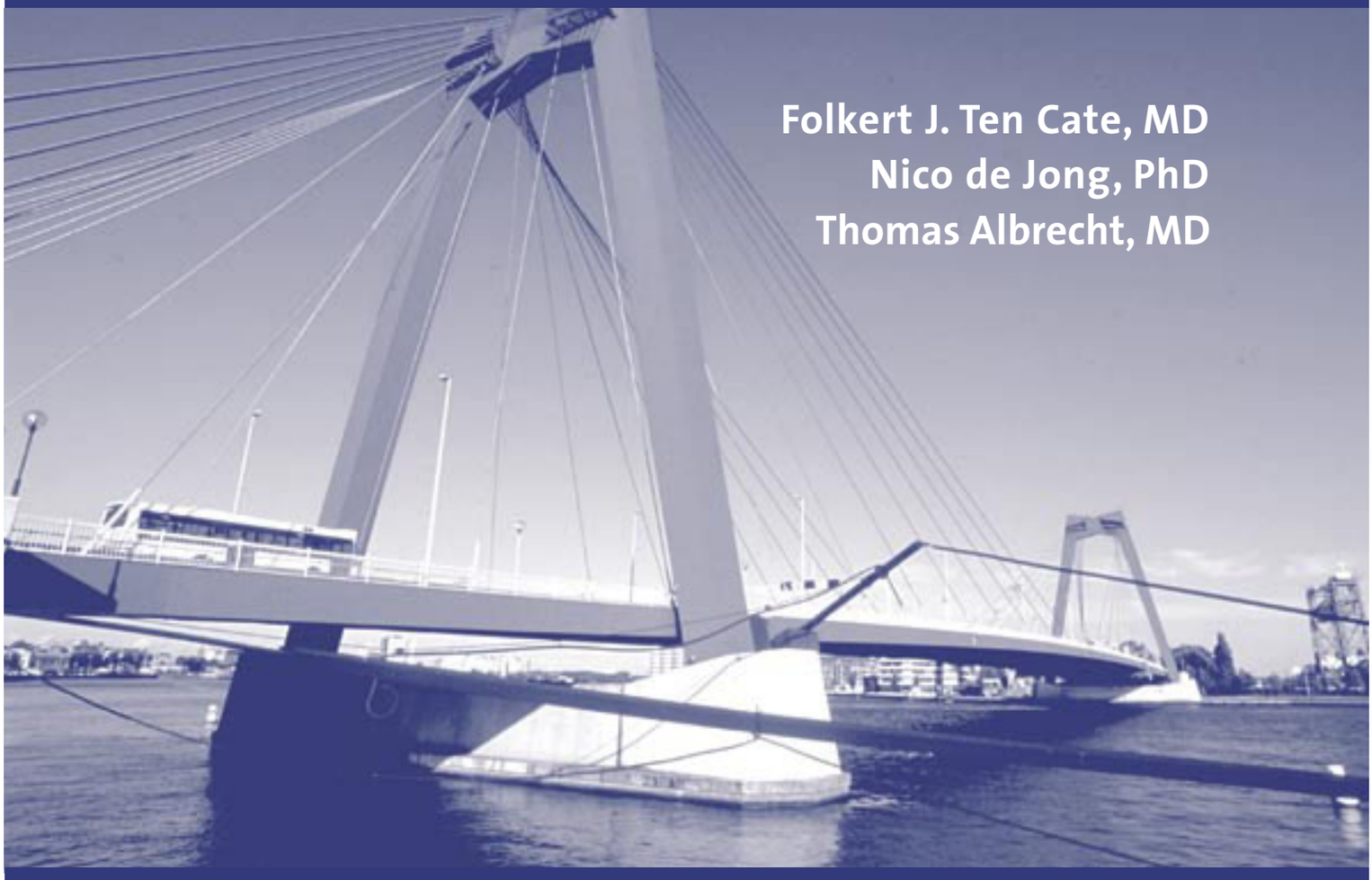
The Thirteenth European Symposium on Ultrasound Contrast Imaging



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Abstract book

January 24-25, 2008, Rotterdam, The Netherlands

13th EUROPEAN SYMPOSIUM ON ULTRASOUND CONTRAST IMAGING
24-25 JANUARY 2008, Rotterdam, The Netherlands

WEDNESDAY, 23 January 2008

18.00 - 20.00 **Registration - Welcome Drinks - Posters** ..... **Hilton Hotel**

THURSDAY, 24 January 2008

08.00 - 09.00 **Registration**

09:00 - 09:15 **Opening** .....
Folkert ten Cate — Message from the President of the European Association of Echocardiography of the European Society of Cardiology concerning Echo Contrast Agents

09.15 - 10.30 **SAFETY AND NEW CONTRAST APPLICATIONS** ..... *Chairpersons: K. Tiemann/T. Albrecht*
Peter Burns — Safety of UCA: Facts and nonsense .....
Mark Monaghan — 3D Echo and Contrast: A perfect marriage? .....
Thomas Porter — Stress MRI versus stress MCE: Evolving high resolution perfusion techniques to detect CAD .....
Andrew Needles — Tracking Contrast Agent Enhancement Dynamics in Mice with Subharmonic Micro-Ultrasound and Constant Infusion .....

10.30 - 11.00 **Intermission**

11.00 - 12.30 **TECHNOLOGY I** ..... *Chairpersons: A. Bouakaz/M. Averkiou*
Dave Goertz — Bubbles for vaso vasorum imaging .....
Alexander Doinikov — Current simulation models of medical bubbles .....
Rik Vos — Radial manipulation of single microbubbles .....

Svein-Erik Måsøy — SURF Imaging – High-frequency ultrasound contrast agent imaging in patients with prostate cancer .....
Meng-Xing Tang — Automatic Attenuation correction in Ultrasound Contrast Agent Imaging .....

12.30 – 14.00 **LUNCH**

14.00 - 14.30 **ICIN*-Lecture** ..... *Chairperson: N. de Jong*
Steve Feinstein — Contrast in vascular medicine .....

14.30 – 15.45 **VASO VASORUM, CARDIOVASCULAR IMAGING AND THERAPY** *Chairpersons: P. Burns/M. Monaghan*
Juan Granada — *Vaso Vasorum using with IVUS* .....
Klaus Niemann — Ultrasound therapeutic applications: Image-target-therapy: Local gene-delivery in cardiovascular structures .....
Joshua Rychak — Contrast Ultrasound Imaging of Inflammation .....
Linsey Phillips — In vivo Ultrasound-mediated Gene Transfection from Microbubble Carriers to Site of Vascular Injury .....
Lynda Juffermans — Mechanisms of delivery of therapeutic compounds by ultrasound and microbubbles .....

15.45 – 16.15 **Intermission**

16.15 – 17.30 **COMPETING TECHNOLOGIES** ..... *Chairpersons: T. Porter/O. Kamp*
Thomas Albrecht — CEUS for monitoring and follow-up of radiofrequency ablation of the liver .....
Riccardo Lencioni — Clinical role of CEUS of the cirrhotic liver in comparison to other modalities (CT, MRI) .....
Eddie Leen — Clinical role of CEUS of the non-cirrhotic liver in comparison to other modalities (CT, MRI) .....
Nathalie Lassau — Dynamic contrast-enhanced ultrasonography (DCE-US) with quantification for the early evaluation of anti-angiogenic treatments in preclinical and clinical studies .....
Hans Peter Weskott — Volume Calculation in Hyper-vascular Liver Tumors: Comparing B-Mode Ultrasound to Different Phases uring Contrast Enhanced Ultrasound imaging (CEUS) .....

18.30 - 22.30 **SOCIAL EVENT (Incl. Dinner buffet)** .....

***ICIN = The Interuniversity Cardiology Institute of the Netherlands**

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FRIDAY, 25 January 2008

07.30 - 08.00 **Registration**

07.30 - 09.00 **POSTER DISCUSSION A** …….. .....*Moderator: Folkert ten Cate*

1a)	Klazina Kooiman	A new drug delivery system for liphophilic drugs using polymeric ultrasound contrast agents.....
2a)	Raffi Bekeredjian	Augmentation of AAV-mediated cardiac gene transfer using ultrasound targeted microbubble..... destruction in rats.....
3a)	Christian Mayer	Spatial distribution of ultrasound targeted microbubble destruction increases cardiac transgene expression ....
4a)	Kadija Kaddur	Transmembrane RELEASE OF Green fluorescent proteins with Ultrasound and BR14 Microbubbles .....
5a)	Gord Lueck	Images of Hepatic Perfusion Index using Microbubble Contrast and Factor Analysis.....
6a)	Aman Thind	Ultrasound contrast agents for accelerating collagenase activity in Chronic Total Occlusion .....
7a)	John Hudson	Quantitative Parametric Images of Disruption-Replenishment: Application to an Anti-Angiogenic Clinical Trial .

07.30 - 09.00 **POSTER DISCUSSION B** .....*Moderator: Nico de Jong*

1b)	Marlies Overvelde	Non-linear resonance behavior of ultrasound contrast agent microbubbles.....
2b)	Valeria Garbin	Secondary Bjerknes forces between ultrasound contrast agent microbubbles .....
3b)	Francesco Conversano	Exploiting Dissolution Behavior of Phospholipidic Microbubbles for diagnostic and therapeutical purposes .....
4b)	Ceciel Chlon	Experimental Investigation of the Effect of Shell Compositions on Acoustic Properties of PolymerMicrocapsules
5b)	Mirza Pasovic	Local Reduction of second Harmonic due to Ultrasound Propagation: mulation and feasibility.....
6b)	Nikos Pelekasis	Characterization of UCA Behaviour - Stability and Simulations…… .....
7b)	Olivier Couture	Time-reversal for the correction of non-linear propagation in pulse-inversion imaging .....
8b)	Guillaume Matte	Benefits of superharmonic techniques for contrast imaging .....

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FRIDAY, 26 January 2007

09.00 - 10.30	**TECHNOLOGY II**	*Chairpersons: M. Versluis/A. van Wamel*
Mike Averkiou	Quantification of tumor angiogenesis with ultrasound contrast agents: Measurement of tumor response to Avastin	
Alexander Klibanov	Microbubbles Targeting in fast flow conditions: Molecular Biomechanics Influences Targeted Imaging	
Eleanor Stride	Microbubble Engineering	
Massimo Mischi	Imaging effects of Ultrasound nonlinear propagation through varying contrast concentrations	
Jeroen Sijl	Acoustical resonance behavior of single ultrasound contrast agent microbubbles	
10.30 - 11.00	**INTERMISSION**	
11.00 - 12.30	**TUMOUR APPLICATIONS OF MICROBUBBLES**	*Chairs: A. Klibanov/P. frinking*
Hessel Wijkstra	Prostate imaging: What is the best modality?	
Heinrich von Busch	Prostate imaging - the case for contrast ultrasound	
Beat Kaufmann	Molecular Imaging of Inflammation in Cardiovascular Diseases	
Annemieke van Wamel	Molecular mapping of murine tumor vasculature biomarkers using targeted contrast high-frequency ultrasound imaging	
James Yeh	Quantification of E-selectin Expression in the Mouse Heart Using Targeted Microbubble Contrast Enhanced Echocardiography	
12.30 - 13.45	**LUNCH**	

Announcement of the winners of the Martin Blomley poster prize and the technical poster-prize.

13.45- 15.15	**CLINICAL CASES**	*Chairpersons: F. ten Cate/P. Burns*
Otto Kamp	Adverse reactions to ultrasound contrast agents: is the risk worth the benefit?	
Steve Feintstein		
Pavel Taimr	Liver imaging	
Osama Soliman	Adenosine contrast and fractional flow reserve	
15.15 - 15.30	**DISCUSSION AND CONCLUSIONS**	*Folker ten Cate-Nico de Jong*
15.30	**ADJOURN**	

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13th European Contrast Symposium
Rotterdam, 24-25 January 2008

Safety of Ultrasound Contrast: Sense and Nonsense

Peter N. Burns
Professor and Chairman of Medical Biophysics
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Microbubble contrast agents have been approved for clinical use in more than 60 countries; both clinical and basic science work continues to elucidate new applications and ever-broader potential contributions that they offer to both medical care and basic science. Yet their clinical and commercial future continues to be dominated by the decisions of a single organistion: the US Food and Drugs Administration (FDA). Unlike the reasoning presented in the rest of this Symposium, that of the FDA is not subject to peer-review, public discourse or even disclosure. Scientists, clinicians, consumers and companies are can only attempt to piece together the logic of their directives by inference. Here, we strive to make sense of their latest pronouncements.

Background: The past 15 years

The FDA approved the first contrast agent (*Albunex*, Molecular Biosystems Inc) for human use in 1994. Subsequently *Optison* (Mallinkrodt Inc) was also approved for cardiac indications around 1999. It may be significant that these were both considered by the FDA as devices, rather than drugs, and that for *Optison* there was an associated legal action against the FDA initiated by a bubble company. *Definity* (Bristol-Myers Squibb Medical Imaging (BMS)) was the first contrast agent to be approved by the FDA as a drug, in 2001, again for LV opacification indications only. Many subsequent attempts have been made, with numerous agents, to gain approval from the FDA for indications involving imaging of the abdominal organs or of myocardial perfusion, but none have been successful. In the meantime, comparable national regulatory agencies in the European Union, Canada, Japan, Australia, China and South American countries have approved microbubble agents for radiological, as well as cardiac, indications.

In 2006, The American Institute of Ultrasound in Medicine (AIUM) founded a contrast task force under the leadership of the President, Dr Len Greenbaum, to try to overcome the apparent impasse in the US. The AIUM wrote to the FDA, stating that the "*… the lack of availability of CEUS for non-cardiac imaging in the United States hinders the delivery of optimal diagnostic imaging services to our patients. As a result, we lag behind the rest of the world in the appropriate and proven uses of contrast agents for liver mass diagnosis. The AIUM believes that this is having an adverse impact on clinical care.*" (1) The FDA agreed to a series of meetings with members of the Task Force to discuss a way forward. The FDA stressed during these meetings that it had no safety concerns about currently approved agents, but that it did not consider that efficacy data emerging from pivotal clinical trials were adequate. The outcome of

these meetings was a decision taken by the AIUM, encouraged by senior FDA representatives, to initiate an investigator-led clinical trial with the aim of gaining approval for an indication in radiology. ACRIN, the NIH-funded clinical trials branch of the American College of Radiology, a group with enormous experience in clinical trials in medical imaging, agreed to participate. Protocols were drafted and circulated.

The past three months

Then, on 10 October 2007 the FDA issued a "black box" warning for perflutren-containing ultrasound contrast agents, quoting post-marketing reports of four deaths occurring within 30 minutes of administration of *Definity*. The new label contraindicates its use in patients with acute coronary syndromes, acute myocardial infarction, and worsening or clinically unstable heart failure. The label also stipulates monitoring ECG and oxygen saturation for 30 minutes *for all patients* following administration (2). Other regulatory agencies have passed on a letter from BMS to clinical users informing them of these recommendations (3) but at the time of writing, neither Health Canada nor the EMEA in Europe (where *Definity* is approved under the name *Luminity*) have announced a change to the labelling.

There has been a vigourous response to the decision by the community of contrast agent users, best summarized in the opinion paper by Main et al published by expedited peer-review in the week before Christmas in the Journal of the American College of Cardiology (JACC) (4). They point out that for the four deaths occurring within 30 minutes of administration of Definity, no causal relationship is posited, in fact, these patients had such serious co-morbidity (#1: ischemic cardiomyopathy and stress; #2: acute MI in the ICU; #3: acute heart failure, DVT and PE; #4: cardiomyopathy and acute PE) that at least some of the deaths could be attributed to 'pseudo-complication' (4). Post marketing surveillance has covered more than 2 million injections, so even if their deaths were directly attributable to the injection of the contrast agent, the aggregate risk of death would be 1:500,000. The risk of death in angiography is 500 times greater, and in treadmill testing 20 greater than this figure (4). Other studies, including extensive preclinical studies and the Phase III trials for *Definity* on 1,700 patients showed no safety concerns (5). Post marketing surveillance of *Definity* and its European relative *SonoVue* (Bracco) show that the predominant cause of severe adverse events is anaphylactoid reaction, with an estimated rate of 1 in 7,000 for both drugs. This rate is comparable to that of most analgesics and antibiotics and lower than that of other imaging contrast agents, such as those used in, for example, CT scans (6). A 2006 study of more than 23,000 injections of *SonoVue* showed no deaths and 2 serious adverse events, giving a measured serious adverse event rate of less than 1:10,000 (7). All adverse events were detected within 3 minutes of injection (8).

In view of these data, it is very hard to understand the 30-minute monitoring requirement stipulated by the FDA for all patients, even those not within risk categories defined in the label. Here some insight might be provided by the substance of a 2-hour meeting held during the RSNA Assembly in Chicago in the last week of November with the (Acting) Deputy Head of the Medical Imaging Division of the FDA, which is responsible for this decision. In it, he stressed that the FDA has access to data that is not publicly available. Among these, he stated, is evidence from both Phase 1-3 trials and post-marketing surveillance that large numbers of patients (of the order of 1 in 20) suffer acute cardiopulmonary symptoms following injection of microbubble contrast. He declined to substantiate this and other extraordinary assertions.

There have been numerous requests to the FDA to disclose and discuss the scientific basis of their labelling decision by convening a panel of experts to assess the data and establish the best corrective action. The AIUM has stated: *"The Bioeffects Committee of the AIUM has announced*

that it cannot comment on the FDA statements concerning the safety of ultrasound contrast unless it can review that data. A multi-specialty group is considering whether to pursue the investigation of the four deaths independently or in conjunction with the FDA". In the circumstances, one might reasonably expect that the evidence for such a decision made by a publicly accountable agency be presented to the scientific literature for peer-reviewed publication. In fact, without such a panel, the FDA is under no obligation to reveal evidence upon which its decision was made to anyone.

Conclusion

We conclude that common sense dictates that the regulatory authorities of jurisdictions outside the US should hold a public advisory panel to discuss the scientific evidence before deciding on a change of labelling for this or any microbubble contrast agent. It is appropriate that members of this Symposium should encourage, and if necessary be willing to participate in, such a process.

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Real Time Three-Dimensional Myocardial Contrast Echocardiography: A perfect marriage of two technologies.

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A Bhan, S Kapetanakis, BS Rana, E Ho, K Wilson, P Pearson, S Mushemi, J Deguzman, J Reiken, MD Harden, MJ Monaghan

Background- 2D low mechanical index (LMI), contrast specific, myocardial perfusion imaging is now an accepted technique. We evaluated the clinical feasibility of a real time three-dimensional echocardiography (RT3DE) LMI implementation.

Methods- 46 patients undergoing contrast enhanced dobutamine stress echo were imaged using a Philips iE33 system, with novel 3D LMI power modulation software. All patients underwent contrast enhanced 2D and RT3DE image acquisition, in left ventricular opacification, and LMI perfusion modes.

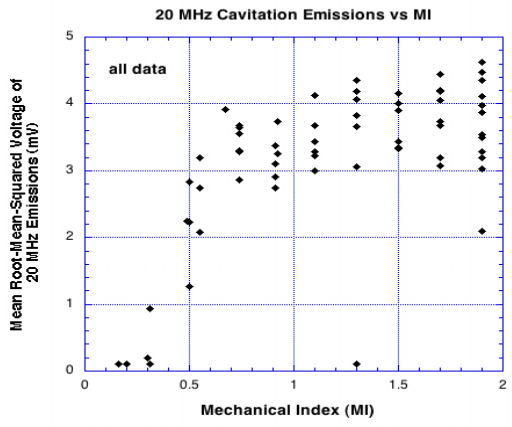
Results- Of the 736 evaluated segments, wall motion could be assessed in 98.6% and perfusion in 96.2% of 2D, and 98% and 95.2% in 3D. 661 were normal in 2D and RT3DE demonstrated normal myocardial opacification in 83.8%. 34 were akinetic, with no perfusion in 2D and of these RT3DE revealed a perfusion defect in 31 (91%, p= NS).

Conclusion- LMI RT3DE evaluation of myocardial perfusion is feasible. It has the potential to accurately locate and possibly quantify perfusion defects.

Detection of Intravascular Cavitational Activity during Treatment of Deep Vessel Thromboses with Diagnostic Ultrasound and Intravenous Microbubbles

Feng Xie[1], Carr Everbach[2], Terry Matsunaga[3], John Lof[1], Anming He[4], Richard M Bennett[4], Thomas R Porter[1]. [1]University of Nebraska Medical Center, Omaha, NE;[2]Swarthmore College, Swarthmore, PA;[3]ImaRx Therapeutics Inc, Tucson, AZ;[4]Siemens Medical Solutions, Mountain View, CA

Background. Although ultrasound and microbubbles are able to recanalize acutely thrombosed vessels, the mechanism for thrombus dissolution is unknown. We have hypothesized that cavitation of microbubbles may play a role, but it is unknown whether cavitation occurs within thrombosed vessels in living systems. The purpose of this study was to determine whether cavitation within a thrombosed vessel could be documented during transcutaneous ultrasound and intravenous microbubble treatment of intravascular thrombi.
Methods. In two dogs with acutely thrombosed arteriovenous grafts (duration of occlusion 150 minutes), a low frequency (1.5 MHz) diagnostic transducer was applied transcutaneously through a six centimeter thick tissue-mimicking phantom (attenuation 0.49 db/cm/MHz) during a continuous intravenous infusion of microbubbles (MRX 801: Imarx Therapeutics Inc). In the first phase of the study, a 20 MHz cavitation detector was placed adjacent to the ultrasound transducer so that its focal point was within the intravascular thrombus. Recordings of cavitational activity (CA) within the vessel were recorded at specific time intervals of treatment (0, 6, 12, 20, 25, 30, 40, and 45 minutes), as well at different MI settings (ranging from 0.3 to 1.9). These conditions were then correlated with the degree of recanalization achieved by conventional angiography. In the second phase of the study, 21 thrombosed grafts were divided into two groups for treatment: (a): low MI ultrasound alone (<0.4 using Contrast Pulse Sequencing or CPS; Siemens Acuson) during microbubble infusion, and (b) intermittent high MI ultrasound impulses delivered when low MI CPS detected microbubbles within the thrombus. **Results.** CA was detected at the 1.9 MI impulses when microbubbles were visualized within the thrombus, with the degree of CA increasing as flow within the vessel increased. When flow was restored, CA was evident within the vessel down to an MI of 0.5, below which virtually no cavitation could be observed (Figure). The angiographic success rate was 69% at 30 min and 77% at 45 min in high MI ultrasound guided by low MI CPS, compared to only 22% at 30 min and 33% at 45 min in low MI ultrasound alone group ($p<0.05$).
Conclusion. Intravascular cavitation occurs with ultrasound induced sonothrombolysis, and appears to be essential for successful recanalization.



Tracking Contrast Agent Enhancement Dynamics in Mice with Subharmonic Micro-Ultrasound and Constant Infusion

A Needles[1], J Sun[1], J Graba[1], C Bilan[1] and FS Foster[2]
1. VisualSonics Inc., Toronto, Canada
2. Sunnybrook Health Sciences Centre, Toronto, Canada

We have previously presented a real-time micro-ultrasound 2D/3D Subharmonic (SH) imaging mode with the capability of high speed sequential image acquisition, used for tracking enhancement dynamics (Needles et al. IEEE UFFC Symposium, 2007). This talk will provide an overview of the current state of high-frequency contrast imaging using micro-ultrasound. A summary of current fundamental frequency-based image subtraction techniques will be given, along with an update of the newly implemented SH mode and recent experiments utilizing the constant infusion of contrast agent. *In vivo* results will compare both fundamental and SH real-time image sequences and 3D data sets, while outlining the relative advantages and limitations of both modes.

A modified HF ultrasound scanner (Vevo770^{TM},VisualSonics, Toronto) was operated at 30 MHz in fundamental contrast mode (F30), as well as in SH imaging mode (SH15) with transmit/receive at 30/15 MHz. A 25 MHz centre frequency transducer (RMV-710B, f-number 2.1, 15 mm focal length) was used at 25-70 fps. For 3D imaging the transducer was mounted on a linear stepper motor and translated over an imaging region (~10-15 mm) in less than 20 s. *In vivo* mouse imaging was conducted in xenograft human melanoma (MeWo) tumours in nude mice, and in the hind limb and kidney of healthy CD-1 mice. MicroMarker^{TM} (VisualSonics, Toronto) contrast agent was injected either by using a 50 µL bolus ($2x10^8$ bubbles/mL) or through constant infusion driven by a syringe pump ($1 x10^9$ bubbles/mL at 20 µL/min). With the constant infusion method, a 10 MHz disruption pulsing sequence was used intermittently to remove contrast agent from the image plane while subsequently tracking the replenishment of agent into the region-of-interest (ROI). Video intensities of both the F30 and SH15 image sequences were linearized, an ROI was selected at the focus, and the signal power as a function of time was calculated to generate a Time-Intensity-Curve (TIC).

All of the *in vivo* models contained networks of microvessels that were not detected with the F30 imaging mode but were detected with the SH15 mode due to its improved contrast-to-tissue-ratio (up to 7.5 dB over F30 mode). SH15 imaging, however, was limited in its ability to detect microbubbles in deeper structures (> 6 mm below skin line) depending on the path-length attenuation of tissue and bubbles. Analyzing the TIC of bolus injections showed that the amplitude of the SH15 signal dropped to -6dB, relative to the peak, on the order of 2 minutes compared with much longer periods of agent detection (> 5 min) in F30 mode. Bolus injections did provide the best image quality, but only for the first 30 s after the injection and with decaying signal amplitude over that period. By using the constant infusion method, the SH15 signal level was held constant (< 10% variation) in the plateau region, and the replenishment phase of the disruption-replenishment TIC was clearly identifiable. Constant infusion was, therefore, also appropriate for 3D imaging where a consistent signal level was critical. Future studies should focus on using the SH15 mode with constant infusion and disruption-replenishment, for deriving relevant physiological parameters of tissue perfusion.

Contrast Intravascular Ultrasound for Vasa Vasorum Imaging

David Goertz[1,2], Martijn Frijlink[1,3], Nico de Jong[1,4], Ton van der Steen[1]

1- Erasmus Medical Centre, Rotterdam; 2- Dept Medical Biophysics, University of Toronto; 3- Biomedical Engineering, Norwegian Technical University; 4- Physics of Fluids, University of Twente

Intravascular ultrasound (IVUS) is an established clinical tool for assessing coronary artery atherosclerosis. Its use has contributed to an improved understanding of the natural history of atherosclerosis and, increasingly, IVUS data are used as an endpoint in therapeutic trials. For diagnostic purposes, it is employed as an adjunct to angiography to provide additional insight into the extent and severity of atherosclerosis and frequently reveals the presence of angiographically occult lesions. Such “nonculprit” lesions are now recognized to be responsible for a high proportion of ensuing cardiac events resulting in either fatalities or requiring further interventional treatment. A significant issue in cardiology is, therefore, to develop imaging methods to identify specific atherosclerotic lesions that are vulnerable to rupture. Candidate markers of lesion vulnerability currently under investigation include plaque volume, mechanical integrity and composition. More recently, there is a growing recognition of the significance of vasa vasorum in plaque development and instability. Neovascular vasa vasorum are part of an apparent positive feedback loop of inflammation and angiogenesis (Moulton et al 2003) and are associated with intraplaque hemorrhage and, thereby, to rupture.

While the ultrasound imaging has been successful in detecting carotid artery vasa vasorum (Feinstein 2004), there are at present no established in vivo methods of imaging vasa vasorum in coronary arteries. IVUS is one candidate imaging modality for accomplishing this. Due to high levels of relative tissue-catheter motion, it is unlikely that conventional high frequency microvascular flow imaging methods will be effective. There have been several reports of extra-luminal image enhancement following the bolus injection of contrast agent, which has been attributed to the presence of vasa vasorum (Kakadiaris et al 2006). The basis of this approach is to compare post-contrast injection images with a baseline image derived from a single point in the cardiac cycle, which is potentially susceptible to acyclical catheter-vessel motion or non-uniform rotation of the transducer element. This motivates the development of contrast IVUS detection techniques based on bubble-specific signatures, which are dominant at lower ultrasound frequencies.

To this end, we are investigating the use of nonlinear contrast intravascular ultrasound techniques for vasa vasorum imaging. A prototype nonlinear IVUS contrast imaging system has been developed which employs mechanically scanned single element transducers (Frijlink et al 2006; Goertz et al 2006a). Conventional commercial catheters as well as catheters modified to incorporate custom dual frequency transducer elements have been assessed. The system has been evaluated at transmit frequencies in the 20 to 40 MHz range in second harmonic (H) and subharmonic (SH) imaging modes. The feasibility of improving contrast-to-tissue ratio (CTR) in H40 and SH20 modes relative to fundamental imaging was demonstrated in phantom experiments using both free and targeted microbubbles. The contrast agents employed include experimental micron to submicron sized lipid encapsulated agents as well as the commercial agent Definity™

(Bristol-Myers Squibb Medical Imaging). These agents have been shown to be capable of exhibiting a rich variety of nonlinear behaviours in the IVUS frequency range.

The feasibility of using nonlinear contrast IVUS imaging to detect the vasa vasorum was investigated in atherosclerotic rabbit aortas (Goertz et al 2006b; Goertz et al 2007) using both H40 and SH15 imaging modes. Following the bolus injection of Definity™, a significant enhancement was observed within the adventia. An example image is shown in Figure 1 below. Histology confirmed the presence of microvessels in the enhanced regions, indicating the potential of nonlinear contrast IVUS as a new technique for imaging vasa vasorum.

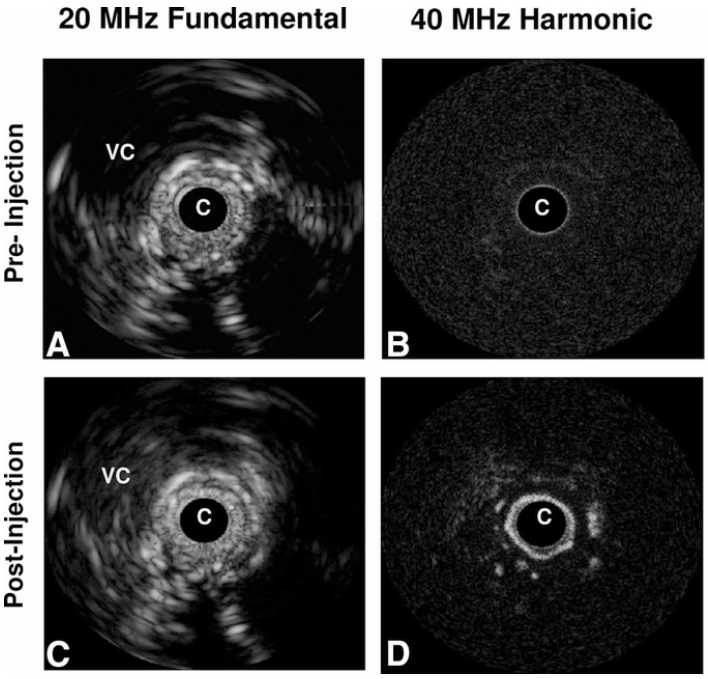


Figure 1. In vivo results in an atherosclerotic rabbit aorta using decanted Definity. A, Fundamental mode before agent injection, where “C” is the catheter and “VC” is the vena cava. B, Fundamental mode 10 seconds after injection where changes in adventitial enhancement are not evident, except for in a region at 4 o'clock and within the vena cava. C, Harmonic mode before injection shows the tissue signals to be largely suppressed. D, At 10 seconds after injection, the harmonic mode shows significant adventitial enhancement, consistent with the detection of adventitial microvessels. Scale of images is 12 mm across. The dynamic range of the fundamental and harmonic images are 40 and 25 dB, respectively.

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Figure 1.

Current simulation models of medical bubbles

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Review of existing theoretical models for ultrasound microbubble contrast agents will be presented. Emphasis will be placed on models for lipid-shelled bubbles. A general theoretical approach to the development of zero-thickness encapsulation models with different shell rheological laws will be proposed. Based on experimental results, analysis of the rheological behavior of lipid shells will be given. The problems of the existing models, such as the dependence of shell parameters on the initial bubble radius and the “compression-only” behavior, will be discussed, as well as possible directions for their solution.

Radial manipulation of single microbubbles

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Introduction

In recent years, the so-called radial modulation technique (also known as SURF imaging) has been introduced to enhance the efficacy of ultrasound contrast agents [1, 2]. A low frequency pulse manipulates the microbubbles, thus modulating the response to a high-frequency 'detection' pulse. The difference between two imaging pulses, transmitted at the expansion and rarefaction phase of the low-frequency pulse, is detected by regular correlation techniques.

In literature, most contrast enhancement is expected from either microbubbles that are resonant near the modulating low frequency [1, 2] or near the imaging high frequency [3]. However the microbubble behavior that results in most contrast enhancement is not known. We believe that non-linear phenomena like threshold and compression-only behavior [4] might also influence the effect of radial modulation. In this study, the behavior of single phospholipid-coated microbubbles in a dual-frequency ultrasound field was investigated. Bouakaz et al. [3] have optically studied a single microbubble that increased its high-frequency radial excursion during the compression-phase of the low-frequency manipulation pulse. We extend their study to a wide range of bubble sizes and lower acoustic pressures, where we expect nonlinear bubble behavior like compression-only to be significant.

Method

The radial excursions of single SonoVue microbubbles (Bracco, Geneva, Switzerland) were recorded with the high-speed camera Brandaris-128. The ultrasound consisted of a 2.5 cycles pulse at 0.5 MHz and a 32 cycles pulse at 3.75 MHz, having respective amplitudes of 30 kPa and 80 kPa. The pulses were simultaneously transmitted by two separate transducers placed confocally at a mutual angle of 90°. We also recorded the response of the bubbles to the separate pulses.

Results

The studied bubbles had diameters ranging from 1.1 – 5.2 μm and their behavior could be categorized according to their size; see Table I and examples of behavior in Fig. 1. Microbubbles of mid-range diameter (1.4 μm < D < 2.6 μm) showed a combination of compression-only and amplitude modulation (up to 25 dB), where a 6 dB modulation-depth of the high-frequency oscillation was considered significant. Compression-only behavior was defined by a relative compression of more than two times the relative expansion. Smaller bubbles frequently showed shrinkage, defined by having a final diameter < 85% of the initial diameter. Larger bubbles showed low (< 8 dB) or no amplitude modulation of the high-frequency pulse. During the single pulse exposure, bubbles of 2.5 μm diameter showed highest radial excursion, which is an indication for the resonant size at 3.75 MHz.

Table I, Categories of microbubble behavior during dual-frequency insonification

Bubble size (# bubbles)	Number of intact bubbles	Percentage showing amplitude modulation	Percentage showing compression-only
< 1.4 μm (8)	1	100 %	100 %
Mid-range (39)	27	74 %	89 %
> 2.6 μm (31)	30	27 %	70 %

Discussion

The effect of radial manipulation depended on the initial size of the bubble. Microbubbles smaller than the resonant size at 3.75 MHz showed compression-only behavior and very strong amplitude modulation, which would be beneficial for contrast detection. However, the echo levels of such small bubbles might be low compared to that of larger bubbles. Future studies have to reveal the optimal size distribution of a contrast agent for radial modulation imaging.

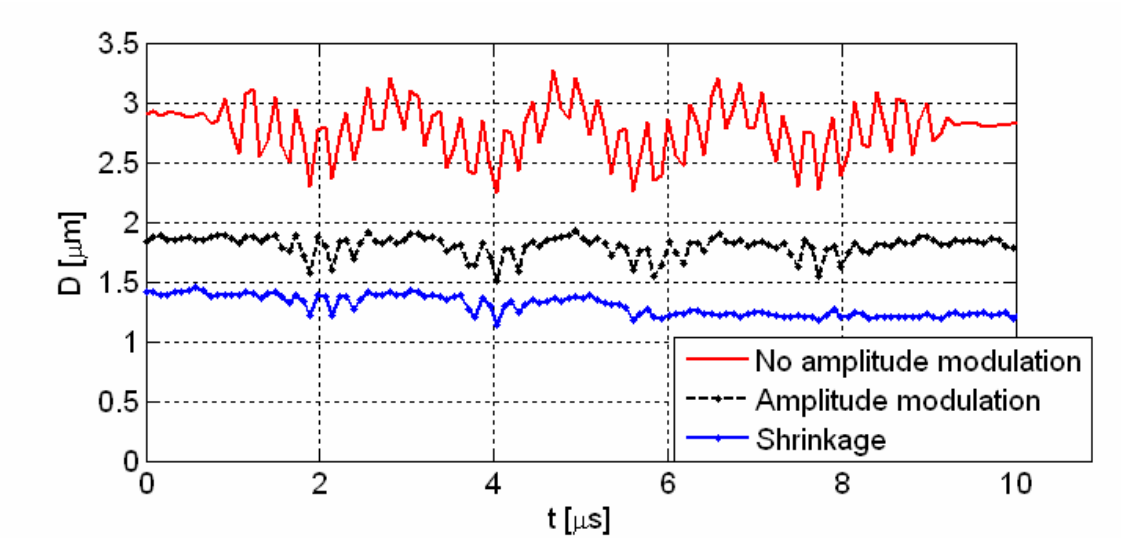

Fig 1, Examples of diameter-time curves of three single microbubbles showing either shrinkage, amplitude modulation, or no amplitude modulation.

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SURF IMAGING – High-frequency ultrasound contrast agent imaging in patients with prostate cancer

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A dual-band method for ultrasound contrast agent detection is demonstrated *in vivo* in patients with prostate cancer. The method is named Second-Order UltRasound Field Imaging, with the acronym SURF Imaging. It relies on simultaneously transmitting two ultrasound pulses with a large separation in frequency. Here, a low-frequency pulse of 0.9 MHz is combined with a high-frequency pulse of 7.5 MHz. The low-frequency pulse is used to manipulate the properties of the contrast agent, and the high-frequency pulse is used for high-resolution contrast imaging. An annular array capable of transmitting the low- and high-frequency pulses simultaneously was constructed and fitted to a mechanically scanned probe used in a GE Vingmed System 5 ultrasound scanner. The scanner was modified and adapted for the dual-band transmit technique. In-house software was written for post-processing of recorded IQ-data, thus real-time imaging was not possible.

Generally, the method decouples the contrast agent detection frequency from the resonance frequency of the microbubbles, allowing imaging at clinically-high frequencies. The low-frequency pulse is used (*e.g.* 0.5-2 MHz) to manipulate the properties of the contrast agent around its resonance frequency, changing the back-scattering from the high-frequency pulse (*e.g.* 3-14 MHz) which is used for high-resolution detection. The general principle of insonifying ultrasound contrast agents with the above described pulse complex is also known as Radial Modulation Imaging in the literature. Here, the name SURF Imaging is used to recognize the fact that transmitting such a pulse complex also infers changes in the forward generated tissue nonlinearities which needs to be accounted for. The low-frequency manipulation pulse generates a local change in the speed of sound experienced by the high-frequency pulse. In the data presented here, this effect is estimated and compensated in the obtained images.

The results present contrast-processed B-mode images from patients enrolled in an ongoing pilot study aimed at imaging prostate cancer using ultrasound contrast agents. The study is approved by the local ethics committee and the patients provide written informed consent. The obtained images show contrast agent detection in the prostate with a transmit frequency of 7.5 MHz, demonstrating that SURF imaging works well in a clinical setting. Due to scanner limitations in IQ-data storage, wash-in curves were not possible to obtain. Images displaying Maximum Intensity Projections (MIP) are presented, demonstrating areas of high contrast activity in regions with positive biopsy findings in patients with locally advanced prostate cancer (T3) and aggressive growth patterns (Gleason score 9).

The results demonstrate the potential of SURF Imaging as an ultrasound contrast detection technique in a clinical setting for high ultrasound frequencies.

AUTOMATIC ATTENUATION CORRECTION IN ULTRASOUND CONTRAST AGENT IMAGING

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Abstract:

Although microbubble contrast agents have been widely used in ultrasound imaging to visualise tissue perfusion, quantification of such tissue perfusion has been undermined by the lack of an effective automatic attenuation correction technique. Image intensity is a function of not only microbubble concentration, but also a number of other factors. Among these factors is the attenuation due to overlaying tissue and bubbles, which is frequency and pressure dependent. Further more, bubbles generate additional frequency components in the echoes which has different attenuation to the transmission pulse. Other factors include ultrasound (US) focusing during transmission and receiving and system Time Gain Compensation(TGC) etc.

In this paper, a model of the US attenuation processes for microbubble contrast enhanced imaging is developed(see Figure1). In the model, factors such as nonlinear bubble scattering, nonlinear attenuation, attenuation to both fundamental and harmonic, and US focusing during transmission and receiving are considered. The model is then used to develop a new attenuation correction method and to interpret the correction results.

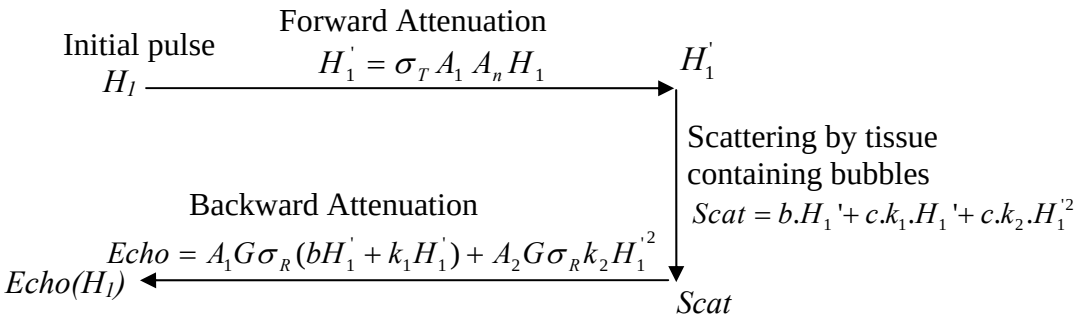


Fig. 1. Diagram of US echo formation.

H_1 : initial amplitude of the US pulse; ω_0 : initial frequency of the US pulse

A: forward attenuation, a produce of linear attenuation A_l and nonlinear attenuation A_n

σ_T : the beam profile due to transducer geometry and transmission beam-forming

H_1' is the amplitude of the attenuated US pulse.

b: the scattering coefficient of the tissue at frequency ω_0

$c(x)$ is the concentration of bubbles;

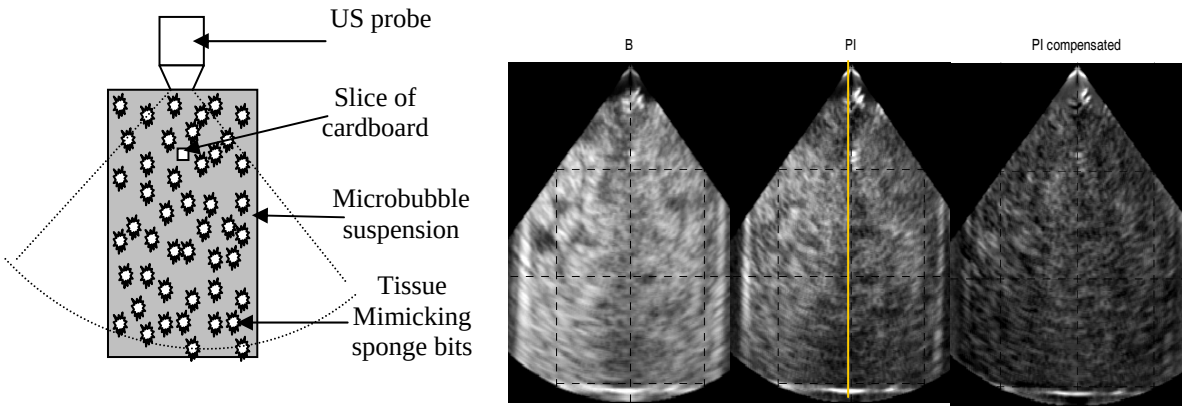
k_1 and k_2 : coefficients of the polynomials for bubble scattering

A_2: attenuation for the 2nd harmonic signal generated by bubble scattering

G : the gain in scanner postprocessing, including any default TGC

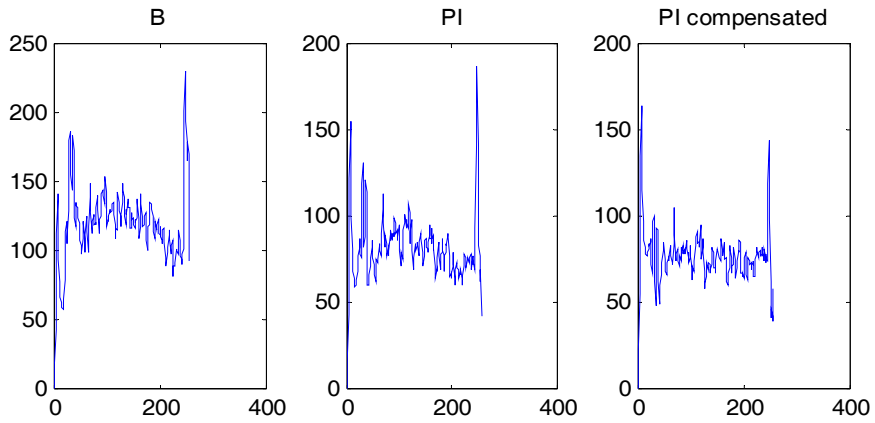
σ_R : the receive sensitivity profile due to receive focusing.

In order to separate the bubble concentration from other quantities in the echoes shown in our model, a new method is proposed. In the method linear tissue echoes are extracted and filtered, and then used to compensate for the attenuation in nonlinear bubble echoes at the same location to produce quantities that, based on model analysis, are a truer representation of bubble concentration. The technique does not require additional measurements and could potentially be implemented in real time. Experiments on laboratory phantoms consisting of bubbles and tissue-mimicking materials are presented and these demonstrate the effectiveness of the proposed methods; showing marked improvements in image quality compared with unprocessed data (Figure2&3). This development is an important step towards real-time quantitative contrast US imaging.



(a) phantom (b)B-mode (c) Pulse inversion (d) corrected

Figure 2: phantom and imaging results



(a) B-mode (b) PI (c) corrected

Fig. 3. Image intensity on a single vertical line in the images shown in Fig. 2(b)(c)(d).

Non-invasive Imaging of Atherosclerosis

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Goal: World–wide Cardiovascular Prevention
The pan-epidemic of obesity, metabolic syndrome and diabetes presage the incidence of accelerated cardiovascular (CV) mortality and morbidity in world-wide populations. Absent coordinated CV preventive programs among academic and governmental agencies, public health care policies currently provide only limited prevention opportunities and are unlikely to effectively provide long term solutions for the CV pan-epidemic.

Proposal: Identify preclinical atherosclerosis through the development of programs which include prudent administration and distribution of available resources, enhanced utilization of technology and improved public education.

Technology developments: There is an ever-increasing utilization of non-invasive imaging modalities that includes: 3-D echo with speckle tracking for the detection of regional contractile abnormalities (including sub endo-sub epicardial differences in disease states), carotid IMT and vasa vasorum imaging, automated arterial compliance testing, coronary artery calcification (CAC) testing, CT-angiography, and a host of biochemical surrogate markers of inflammation (HS-crp, Lp(a), LpPLA2, fractionated cholesterol testing, HgbA1C, etc.).
It is important to incorporate these technologies into our current health care systems with a special focus on the current and future uses of ultrasound contrast agents for the detection and monitoring of atherosclerosis. For over 20 years, the measurement of carotid intima-media-thickness (c-IMT) has served as a surrogate marker of atherosclerosis; first described by Pignoli in 1986. Subsequently, the c-IMT method has become an accepted standard for clinical trial endpoints. (A listing of the title: “carotid intima-media-thickness 2007” resulted in 1277 entries; Entrez Med Dec 23, 2007). Increasingly there is a trend toward using the ultrasound derived c-IMT as a clinical screening tool for preclinical disease in at risk populations.

Improvement in c-IMT imaging: The use of an ultrasound contrast agents when applied to carotid imaging result in enhancement of the carotid lumen, thus permitting improved precision of the c-IMT. The contrast-filled, lumen provides a superb backdrop for highlighting the luminal irregularities and importantly, provides direct visualization of angiogenesis within the carotid artery wall, including atherosclerotic plaques and vessel wall neovascularization (vasa vasorum).

Recently several investigators have published work corroborating these initial findings, that is the investigators have similarly described that the use of ultrasound contrast to identify vessel wall and intraplaque angiogenesis in patients with CV disease.

Future: The future applications of non-invasive imaging of atherosclerosis may well include the uses of ultrasound contrast agent and real-time 3-D quantification of the c-IMT and vasa vasorum for diagnostic and therapeutic interventions in patients identified as “high risk” for

premature CV disease. If validated clinically, these innovative, non-invasive, technologies may become an integral aspect of the universal program for the prevention of CV diseases.

Ultrasound Imaging of Inflammation using Microbubble Contrast Agents

Joshua Rychak, Scott Acton, John Hossack, Klaus Ley, Alexander Klibanov

This presentation will explore molecular markers of various inflammatory processes, previous work in contrast ultrasound imaging of inflammation, and new developments in the field. Inflammation is the generalized reaction of the body to injury or infection, and is broadly involved in numerous acute and chronic pathophysiological mechanisms. A crucial component of the inflammatory process is the expression and up-regulation of endothelial surface markers, particularly selectins and members of the immunoglobulin superfamily. These proteins serve as adhesion and signaling molecules, and recruit circulating leukocytes to the inflammatory focus. As conventional microbubble contrast agents are confined to the vascular lumen, the presence of endothelial molecular markers makes inflammation an especially attractive application for contrast ultrasound imaging. Additionally, the phases of the inflammatory process are mediated by distinct endothelial surface molecules, suggesting that molecular imaging of endothelial targets may enable analysis of a patient's inflammatory state. Many acute and chronic diseases have inflammatory components, which often determines the disease progression and prognosis.

A wide variety of experimental animal models of inflammation recapitulate the human diseases. Inflammation can be rapidly induced in skeletal muscle by cytokine injection or administration of histamine, bacterial LPS, or other noxious substances. Such models of acute inflammation were used in early leukocyte trafficking studies, and present a well-validated and reproducible tool for assessing targeted microbubble adhesion in the various phases of the inflammatory process. More complex injury models, including myocardial infarction and ischemia-reperfusion injury, are also possible and have been described in contrast ultrasound studies. Several chronic inflammatory disorders have also been investigated in this context. Gene knock-out mouse models of atherosclerosis are widely available, and allow characterization of this multi-faceted disease at various stages. Several models of inflammatory bowel disease have been described in small animals and recapitulate some aspects of the human disease. Transplant rejection has also been investigated using contrast ultrasound imaging.

Leukocyte adhesion to inflamed vasculature is a finely-tuned biophysical process, with several similarities to targeted microbubble adhesion. Recently, several aspects of the leukocyte adhesion cascade have been appropriated to the design of microbubble contrast agents with enhanced targeting efficiency. Deformation, microvillus projection, and rapid bond kinetics are components critical to leukocyte adhesion that have been explored in the context of increasing microbubble targeting efficiency.

In vivo Ultrasound-mediated Gene Transfection from Microbubble Carriers to Site of Vascular Injury.

Linsey C. Phillips, Ramin Zargham, Alexander L. Klibanov, Brian R. Wamhoff, John A. Hossack

Balloon angioplasty and stent placement are currently used to restore blood flow to an atherosclerotic artery. However, in many patients, the vascular smooth muscle cells (SMCs), which compose the vessel wall, undergo proliferation and subsequent migration in response to the procedure leading to re-narrowing of the vessel wall; a process termed restenosis. Current therapies include drug eluting stents (DES) which release anti-proliferative agents to prevent restenosis. Although effective, patients that receive DESs are highly susceptible to late in-stent thrombosis, which often results in death, and patients require long term anti-platelet therapy (e.g. Plavix).

Ultrasound contrast agents, similar to those that are clinically approved, can be modified to carry therapeutic agents. Herein, we propose that the application of ultrasound-mediated drug/gene delivery has potential for the treatment of restenosis following balloon angioplasty. Such a procedure would circumvent the need for polymer coated DESs and rely solely on bare metal stents which do not put the patient at high risk for late in-stent thrombosis. To demonstrate the feasibility of *in vivo* localized drug/gene therapy to a vessel wall we investigated the use ultrasound-mediated gene delivery to a rat carotid artery following balloon angioplasty.

Lipid monolayer microbubbles were formed by self-assembly during sonication of decafluorobutane gas in an aqueous micellar mixture of phosphatidylcholine, PEG stearate, and distearyl trimethylammonium propane. CMV-RFP plasmids (expressing a red fluorescent protein, as a marker) were conjugated to the positively charged lipid-shelled microbubbles by charge coupling. Plasmid-to-bubble binding was quantified via spectrophotometry at 260nm. Ultrasound parameters were optimized *in vitro* for transfection in rat aortic SMCs with minimal cell damage. Using a focused, 1 MHz, single element transducer a 100% -6 dB bandwidth pulse was emitted with a duty cycle of 20% for one second every 2 seconds at a pressure of 300 kPa. *In vitro* studies revealed visibly fluorescent cells, in cells which were insonated in combination with plasmid carrying microbubbles, indicating successful transfection.

A linear array clinical scanner (15L8, Sequoia, Siemens), was used for in vivo studies to locate the left carotid artery in a rat which had previously (3 days prior) undergone balloon angioplasty. Microbubbles coated with 40μg of CMV-RFP plasmids were infused at a rate of $2x10^7$ bubbles/min for 5 minutes into the right jugular vein of the rat. Ultrasound-mediated release of CMV-RFP from bubbles was performed under similar ultrasound conditions as those applied *in vitro* (except at 8MHz, MI=1.9). Three days after insonation histological cross sections of the injured and contralateral vessels were imaged. Fluorescent microscopy revealed RFP expression in the innermost SMC layer of only the injured vessel which received both ultrasound and plasmid coated microbubbles (Fig. 1).

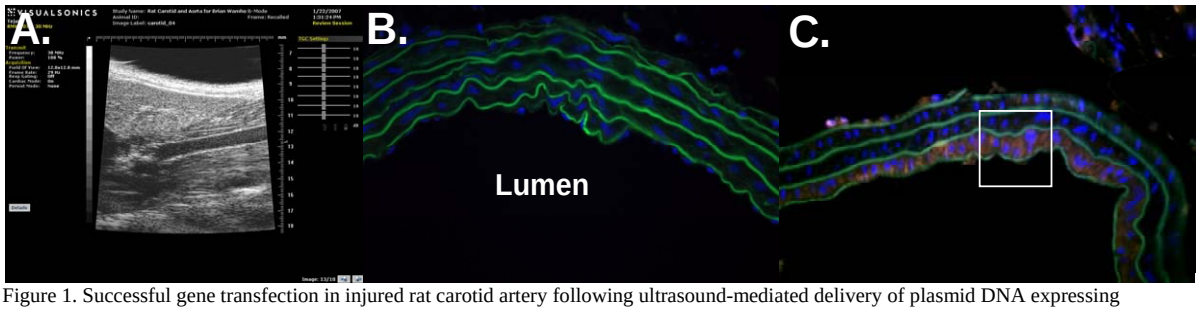

Figure 1. Successful gene transfection in injured rat carotid artery following ultrasound-mediated delivery of plasmid DNA expressing RFP. A) 14 MHz B-mode image of a rat carotid artery, post balloon injury, perfused with plasmid DNA-coated microbubbles. B) Uninjured contralateral control carotid artery (no RFP evident). C) Injured blood vessel (RFP evident -white box).

We demonstrate that CMV-RFP can be delivered to a blood vessel following angioplasty and that this method may be useful for clinical gene therapy as well as drug delivery to prevent in-stent restenosis. Based on preliminary work with a single element IVUS transducer at 200 kPa and 11 MHz, which revealed successful ultrasound microbubble-mediated transfection of CMV-RFP to SMCs *in vitro*, a potential for

drug delivery via IVUS (or a modified IVUS transducer/system) immediately following stenting exists. Preliminary *in vitro* studies using an anti-proliferative drug, rapamycin, conjugated to microbubbles resulted in significant reduction in SMC proliferation after application with ultrasound. Taken together these results show promise for *in vivo* therapy to prevent SMC proliferation via ultrasound-mediated delivery from microbubbles.

Mechanisms of delivery of therapeutic compounds by ultrasound and microbubbles.

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Background It has been demonstrated that ultrasound (US) and microbubbles are a promising non-viral tool for local delivery of drugs and genes. However, the exact mechanisms underlying increased uptake of therapeutic compounds are still not completely understood. We hypothesize that alternate mechanisms of uptake are involved for different-sized compounds.

Material and Methods Bovine aortic endothelial cells (BAECs) were cultured on US transparent membranes. US exposure consisted of bursts of 1 MHz with a peak negative pressure of 220 kPa. Pulse repetition frequency was set to 20 Hz, with a duty cycle of 6%. Cells were exposed during 30s in the presence or absence of Sonovue microbubbles. Pore formation was measured during US exposure by a Ca^{2+} influx with the fluorescent dye Fluo4. Fluorescent dextrans (4.4, 70 and 155, 500 kDa) were used as a model for drug delivery. For blocking the three main routes of endocytosis, the specific inhibitors chlorpromazine (clathrin-mediated endocytosis), filipin (caveolae-mediated endocytosis) and wortmannin (macropinocytosis) were used. Number of dextran positive cells was counted, and the amount of dextran uptake by the cells was quantified. Immunofluorescent stainings were performed to detect possible co-localisation of clathrin and caveolin-1 with 500 kDa dextran.

Results After US exposure in the presence of microbubbles, 4.4 kDa en 70 kDa dextrans were mainly homogenously distributed throughout the cytosol, with the 4,4 kDa also being localized in the nucleus. Together with the influx of Ca^{2+}, which was abolished when using medium without Ca^{2+} and not affected by verapamil (L-type channel blocker) and lanthanum (general Ca^{2+} channel blocker), these results indicate that pore formation is one of the mechanisms of uptake of at least 70 kDa molecules. Dextran molecules of 155 kDa en 500 kDa were mainly localized in vesicles after US exposure in the presence of microbubbles. Individual inhibition of the three main routes of endocytosis showed

that clathrin- and caveolae-mediated endocytosis, as well as macropinocytosis, all play a role in the delivery of all sizes of dextrans. The uptake of the 500 kDa dextran via endocytosis is further supported by co-localization with both clathrin-coated pits and caveolin-1.

Conclusions These results show that US and microbubbles mediated delivery of macromolecules to endothelial cells is mediated both via pore formation and different endocytotic pathways. This uptake of especially the larger molecules via endocytosis, which also holds for the uptake of plasmid DNA, implicates that another barrier after the cell membrane has to be overcome before efficient gene delivery will result in efficient gene expression.

CEUS for monitoring and follow-up of radiofrequency ablation of the liver

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Image guided radiofrequency ablation (RFA) of liver tumours is an established local treatment option for patients with metastases limited to the liver, mainly from colorectal carcinoma, and HCC. The goal of RFA of liver tumours is to fully ablate the entire tumour including a safety margin of at least 0.5 to 1 cm. The safety margin is required to include microscopic tumour extensions and microscopic satellite tumours. Unfortunately, local tumour progression / recurrence after RFA, which indicates incomplete ablation, is reported to be as high as 60% in the literature. The good news is that local tumour progression can be as low as 7%, if a circumferential safety margin of > 0.5 cm is achieved.

The success of image guided RFA is crucially dependent on high quality imaging. The tasks of imaging include treatment planning, guidance of probe placement, monitoring of treatment effects during or immediately after the procedure and post-interventional follow-up. For these ends, CT, MRI or ultrasound (US) can be used.

Conventional US is the ideal modality for guidance of probe placement due to its bed-side and real time nature. However, monitoring of treatment effects is not possible with conventional US including Doppler modes, since it cannot differentiate properly between viable and necrotic tissue. Conventional US is therefore considered inappropriate for RFA monitoring.

The use of US contrast agents has changed this. On contrast enhaced US (CEUS), accurate assessment of treatment effects is possible at approximately 10 min after the RF-device has been switched off – before this, however, sonographic vision is impaired by gas formation in the liver during the ablation. This gas is usually sufficiently absorbed after 10 min to allow for acceptable image quality. The thermal necrosis is displayed as an enhancement defect in an otherwise normally enhancing liver. The size of this enhancement defect correlates well with the results of MRI performed 24h after ablation. If the thermal necrosis shown on CEUS immediately after the ablation is not suffitiently large, further ablation and sometimes probe repositioning is required.

In a preliminary trial performed at our institution, we could show that the primary effectiveness rate and the rate of local tumour progression on follow-up of CEUS guided liver RFA are not inferior to those of CT-guided RFA.

Further applications of CEUS in liver for RFA are treatment planning (for detection of liver tumours), probe guidance in case of lesions not or poorly visible on conventional US and follow-up imaging, if CT or MRI are not available. These indications are included in the EFSUM guidelines on the use of CEUS of 2004 and 2007.

Clinical role of contrast ultrasound of the cirrhotic liver in comparison to other modalities

Prof. Dr. Riccardo Lencioni, University of Pisa, Italy

In view of the high risk to develop hepatocellular carcinoma (HCC), patients with liver cirrhosis are carefully followed with ultrasound examinations repeated at 6-month intervals (1). While the detection of a focal lesion in cirrhosis should always raise the suspicion of HCC, it is well established that the pathologic changes inherent in cirrhosis may simulate HCC in a variety of ways, especially because non-malignant hepatocellular lesions, such as regenerative and dysplastic nodules, may be indistinguishable from a small tumor. One of the key pathologic factor for differential diagnosis that is reflected in imaging appearances is the vascular supply to the nodule. Through the progression from regenerative nodule to dysplastic nodule to frank HCC, one sees loss of visualization of portal tracts and development of new arterial vessels, termed nontriadal arteries, which become the dominant blood supply in overt HCC (2). It is this neovascularity that allows HCC to be diagnosed with contrast-enhanced computed tomography (CT) or dynamic magnetic resonance (MR) imaging (3). Owing to the ability to display contrast enhancement in real-time, contrast ultrasound appears to be a tool to show arterial neoangiogenesis associated with a malignant change, and, therefore, to help establish the diagnosis of HCC (4). Hence, performing a contrast-enhanced study is recommended in all lesions or suspected lesions detected at baseline ultrasound in patients with cirrhosis or chronic hepatitis undergoing surveillance programs (5). The use of contrast ultrasound as a reliable alternative to CT or MR imaging in characterizing nodular lesions detected by ultrasound surveillance has been recently endorsed by the conclusions of the 2005 Monothematic Conference on HCC of the European Association for the Study of the Liver and the practice guideline document published by the American Association for the Study of Liver Diseases (6). The recommended diagnostic protocol is structured according to the actual risk of malignancy and the possibility to achieve a reliable diagnosis. Since the prevalence of HCC among ultrasound-detected nodules is strongly related to the size of the lesion, the work-up depends on the size of the lesion. Lesions smaller than 1 cm in diameter have a low likelihood of being HCC. However, minute hepatic nodules detected by US may become malignant over time. Therefore, these nodules need to be followed-up in order to detect growth suggestive of malignant transformation. A reasonable protocol is to repeat US every 3 months, until the lesion grows to more than 1 cm, at which point additional diagnostic techniques are applied. It has to be emphasized, however, that the absence of growth during the follow-up period does not rule out the malignant nature of the nodule because even an early HCC may take more than one year to increase in size. When the nodule exceeds 1 cm in size, the lesion is more likely to be HCC and diagnostic confirmation should be pursued. It is accepted that the diagnosis of HCC in cirrhosis can be made without biopsy in a nodule larger than 1 cm that shows characteristic vascular features of HCC - ie, arterial hypervascularization with washout in the portal venous or delayed phase - even in patients with normal alpha-fetoprotein value. Such lesions should be treated as

HCC, since the positive predictive value of the clinical and radiological findings is as high as 100%, provided that examinations are conducted by using state-of-the-art equipment and interpreted by radiologists with extensive expertise in liver imaging (7). For lesions ranging 1-2 cm, current guidelines require typical imaging findings to be confirmed by two coincident dynamic imaging modalities to allow a non-invasive diagnosis. For nodules above 2 cm, a single imaging technique – out of contrast-enhanced US, multidetector CT, and dynamic MR imaging – showing the characteristic vascular profile of HCC mentioned above may confidently establish the diagnosis. It has to be pointed out, however, that if the diagnosis is made by using contrast-enhanced US, additional investigation with multidetector CT or dynamic MR imaging is required to provide a comprehensive assessment of the liver parenchyma and rule out additional tumor foci. Moreover, it has to be stressed that non-invasive criteria based on imaging findings can be applied only in patients with established cirrhosis. For nodules detected in non-cirrhotic livers, as well as for those showing atypical vascular patterns, biopsy is still recommended.

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Rotterdam Abstract 2008
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Clinical Role of CE-US of the Non-Cirrhotic liver in comparison with other modalities such as CT and MR

For the last two decades contrast enhanced CT and MR have been well recognised to be the imaging modality of choice in the staging of cancer patients given their accuracy in assessing the extent of both intra- and extra-hepatic disease. Unenhanced conventional US have been the prefered initial modality for the assessment of patients presenting with suspicious biliary disease, non-specific upper abdominal symptoms or with abnormal liver function tests; its role as a guidance tool for biopsy, drainage and local ablative therpay is well established. However with the advent of echo-enhancers and non-linear imaging modes, CEUS has now emerged as a modality with sensitivity and specificity matching or even exceeding those of the apparently more established techniques in the evaluation of focal liver disease and is redefining its role in clinical practice. Cost, accessibility, mobility with combined real-time & functional capabilities are other key elements making it competitive in specific clinical oncological areas. CEUS has extended its role in the assessment of the cancer patients from both diagnostic and interventional aspects fulfilling all the clinically distinct tasks in imaging the non-cirrhotic liver. Improved global detection of liver metastases has led some European centres in its routine use for the surveillance of patients following surgical resection of primary tumours such as colorectal cancer, breast, gastric and pancreatic carcinoma. In the characterisation of focal liver lesion, CEUS is now considered at least as an equal or even superior than MR such that it is ultimately used in the evaluation of incidental and coincidental lesions. CEUS has also improved its intra-operative application in the resection of liver metastases altering surgical management in up to 29% of cases and establishing itself as the standard of reference. From the interventional aspect, the advent of 3D with CEUS, it provides the whole imaging and guiding package in terms of staging, planning, guidance, earlier assessment of response as well as surveillance. Future functional developments with 3D quantification will further extend CEUS role in the monitoring of early response to new biological agents in oncology as well as the treatment of diffuse inflammatory conditions.

Dynamic Contrast Enhanced-ultrasonography (DCE-US) with Quantification of Tumour Perfusion for the early evaluation of Anti-angiogenic Treatments.

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New treatments based on antiangiogenic substances are developed in order to destroy tumor vessels and are the object of promising clinical research for cancer treatment. Considering the large number of new targeted drugs under development, there is a great need for early reliable imaging indicators of tumour responses, and identification of a recommanded modality of drug administration to guide further steps in the clinical development. The response rate remains the best objective parameter of efficacy of new treatments tested in Phase 1 2, or 3 or approved by the FDA but this parameter is obtained very late in the clinical development, while the effect on the tumour must be determined as soon as possible in order to optimise the schedule. This early functional evaluation of new treatments is a main goal.

At present, technical advances in DCE-ultrasonography using contrast agent (SonoVue®, Bracco) and perfusion software (Aplio, CHI-Q, Toshiba) allow the detection of microvascularization and perfusion for superficial and deep malignant tumors. Thus, it becomes possible to early evaluate the efficiency of antiangiogenic or anti-vascular molecules in pre-clinical and clinical studies. Treatment response can be early predicted according to modifications of this vascularization before any volume modification. The acquisition of raw linear data affords the precise quantification (peak intensity, time to peak intensity, slop of wash-in, area under the curve….) of the perfusion, in particularly using time tracking of region of interest. Our results using this methodology are focused on metastasis of RCC, HCC, and GIST, with different molecules at different doses and with different schedules.

Volume Calculation in Hyper-vascular Liver Tumors: Comparing B-Mode Ultrasound to Different Phases During Contrast Enhanced Ultrasound imaging (CEUS)

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PURPOSE: To evaluate the tumor volume of hyper-vascularized benign and malignant liver lesions using 3-D in conventional and Contrast Enhanced Ultrasound (CEUS 3D)

METHOD AND MATERIALS: US devices: Voluson 730 E, LOGIQ 9. Motor driven 3-D probe: RAB 2-5, 4D3C (GE, Milwaukee, WI, USA). 37 patients with hyper-vascularized liver lesions which enhanced ahead of liver tissue, were examined in a 3-D CEUS technique using a 2.4mL bolus injection of contrast agent Sonovue™ (Bracco, Milan, Italy): Focal nodular hyperplasia (n=17), hemangioma (n=5), hypervascularized metastases (n=15) and HCC (n=5) were examined with US during early arterial phase. Scan direction followed the flow direction of the tumor supplying arteries. In order to keep the acquisition time low the ROI only included the liver segment of the lesion. Tumor diameters ranged from 10mm to 110mm. B-Mode echogenicity of lesions was characterized as dominantly echo-poor, echo-rich or iso-echoic, and their volumes except the latter were calculated. With bubble arriving in the tumor supplying artery volume acquisition started and was repeated 3 times covering the lesion and the supplying vessel. In all metastases a late phase CEUS 3D was performed. Only lesions that were well defined in CEUS were evaluated for the study. FLL were characterized using the guidelines of the EFSUMB.

RESULTS: Volume calculation of small lesions (<3mL) varied greatly between B-mode and CEUS (-45% to +225%) in all type of malignant lesions. Volume of iso-echoic lesions in B-mode studies could only be calculated in CEUS (>3mL): 9/17 FNH (31.3mL±29.9mL), 3/5 hemangiomas and 3/5 HCC only during arterial phase, 5/15 metastases. While all other FNH and hemangiomas showed only little differences between B-mode and contrast enhanced US (+0.2% to + 15%), all malignant lesions showed a higher volume during arterial phase (+ 26% to 61%). Compared to arterial phase, late phase volumes were smaller again (-5.6% to -110%). CD34 staining of 5 histological specimen, peritumoral liver tissue showed dilated vessels. In 5 cases a follow up under chemotherapy was performed. In two the lesion the tumor borders could not be clearly defined any more.

CONCLUSION: In well defined benign and malignant liver lesions during the arterial phase of CEUS, the volumes of malignant lesions were -in contrast to benign lesions- markedly higher when compared to B-mode images or late phase CEUS. Peri-tumoral enhancement of two liver metastases shrunk under successful chemotherapy while the tumor volume calculated in the late phase remained unchanged.

Quantification of tumor angiogenesis with ultrasound contrast agents: Measurement of tumor response to Avastin

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Introduction-Objective

Blood flow at the microcirculation level is imaged with ultrasound contrast agents and modern diagnostic ultrasound systems. Quantification of the perfusion characteristics of organs and tumors is now possible. Our objective is to measure perfusion changes including those caused by tumor angiogenesis and by therapies targeting anti-angiogenesis available today and thus provide a means of assessing therapy.

With low Mechanical Index (MI) imaging (to avoid microbubble destruction) and nonlinear pulsing schemes (to suppress the tissue response) tumor perfusion is imaged in real-time. Over the years ultrasound imaging schemes and methods have greatly improved and have reached the point where we can image the microcirculation reliably and repeatably. However, to assess tumor therapy response and detect even the smallest changes, there is a need to quantify the image data accurately.

Materials and Methods

Time-intensity curves of the wash-in and wash-out of microbubble contrast agents in a region of interest (ROI) were formed from linear (absolute scale) ultrasound image data where the logarithmic compression of the scanner has been removed. This is a very important step in perfusion quantification because in order to measure small changes and perform data operations, the highly nonlinear image data compression must be undone, as suggested by other researchers as well [1]. A curve-fitting algorithm developed with Matlab was used to fit the linear data to a Supper-Gaussian function defined as $SG(t) = a * \exp\{-[(t - t_0)/T]^{2m}\} + b$, where t is the time, t_0 is a time offset, T is a constant for the maximum duration of the wash-in, a is the maximum intensity, b is a DC amplitude offset, and m is the super-Gaussian exponent which we call the *Wash-in Index* (*WI*). All image data where collected on a Philips iU22 scanner in the *contrast-side-by-side* mode [where the microbubble contrast image and the tissue image both at low MI are displayed as shown in Fig. 1(a) and (b)]. The machine *native data* which include the linear data without the logarithmic compression were saved and later on processed on QLAB software to extract the time-intensity curves.

In order to establish the repeatability and robustness of the method first, 3 healthy volunteers (1 had a benign lesion and the other 2 did not) were scanned 3 times over a period of 3 weeks with 2.4 ml of SonoVue. Data from more healthy volunteers are currently being collected. It is important to establish that in cases where no perfusion changes take place the measured *WI* stays the same over time.

Seven (7) patients undergoing chemotherapy treatment for liver metastasis from colorectal cancer with a regimen containing Avastin (6 sessions each, 3 weeks apart) were scanned with two 2.4 ml SonoVue injections at the beginning of every therapy

session. For all patients loops of 120 seconds (when possible) were collected and ROIs in the metastases and the normal parenchyma were placed in QLAB (as shown in Fig. 1) The resulting linear data for time-intensity curves were extracted for further processing including curve fitting in Matlab.

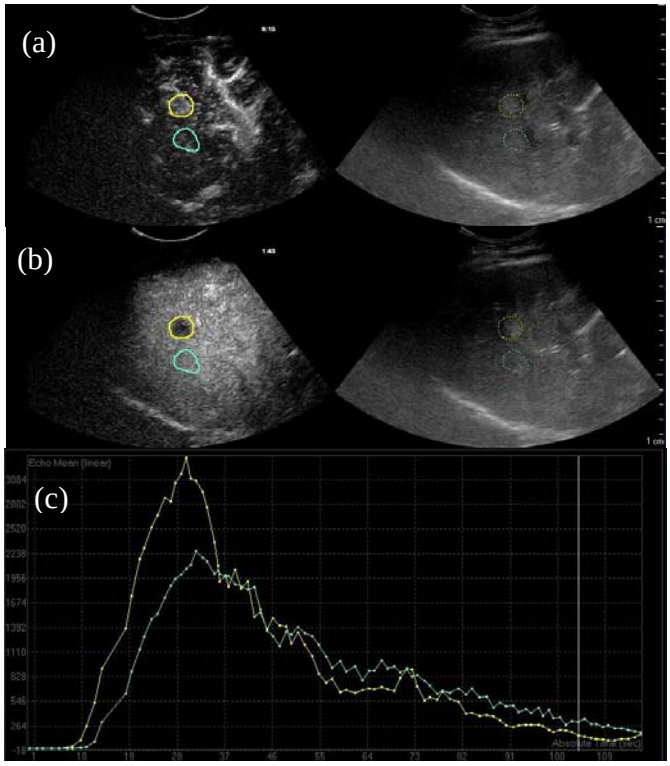

Figure 1: Images showing the ROIs for the time intensity curves (c), one for the metastasis and one with normal parenchyma. (a) is taken in the arterial phase and (b) in the late portal phase.

A typical example of curve fitting is shown in Fig. 2. For this specific case, the Wash-in Index (*WI*) for the lesion was 6 and for the normal parenchyma was 4.

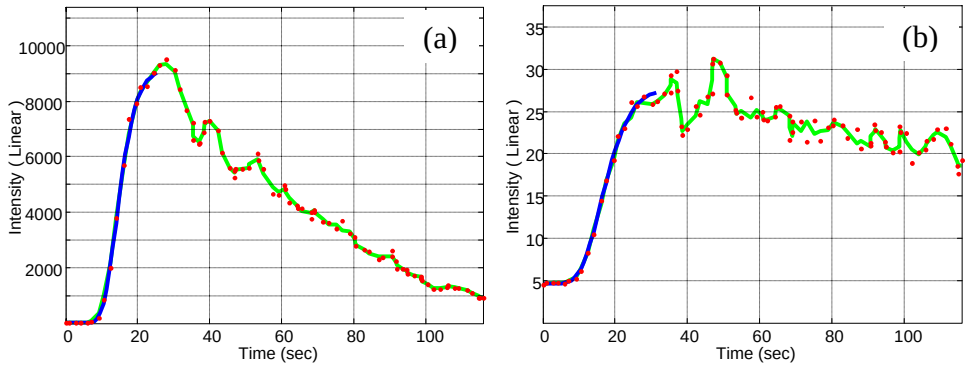

Figure 2: Time-intensity curves for a metastatic lesion (a) and for normal parenchyma (b). Red dots are actual data, green lines are smoothed data, and blue lines are curve fits for data. The wash-in index in (a) is 6 and in (b) is 4.

Often due to breathing motion the lesion did not stay in the image plane thus making it difficult to accurately observe the intensity of the lesion as a function of time. This is an inherent problem of 2D imaging and it can only be completely solved with 3D real-time imaging. However, in 2D imaging either motion compensation [2] (only when the motion is in-plane) or respiratory gating may solve the problem of breathing motion. Since there is often out-of-plane motion, we decided to use respiratory gating as a post-processing scheme. Our approach consisted of selecting a ROI that encloses a part of the diaphragm and removing all frames (not considering them for quantification) where the diaphragm moved outside the ROI (see Fig. 3).

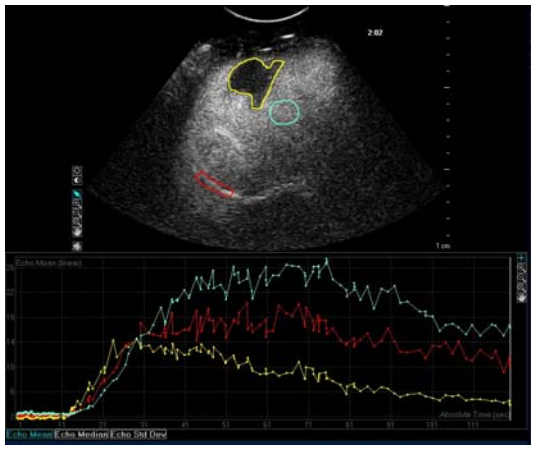

Figure 3: Demonstration of respiratory motion gating. A ROI (red) is placed at the diaphragm and all frames that show the diaphragm outside the ROI are rejected.

This approach has enabled us to use exams (loops) that would otherwise be rejected for quantification. Without the respiratory gating the curve fitting algorithm would often fail. In Fig. 4 below we show an example of curve fitting with respiratory gating. The image data of 3 out of the 7 patients would have to be totally thrown out if respiratory gating was not applied and in almost all patients it has been applied to loops with large out of plane motion.

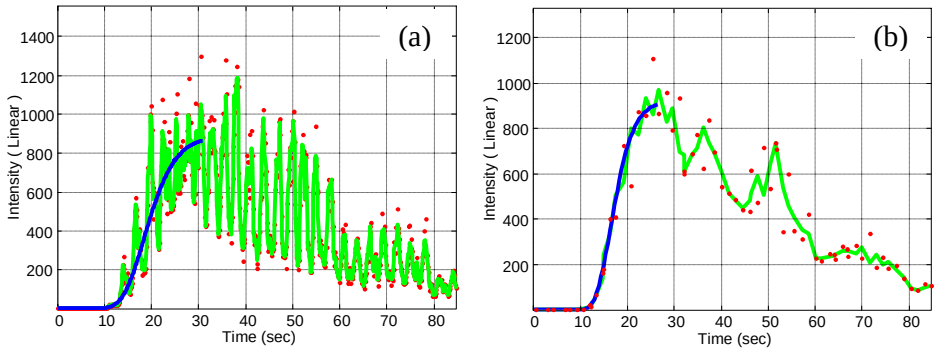

Figure 4: A time-intensity curve with large breathing motion (a) and with respiratory gating (b).

Results

In the healthy volunteers we have found that when no lesion was present *WI* stays roughly the same (with ± 0.5 variation). The same was found when benign lesions were present. In the patients with colorectal metastases undergoing therapy with Avastin we have observed that the lesion always had higher *WI* than the normal parenchyma before

treatment. During treatment, *WI* of lesion generally goes down where for the normal liver stays roughly the same. However, there were times that the *WI* of both the lesion and the normal parenchyma increased in subsequent therapies. Possible explanation for this may be the variations in bolus injections or even changes in the cardiac output of the patient. To minimize the effect of these variations we have formed the *WI-Ratio* which is defined as $WI_{\text{lesion}}/WI_{\text{parench}}$. The *WI-Ratio* decreased more for patients with better response to therapy. Table 1 below tabulates all the results for the patients considered in the study.

	Wash-in Index WI		WI-ratio	Comments (oncology)
Patient	Lesion	Norm. Parench.		
EL1	10	4	2.5	stabilized, surgery (resection)
EL2	6	4	1.5	
EL3	9	3	3	
EL4	4	5	0.8	
NP0	8	6	1.33	stabilized (based on CT), reduced blood neoplastic markers
NP1	7	4	1.75	
NP2	-	-		
NP4	4	4	1	
NP5	-	-		
AS1	9	4	2.25	improvement (on MR)
AS2	4	2	2	
AS3	6	4	1.5	
AS4	6	2	3	
AS5	4	3	1.33	
DRa0	6	2	3	overall improvement and reduction of neoplastic markers
DRa1	10	4	2.5	
DRa2	5	5	1	
DRa3	2	3	0.67	
DRa4	2	4	0.5	
DRb0	6	2	3	Liver function improvement and reduction of neoplastic markers
DRb1	-	4		
DRb2	11	5	2.2	
DRb3	6	3	2	
DRb4	6	4	1.5	
DP1	3	2	1.5	no clinical evaluation yet
DP2	3	2	1.5	on-going
DP3	3	3	1	
DP4	8	8	1	
CD0	5	2	2.5	no clinical evaluation yet
CD1	7	3	2.33	on-going
CD2	6	3	2	
NG1	6	3	2	improvement and reduction of neoplastic markers
NG2	3	2	1.5	
NG3	3	2	1.5	

Table 1: Results for *WI* and *WI-Ratio* for all patients in the study. Entries in light gray indicate problematic loops (e.g., very large out of plane motion) and were discarded.

Patient DR ("*a*" is a large lesion that disappeared during therapy and "*b*" is a small lesion still present). According to our analysis this patient showed the best response and this is in agreement with the clinical observations of the oncologist. Patient AS had some initial response but later on the *WI-Ratio* started increasing again. Evaluation with MRI suggested improvement (at least in the liver). However, the patient after the therapy was completed died from recurrence of the primary in the intestines.

In Fig. 5 *WI-Ratio* as a function of therapy number is shown for all patients. For most patients a reduction in WI-Ratio is observed over time (increasing therapy number). The study is not completed yet and some patients are still undergoing therapy (patients DP and CD). It is our intention to perform one last SonoVue study two months after the therapy cycle is completed.

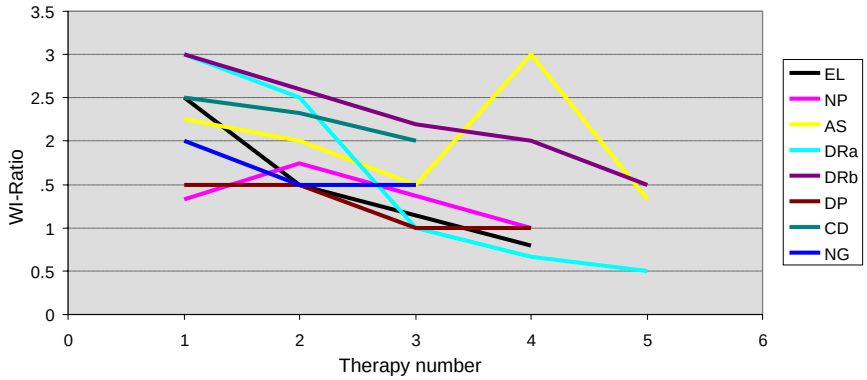

Figure 5: WI changes as a function of therapy number.

Another approach to remove variability caused by the bolus injection rate, the cardiac output of the patient, and also variation in machine settings is to take the difference between normalized lesion data (with respect to max value) and normal parenchyma data and form a new curve as shown in Fig. 6(b). A similar normalization scheme was also used by Rognin et al. [2] in order to differentiate between malignant and benign lesions.

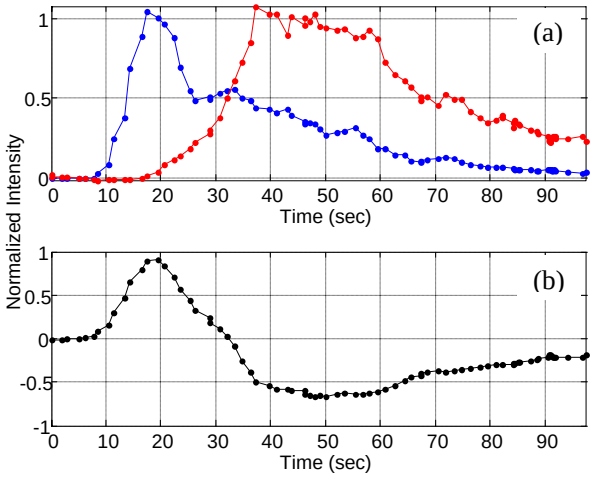

Figure 6: (a) Normalized time-intensity curves for a matastatic lesion (blue) and normal parenchyma (red). (b) The resulting curve after subtracting the intensity of the normal parenchyma (red) from the lesion (blue).

The normalization scheme was applied to all patients and similar curves were observed. The maximum value of the normalized difference curve [Fig. 6(b)] around 20 sec represents the peak of the arterial phase where as the minimum value around 50 seconds represents the peak of the portal phase. During therapy and when response is observed the curve shown in Fig. 6(b) starts to flatten out approaching a straight line around the zero value. This is an expected result since ideally when a lesion is responding it behaves more like normal liver and the difference between the two curves approaches zero.

Conclusions

A method was developed for the quantification of blood flow in the microcirculation with ultrasound contrast agents. This method enables us to measure perfusion changes caused by tumor angiogenesis and its regression during therapies targeting anti-angiogenesis. Time-intensity data were curve-fitted to Super-Gaussian functions and a Wash-in Index (*WI*) was derived. One very important aspect of the present work is the use of linear data (removal of logarithmic compression). The use of linear data enabled us to perform two different normalization schemes in order to treat variations caused by the bolus injection rate, patient cardiac output changes, and machine settings. The normalization schemes investigated were: the Wash-in Index Ratio (WI-Ratio) and the difference between normalized lesion intensity and normal parenchyma intensity. A problem encountered was respiratory motion especially when causing out of plane motion of the lesion and a novel gating scheme was introduced that was applied to the real-time data.

We have found out that our approach is repeatable when used in healthy volunteers. However, a larger cohort of healthy volunteers and patients not undergoing therapy must be studied in order to further validate the approach. In patients with colorectal metastases undergoing treatment we have seen that the *WI-Ratio* is reduced and the shape of the normalized difference curve (lesion intensity minus normal parenchyma intensity) approaches zero when response to therapy was observed and the patient's tumor markers were reduced.

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MICROBUBBLES TARGETING IN FAST FLOW CONDITIONS: MOLECULAR BIOMECHANICS INFLUENCES TARGETED IMAGING.

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Molecular imaging with targeted microbubble contrast agents is becoming a standard technique for preclinical evaluation of receptors within the vasculature, and may eventually become popular in the clinical setting. Intensity of ultrasound backscatter is directly related to the concentration of microbubbles deposited in the tissue, so it is very important to investigate the conditions, which result in the microbubble binding and retention on the target surface. Flow rate may play a critical role in the "binding/no binding" act for every contrast agent particle passing through the interrogated area.

A number of factors influences adhesion of a microbubble to the target. First of all, a bubble should have a chance to reach target vasculature (i.e., the higher the blood flow through the tissue, the higher the chance of bubble targeting). Furthermore, especially in larger vessels, microbubbles may stay away from the vessel wall, and flow by in the center of the vessel, not having a chance to touch the target. Flow irregularities (turbulence) may increase binding opportunity.

The elementary act of bubble "probing" the surface consists of a contact of a bubble with the surface, at which one or several ligands attached to the bubble surface will bind to the specific receptors on vascular endothelium. Whether attachment is firm and bubble will stay at the target, and for how long, will be determined by ligand and receptor surface density respectively on the bubble and on the cell, heterogeneity and clusterization, geometry of ligand and receptor positioning (such as a molecular spacer arm, or supramolecular macrostructures, such as membrane folds, tentacles or glycocalyx coat).

An important factor determining the success of targeting can be the kinetics of interaction of ligand and receptor. Initially, when antibodies were applied for microbubble targeting, it was discovered that adhesion efficacy drops in fast-flow conditions, because antibodies are selected (usually via ELISA) by their ability to bind to the target antigen with overall high affinity with slow detachment rate, not by the ability to bind to the target rapidly. Antibody-targeted bubbles were essentially ineffective in the high flow conditions, e.g., >4 dyn/cm2 wall shear stress.

Nature has evolved specialized ligands (Lewis carbohydrates) to achieve rapid binding to P- and E- selectins that are upregulated on the surface of vascular endothelium during inflammatory events. Use of these ligands, especially in the polymeric form and in combination with antibodies, ensures effective targeting, including slow rolling and firm binding of microbubbles in the conditions of fast flow in vitro, and successful targeted ultrasound molecular imaging of the areas of inflammation in vivo.

Amplifying the non-linear response of microbubbles at low pressure amplitudes

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The effectiveness of microbubbles as contrast agents for ultrasound imaging is, in large part, due to their ability to scatter ultrasound non-linearly. This phenomenon is exploited by techniques which use the non-linear components in the microbubble signal for image reconstruction, thus greatly increasing the contrast between the microbubbles and the surrounding tissue. In order to excite significantly non-linear behaviour, however, it is often necessary to drive the microbubbles with relatively high amplitude pulses. This can lead to bubble destruction, which is undesirable if continuous imaging of a particular region is to be performed. Similarly, if the bubbles are to be used therapeutically, e.g. for drug delivery or gene therapy, bubble destruction could result in reduced efficiency and the risk of unwanted tissue damage. It is therefore desirable to identify ways of exciting non-linear bubble behaviour at low pressure amplitudes. It has been found that certain types of bubble coating can increase the non-linear characteristics of gas bubbles, producing non-symmetrical “compression-only” oscillations. The aim of this work was to determine whether it was possible to reproduce and enhance this behaviour by modifying the microbubble coating. Initially, theoretical modelling was performed to identify the mechanisms contributing to the non-linear behaviour of microbubbles and to determine how the effects could be amplified. The theoretical results were first compared with those from previous experimental studies in which the radial oscillations of individual microbubbles coated with different surfactant compositions were recorded optically using a high speed camera (3 million frames per second) for insonation by a 4-cycle Gaussian pulse (centre frequency 0.5 MHz; peak negative pressure 60 kPa). Subsequently, experiments were carried out in which the acoustic response of suspensions of microbubbles was measured to determine the effect upon the scattered field of both varying the surfactant composition and of doping the coating with gold nanoparticles. It was found that the nanoparticles, in particular, enabled the harmonic content of the scattered signal to be significantly enhanced. The potential for diagnostic and therapeutic applications will be discussed.

Effects of nonlinear ultrasound propagation through varying contrast-agent concentrations

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Introduction: Several methods are being developed for quantitative contrast echography. These methods aim at the assessment of perfusion defects or alterations, caused for instance by the presence of ischemic or cancerous tissue [1, 2]. In order to extract quantitative data from contrast echography, the concentration of the diluted ultrasound contrast agent (UCA) must be accurately measured. To this end, the sensitivity to UCA of ultrasound (US) imaging techniques has been increasingly improved in the past decade by the introduction of several dedicated contrast-enhancement imaging modes, often referred to as harmonic modes, which exploit the nonlinear behaviour of the UCA microbubbles [3].
A common measure for the performance of these imaging modes is the contrast-to-tissue ratio (CTR), which is the ratio between the acoustic backscatter coming from UCA and tissue [4]. The determination of the CTR is usually based on independent measurements of the backscatter from UCA and tissue, while the interaction between the two systems is not considered. In this study, the nonlinear distortion of US propagating through varying concentrations of Definity® UCA (Bristol-Myers Squibb) is measured and modeled. The implications on contrast imaging are also discussed and quantified.

Methodology: For the analysis of nonlinear distortion, a narrow-band US signal was transmitted by a single element circular transducer. After passing through a latex tube filled with different concentrations of UCA, the distorted pressure wave was measured using a calibrated hydrophone (Onda HGL-0400). Two different US sequences were used: a ten period sinusoidal wave was used for the analysis of the harmonic content of the distorted US wave, while a sequence of three pulses of 2 periods, the first and third pulse having half amplitude, was used to determine the implications on power modulation contrast detection modes.
All measurements were performed with a low Mechanical Index (MI) of 0.1 (measured in the centre of the UCA dilutions, corresponding also to the transducer focal distance) and contrast concentrations up to 36μL/L. The concentration is defined in terms of gas volume per liquid volume. The investigated concentration interval is suitable for quantitative measurements because it provides, based on dedicated measurements performed by commercial scanners (Sonos 5500 and iE33, Philips Medical Systems), an approximately linear relationship between UCA concentration and backscattered acoustic intensity. Two US frequencies, 1 and 2 MHz, respectively below and close to the resonance frequency of Definity® UCA, were used.
The measured US signals were fitted by a nonlinear model derived from the Burgers equation (approximated to the second order) and describing the nonlinear sound propagation through a homogenous medium [5]. The results were also compared to numerical simulations of the combined linear-wave and modified Rayleigh-Plesset equations as described by Zabolotskaya and Soluyan [5, 6, 7]. This approach considers the bubble dynamics as the main source of nonlinearity.
Additional in vitro measurements were made to quantify the effects of US nonlinear propagation through UCA on the signal coming from a tissue mimicking phantom (ATS Laboratories) in ultra-harmonic, second harmonic, and power modulation mode by a Sonos 5500 and an iE33 US scanner. Software Q-Lab (Philips Medical Systems) was used for the acoustic quantification.

Results: The measurements showed an average increase of US distortion for increasing UCA concentration at both the investigated frequencies. This distortion can be measured as the fraction of the total power that is transferred to the second harmonic (Fig. 1). The increase of other harmonics (sub-, ultra- and super-harmonic) was less significant. An increase of the attenuation for increasing UCA concentrations was also found. Despite the attenuation, the power modulation measurements revealed an increased energy for increasing concentrations within the investigated range.
The Burgers model fits showed a high correlation coefficient (larger than 0.97 for all concentrations) at 1 MHz. Based on the Burgers model, the nonlinearity coefficient β was also estimated [5]. For the considered range of concentrations, $\beta \in (0, 100)$. For the measurements at 2 MHz (closer to resonance frequency), the

Burgers model could not provide accurate fits. In fact, at this frequency the bubble dynamics seems to be the dominant contribution to the US distortion. Therefore, the numerical simulations integrating the linear-wave and the modified Rayleigh-Plesset equations were adopted to predict the US distortion. The power-spectrum prediction was satisfactory. Obviously, accurate parameter estimations, such as by the Burgers model, cannot be obtained by numerical simulations.
Figure 2 shows two distorted waves at 1 and 2 MHz. The typical saw-shaped curve, explained by classic nonlinear propagation theory and well predicted by the Burgers model, is clearly recognizable at 1 MHz. At 2 MHz, the saw-shaped distortion is replaced by an asymmetry between compression and rarefaction, which is clearly related to the dynamics of coated bubbles. The distortion increases for increasing UCA concentration.
The in vitro measurements by the commercial scanners confirmed the increasing US distortion for increasing UCA concentrations. This effect was especially noticeable for ultra-harmonic imaging.

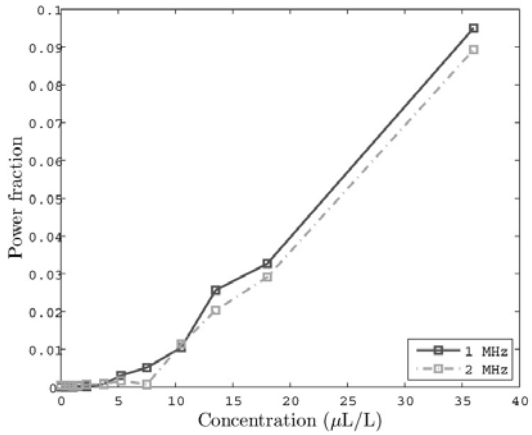

Fig. 1. Second harmonic power fraction.

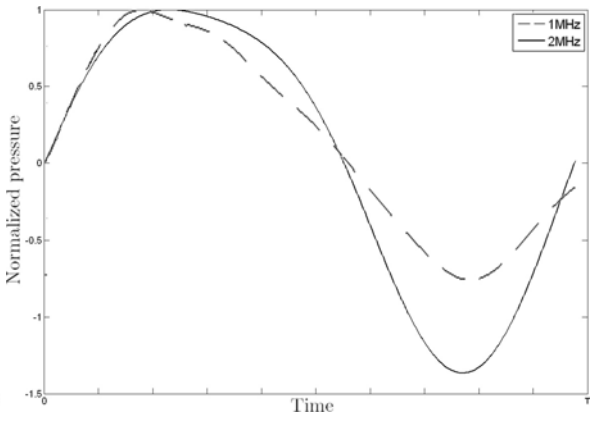

Fig. 2. Distorted US wave, 13.5 $^{\mu L}/_{L}$ Definity®.

Conclusions: The nonlinear distortion of US propagating through UCA dilutions was investigated for different concentrations of Definity®. For the measurements performed at 1 MHz, below resonance, the presented analysis by the Burgers model provides a suitable approach for quantification of the nonlinear distortion. Instead, the Burgers equation is not suitable for modelling nonlinear distortion of US propagating at 2 MHz. As this frequency is closer to the resonance frequency of the adopted UCA, models incorporating the nonlinear bubble dynamics are likely to provide better results.
In general, as confirmed by our measurements with commercial scanners, the US distortion increases for increasing UCA concentration. Therefore, attention should be paid to the nonlinear propagation of US through contrast when evaluating contrast enhancement modes. In particular, the CTR can be negatively affected by the effects of nonlinear propagation through UCA.

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Acoustic measurements of resonance behavior of single ultrasound contrast agent microbubbles

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With an increased interest in more sophisticated ultrasound contrast-specific imaging techniques, scientists are investigating means to accurately and quantitatively study the behavior of ultrasound contrast agents (UCA) in an ultrasound field. Acoustical studies on populations of UCA bubbles with a known size distribution have been widely performed. Several authors have proposed to fit experimentally obtained attenuation spectra with modeled spectra [1-2]. Although these models describe single bubble responses, they predict bulk behavior of a UCA as weighted sums of contributions from different bubble sizes. Bubble-bubble interactions are thus not taken into account although this could play an important role. Furthermore, Gorce et al. [2] showed by considering fractions of the native size distribution of SonoVue, that the shell properties of the bubbles are size-dependent. Therefore, to better understand and describe the behavior of UCA, one should look at individual bubbles.

Several authors have shown that high speed optical measurements provide quantitative information on the dynamical behavior of a single microbubble in a sound field [3]. Furthermore, such measurements have revealed new phenomena, e.g. compression-only behavior [4], threshold behavior [5] and the occurrence of surface modes [6], which have never been measured acoustically. Nevertheless, high speed imaging of microbubbles has some disadvantages. Dedicated high-speed cameras are costly and of limited availability. Moreover, optical studies are limited by the resolving power of the optical system in detecting small radial responses at low acoustic pressures. Furthermore, although optical measurements give quantitative information on the amplitude of oscillation of the microbubble, they do not provide a direct quantitative measure of the sound produced by these microbubbles (which is important for diagnostic applications).

To overcome these shortcomings we present quantitative acoustic measurements on single BR-14 (Bracco Research S.A., Geneva, Switzerland) microbubbles at low acoustic driving pressures, combined with simultaneous high speed imaging of the same bubble to resolve its radial dynamics. Single ultrasound contrast agent microbubbles were insonified at different frequencies, using both chirps and a microbubble spectroscopy approach [3] to investigate their full resonance behavior. Depending on the initial bubble radius, driving pressure amplitude and frequency, either optical or acoustical methods have a better sensitivity to detect the response of a single microbubble to ultrasound. Calculating the sound emission from the measured radius time curves gives excellent quantitative agreement with the directly measured sound emission. This finding gives good confidence in using radial responses as a measure for the acoustic response, and vice versa. An excellent agreement was found between the optical and acoustical response for bubbles displaying ‘compression-only’ behavior, showing for the first time that the compression-only bubbles display strong second harmonic sound emission.

Finally, we will show how ‘threshold behavior’ of a microbubble can be identified in its acoustical response [5].

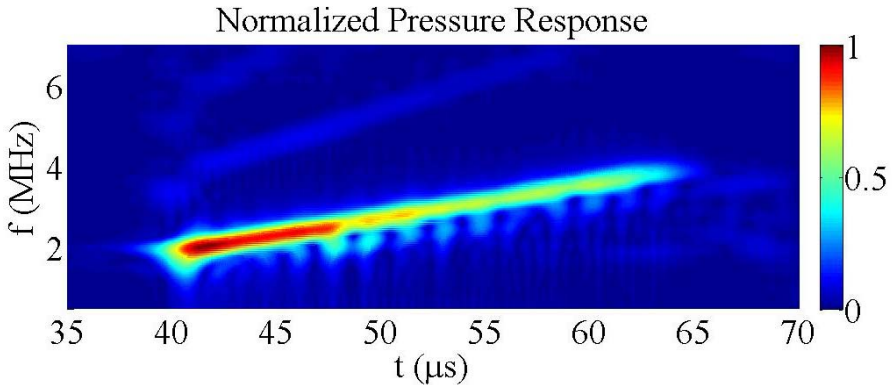

Figure: Typical acoustic response of a single microbubble with a radius of 3 μm to a 40-kPa amplitude chirp with a frequency sweep ranging from 1 to 4 MHz. Both the fundamental and the second harmonic responses are clearly visible.

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Prostate Imaging: What is the best modality?

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INTRODUCTION: THE PROBLEM

In the Western world, one in six men will be confronted with the diagnosis of prostate cancer (PCa) and one in thirty-two will die from it. In 2007 in the USA approximately 30% of all new cases in the male was prostate cancer (PCa), and approximately 10 % of all cancer related deaths was due to cancer of the prostate. With these numbers, PCa is the #1 in new diagnosed cancer cases, and it is #2 in death related cases in the male. Furthermore, based on increased life expectancy and a more aggressive screening of PCa, these numbers will further increase during the next decade. Treatment of PCa very frequently involves severe impairments of quality of life, including problems with urinary, bowel, and sexual function. This makes PCa one of the major cancer related health problems in the Western world.

The diagnosis of PCa is always based on histological systematic biopsy results. The decision to take biopsies is based on three clinical tests: PSA (prostate specific antigen), DRE (Digital Rectal Examination) and TRUS (TransRectal Ultrasound). However, the diagnostic accuracy of these tests is low which results in only 25% positive biopsy procedures.

What do we really need in for an improved diagnosis and treatment?
1) An accurate screening test, by which we can avoid unnecessary biopsies
2) We need to improve false negative results and improve grading. For this we need targeted instead of systematic (‘random’) blind biopsies.
3) In order to treat only the relevant cases we need an accurate grading and staging, to reduce over-treatment and unwanted side-effects.

Currently, all decisions related to patient care are based on rather limited pieces of information on the localization, extent and grade of malignant tissue. Except for bone metastasis imaging in late-stage disease, at this moment, there is no reliable imaging method available. Prostate cancer diagnosis and therapy are essentially blind towards the localization and extent of malignant tissue.

IMAGING

TRUS on its own has a low diagnostic accuracy for detection and staging, and is used only for guiding biopsies. Targeted biopsies directed towards visible lesions on B-mode TRUS have a very limited value. Color and power Doppler techniques increase the sensitivity of TRUS, but can certainly not avoid random biopsies.

Other imaging modalities, such as positron emission tomography (PET), CT and MRI are being evaluated. CT has no additional value in the diagnosis of prostate cancer, except for staging of the pelvic and distant metastases. MRI is replacing CT for this indication.

Reports on the value of endorectal (contrast enhanced) MRI are contradicting, and MRI is only used in selected centers for this application. Furthermore, MRI and certainly the latest MRI techniques are not widely available for routine patient care.

PET can visualize prostate cancer, however for the localization and detection of organ-confined prostate cancer, it is not useful. Recently, it was reported that PET–CT has some value for PCa diagnosis.

In conclusion, at this moment, there is no imaging modality available that can image PCa with the needed diagnostic accuracy.

CONTRAST ENHANCED TRUS (CETRUS)

TRUS is used for guiding biopsies and thus is part of the diagnostic process of prostate cancer all over the world. Sensitivity and specificity of TRUS are low and therefore new techniques are necessary to improve its accuracy. Based on promising results in different centers, we believe that contrast enhanced TRUS imaging may be capable of ‘un-blinding’ the PCa diagnosis and treatment, and therefore potentially is the best modality to image prostate cancer.

In this presentation the following will be discussed:
1) The current state-of-the-art of CETRUS.
2) Correlation between histology and imaging as determined in studies in which CETRUS imaging was performed before radical prostatectomies.
3) Large clinical studies in which CETRUS targeted biopsies were performed.
4) New developments to achieve an objective quantification.

As with all clinical research, new developments start in a few high-experienced centers. To prove the clinical value of these techniques, prospective multi-center studies are needed. The use of contrast enhanced TRUS is now in the phase that these large studies will be performed during the next years, and if the outcome is positive, contrast enhanced TRUS imaging may become a routine clinical diagnostic tool in PCa diagnosis and treatment.

TAKE HOME MESSAGE

Contrast enhanced TRUS has the potential to un-blind PCa imaging, and therefore is (probably) the best modality to visualize PCa.

Prostate cancer imaging – the case for contrast ultrasound

Heinrich von Busch

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To this date, the accurate, non-invasive identification, localization and assessment of malignant prostate lesions constitutes an unresolved clinical challenge. As a result, systematic (i.e., lesion-blind) biopsy and radical (again, lesion-blind) therapy remain the standard of prostate care.

The technical approaches that currently appear to hold the best promise for prostate cancer imaging are multiparametric magnetic resonance and contrast ultrasound. The presentation will discuss the current state, the hurdles in the way, and the implications of a success of contrast ultrasound for prostate cancer imaging from the perspective of a commercial medical equipment manufacturer.

Contrast Ultrasound Imaging of Inflammation in Cardiovascular Diseases

B.A. Kaufmann

Inflammation is a highly complex biological process, during which the emigration of leucocytes from the blood stream into inflamed tissue is tightly regulated by an interplay between adhesion molecules on the endothelial cell surface, integrins on leukocytes, and local cytokine concentrations. Inflammation is central to pathogenetic processes in cardiovascular diseases such as atherosclerosis or inflammation during reperfusion after myocardial ischemia. Targeting of microbubbles to endothelial surface antigens allows molecular imaging of the appearance of specific adhesion molecules. P-Selectin is an adhesion molecule that appears rapidly on the endothelial surface after an inciting inflammatory event and persists for hours even if the inflammatory stimulus subsides. Molecular imaging of the expression of P-Selectin with contrast enhanced ultrasound has been used to detect recent myocardial ischemia after resolution of the perfusion defect and wall motion disturbance in animal models. Such a strategy, also termed ‘ischemic memory’ imaging, could be used in the clinical arena for diagnosing patients with recent chest pain that has resolved at presentation. Vascular cell adhesion molecule 1 (VCAM-1) is another cell adhesion molecule that plays a role in the pathogenesis of atherosclerosis and has been shown to be upregulated early in the development of atherosclerotic plaques. Contrast enhanced ultrasound targeted to VCAM-1 can quantify vascular inflammation that occurs in different stages of atherosclerosis in animal models of atherosclerosis. This method may in the future be useful to risk stratify patients according to vascular inflammatory phenotype.

Molecular mapping of murine tumor vasculature biomarkers using targeted contrast high-frequency ultrasound imaging.

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Background: Diagnosis of the molecular signatures of the vasculature of malignant tumors can aid in the therapy of this life-threatening disease. Molecular imaging of the selective endothelial markers of tumor vasculature will help in understanding tumor development process and designing targeted therapies. In this study, we explored targeted ultrasound contrast imaging of the vasculature markers (P- and E- selectins, VCAM-1 and VEGF receptors) expressed on the surface of tumor endothelium during the course of tumor development in a murine model.

Methods: Decafluorobutane microbubbles stabilized with a lipid monolayer shell carrying biotin-PEG3400 were prepared by sonication. Biotinylated polymeric sulfo Lewis^x (Glycotech, Rockville, MD), biotinylated MVCAM.A antibody (Pharmingen, San Diego, CA), or biotinylated single-chain VEGF (Sibtech, Newington, CT) were coupled to microbubbles via streptavidin link. MC38 colon carcinoma cells were injected subcutaneously in the hind leg of C57Bl/6 female mice (30-35 g body mass) in accordance with IACUC-approved protocol. Ultrasound imaging (vevo 770 system, 40 MHz, contrast package, Visualsonics, Toronto, Canada) was performed 5 min after intravenous administration of microbubble contrast (5-10x10^6 microbubbles). Ultrasound-induced destruction was applied to distinguish targeted microbubbles from residual circulating contrast. Visualsonics contrast software was used to determine mean signal intensity in the region of interest. Repeated contrast imaging was performed as tumor progressed.
Results: For all tumors sizes, ultrasound imaging immediately after contrast agent administration showed microbubbles passing through the tumor vasculature. Most of circulating bubbles cleared at 5 min. Control non-targeted bubbles did not accumulate, regardless of tumor size. Polymeric HSO_3Lex microbubbles successfully targeted to P- and E- selectins present in the vasculature of smaller tumors (20 mm^2 or less, with best accumulation in smallest tumors); as tumors increased in size, accumulation of these bubbles in the tumor vasculature dropped to background level. VCAM-1-targeted bubbles accumulated well in all tumors except largest (31-40 mm^2 cohort). VEGF-carrying bubbles accumulated in all but the smallest tumors (5mm^2).
Conclusions: Targeted ultrasound contrast agents allow molecular imaging of inflammation and angiogenesis related vascular gene expression. Differential accumulation of each contrast agent-type during tumor growth indicates the potential to use ultrasound contrast molecular imaging for tumor development assessment.

Quantification of E-selectin Expression in the Mouse Heart Using Targeted Microbubble Contrast Enhanced Echocardiography

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Background Inflammation underlies important cardiovascular diseases such as coronary heart disease, myocarditis and heart transplantation rejection. The ability to detect the extent and degree of inflammation in the heart at molecular level has potential diagnostic, prognostic and patient management values. E-selectin is an inflammatory molecule expressed on activated endothelial cells, and is involved in the recruitment of leukocytes to sites of inflammation. We designed targeting microbubbles (MBs) as contrast enhancement agents for real-time echo imaging and quantification of E-selectin expressions in the mouse heart.

Methods Phospholipid MBs targeted to E-selectin were prepared by conjugating reduced anti-E-selectin $F(ab')_2$ onto the MB surface using maleimide. The size and concentration of the MBs were determined using electrozone sensing and specific binding was confirmed optically *in vitro* using E-selectin coated plates. Lipopolysaccharide (LPS, a bacterial endotoxin) was used to induce global E-selectin expression *in vivo* in mouse hearts. The expression of E-selectin *mRNA* in the heart at different time points post LPS induction was quantified using real-time reverse transcription polymerase chain reaction (RT-qPCR). This was found to correlate with the amount of E-selectin *protein* measured using radio-labeled antibodies published elsewhere. MB ultrasound imaging to detect and quantify E-selectin in the heart was done on anaesthetised adult male C57Bl6 mice (wild-type, WT) at 4-5h or 5-6h post LPS induction. These time points were chosen as the levels of E-selectin expression differed by ~2 fold between them. Targeting MBs (dose ~$1x10^8$) were injected as a bolus via the tail vein. A Siemens Acuson Sequoia 512 with the 15L8-S transducer in Contrast Pulse Sequencing mode (CPS) at 14MHz was used. The scanner was set at low power (mechanical index, MI = 0.22-0.26) with a fixed gain. LPS treated homozygote E-selectin knock-out (KO) mice on C57Bl6 genetic background were used as negative controls. Ultrasound image signal intensities were log-decompressed, background subtracted and quantified off-line using a dedicated YABCO© software.

Results Targeting MBs were stable; their mean & maximum diameters were 2 & 6um, respectively - allowing non-obstructed flow in the microvasculature. MBs cleared from the blood pool earlier in WT than KO mice, the latter required upto 24min following MB injection. Targeted MB echo detected E-selectin in the myocardium of WT (11/11) but not KO mice (5/5) in real-time, Fig 1. Overlying bone and air caused attenuation of ultrasound signals in the septal and inferior walls of the heart in the parasternal short axis imaging plane (PSA), more marked for CPS than B mode, Fig 1b. However, attenuation in these cardiac walls were absent/less marked when other imaging planes (Fig 1d) or lower US frequencies (eg 7MHz at the same MI & gain) were used. Echo signal intensities for E-selectin in the non-attenuated cardiac walls (eg anterior wall) in the PSA plane, measured at 24min post MB injection correlated with the levels of E-selectin expression, Fig 2.

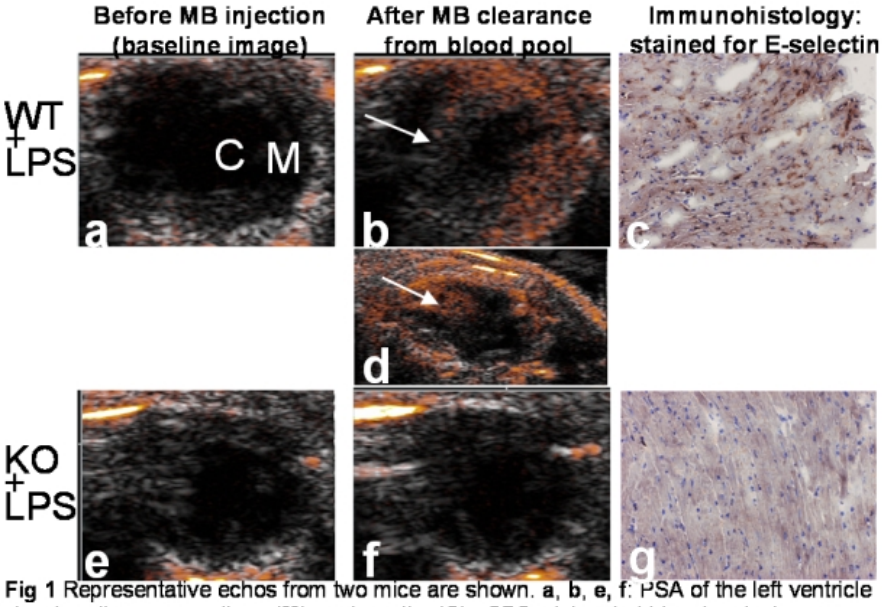

Fig 1 Representative echos from two mice are shown. **a**, **b**, **e**, **f**: PSA of the left ventricle showing the myocardium (**M**) and cavity (**C**). CPS giving bubble signals in orange (heated object scale) was overlaid on B-mode giving mainly tissue signals (grey scale). E-selectin expression was detected in the myocardium of LPS-induced wild-type mouse (**b**) and absent in the E-selectin KO (**f**). Note the attenuation of US signals in the septal wall (**arrow, b**) which was absent/less marked in the parasternal long axis imaging plane (**arrow, d**). Frozen section immunohistochemistry confirmed the presence (**c**, positive brown staining in the microvasculature) and absence (**g**) of E-selectin expression in the wild-type and E-selectin KO hearts, respectively.

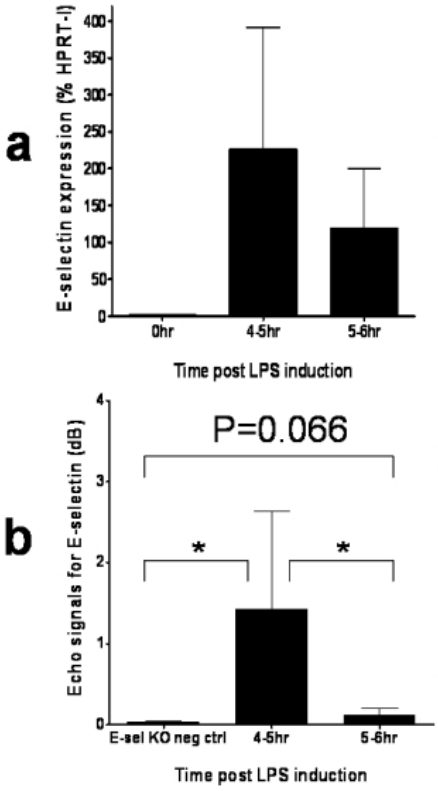

Fig 2

a E-selectin expression in the heart of adult male C57Bl6 mice at 0h (no LPS), 4-5h and 5-6h post LPS induction. Levels of mRNA expressed as % hypoxanthine phosphoribosyltransferase-I (HPRT-I, a house keeping gene). n = 22

b Quantification of targeted MB echo signals for E-selectin in the heart of adult male C57Bl6 mice at 4-5h and 5-6h post LPS induction. US signal intensities (measured at 24min post MB injection) correlated with the levels of E-selectin expression seen in (**a**). n = 16

* p<0.05

Conclusion We believe this is the first report demonstrating that acoustic quantification of molecular expressions in the heart is feasible using targeted MB echo.

Adverse reactions to ultrasound contrast agents: is the risk worth the benefit?

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The introduction of ultrasound contrast agents has led to a markable improvement of diagnostic capabilities in echocardiography. As no serious adverse events were seen during the clinical development phase, ultrasound contrast agents were thought to be safe. Three fatal and nineteen severe, non-fatal adverse reactions were reported in a post marketing analysis of more than 150,000 studies of Sonovue®, which has led to the addition of several contra-indications for the use of this ultrasound contrast agent. Although a strong relationship was established between the non-fatal cases and administration of Sonovue®, a causal relationship between the fatal cases and the use of Sonovue is debatable. Recently, fatalities with Definity® led to a black box warning in the USA.For a balanced risk stratification of the use of an echo contrast agent, the occurrence of adverse events must be compared with other contrast agents such as radiopharmaceuticals, ionics and non-ionics, agents used in magnetic resonance imaging, and other ultrasound contrast agents Besides, the risks and benefits of the use of ultrasound contrast agents should be compared with the value and hazards of that of other tests, such as exercise tests and dobutamine stress echocardiography. Therefore, the risk associated with the use of ultrasound contrast agents should be judged carefully, taking into consideration the prevalence of adverse effects of other contrast media and diagnostic procedures used in cardiology.

Table. Studies assessing the safety of several types of contrast media.

Author/reference	Type of contrast agent	Number of studies	Serious adverse events	Fatal adverse events	Total serious adverse event rate
Niendorf et al.	Gd-GTPA : -Phase IIIb-IV -PMS	13,439 2.000,000	0 (0%) 32 (0.002%)	0 (0%) 7 {0.0004%}	0 (0%) 39 (0.002%)
Katayama et al.	-Ionic contrast media -Non-ionic contrast media	169,284 168,363	370 (0.22%) 70 (0.04%)	{1} (0.0006%) {1} (0.0006%)	371(0.22%) 71 (0.04%)
Shehadi et al.	Intravascular contrast media	302,083	216 (0.07%)	18 (0.006%)	244 (0.077%)
Silberstein et al.	-Radiopharmaceuticals -Non-radioactive pharmaceuticals	783,525 67,835	0 (0%) 1 (0.001%)	0 (0%) 0 (0%)	0 (0%) 1 (0.001%)
Personal communication	-Optison	>500,000	≤10 (≤0.002%)	0 (0%)	≤10 (≤0.002%)
EMEA	Sonovue	157,838	19 (0.012%)	3 (0.002%)	22 (0.014%)

PMS: post marketing surveillance; {…} no causal relationship established.

Non-invasive Imaging of Atherosclerosis

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Goal: World–wide Cardiovascular Prevention
The pan-epidemic of obesity, metabolic syndrome and diabetes presage the incidence of accelerated cardiovascular (CV) mortality and morbidity in world-wide populations. Absent coordinated CV preventive programs among academic and governmental agencies, public health care policies currently provide only limited prevention opportunities and are unlikely to effectively provide long term solutions for the CV pan-epidemic.

Proposal: Identify preclinical atherosclerosis through the development of programs which include prudent administration and distribution of available resources, enhanced utilization of technology and improved public education.

Technology developments: There is an ever-increasing utilization of non-invasive imaging modalities that includes: 3-D echo with speckle tracking for the detection of regional contractile abnormalities (including sub endo-sub epicardial differences in disease states), carotid IMT and vasa vasorum imaging, automated arterial compliance testing, coronary artery calcification (CAC) testing, CT-angiography, and a host of biochemical surrogate markers of inflammation (HS-crp, Lp(a), LpPLA2, fractionated cholesterol testing, HgbA1C, etc.).
It is important to incorporate these technologies into our current health care systems with a special focus on the current and future uses of ultrasound contrast agents for the detection and monitoring of atherosclerosis. For over 20 years, the measurement of carotid intima-media-thickness (c-IMT) has served as a surrogate marker of atherosclerosis; first described by Pignoli in 1986. Subsequently, the c-IMT method has become an accepted standard for clinical trial endpoints. (A listing of the title: “carotid intima-media-thickness 2007” resulted in 1277 entries; Entrez Med Dec 23, 2007). Increasingly there is a trend toward using the ultrasound derived c-IMT as a clinical screening tool for preclinical disease in at risk populations.

Improvement in c-IMT imaging: The use of an ultrasound contrast agents when applied to carotid imaging result in enhancement of the carotid lumen, thus permitting improved precision of the c-IMT. The contrast-filled, lumen provides a superb backdrop for highlighting the luminal irregularities and importantly, provides direct visualization of angiogenesis within the carotid artery wall, including atherosclerotic plaques and vessel wall neovascularization (vasa vasorum).

Recently several investigators have published work corroborating these initial findings, that is the investigators have similarly described that the use of ultrasound contrast to identify vessel wall and intraplaque angiogenesis in patients with CV disease.

Future: The future applications of non-invasive imaging of atherosclerosis may well include the uses of ultrasound contrast agent and real-time 3-D quantification of the c-IMT and vasa vasorum for diagnostic and therapeutic interventions in patients identified as “high risk” for

premature CV disease. If validated clinically, these innovative, non-invasive, technologies may become an integral aspect of the universal program for the prevention of CV diseases.

Case report: Non-invasive diagnosis of hepatic nodule in cirrhosis – from dysplasia to hepatocellular carcinoma.

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Hepatocellular carcinoma (HCC) complicates liver cirrhosis and constitutes the leading cause of death. The sole approach to achieve long-term survival is to detect tumour at an early stage, when effective therapy can be applied. HCC is a result of a multi-step hepatocarcinogenesis process. A regenerative nodule (RN) might be the first step, subsequently developing into HCC via low- and high-grade dysplastic nodule (DN). Early small HCC is usually composed of well-differentiated hepatocytes, and this turns the confident diagnosis through examination of biopsy/FNAB samples into a pathological challenge. At some point during the process the formation of new tumour vessels (neoangiogenesis and capillarization) leads to a gradual change in blood supply. Although complex, the blood supply of the nodule has shown a sequential decrease in the portal venous flow and an increase in the arterial blood supply as the lesion progresses from DN to HCC. Neovascularity within HCC is used for detection and characterisation on several imaging techniques (CT, MRI, US).

A 48 y/o man, suffering liver cirrhosis caused by alcohol abuse and chronic hepatitis B virus infection is presented. Diagnosis was made in 2006 on clinical and laboratory grounds, patient developed several clinical cirrhotic complications as ascites, spontaneous bacterial peritonitis, hepatic encephalopathy and esophageal varices grade III. Screening for liver transplantation was completed in May 2007 and patient was put on a waiting list. Ascites became refractory and TIPS (transjugular portosystemic shunt) was placed in August 2007.

Initial liver imaging was done in the end of May 2007 and a focal lesion in segment 5 was found which was regarded on MRI as regenerative nodule. On contrast-enhanced ultrasound (CEUS), except an impressive macro-nodular liver parenchyma, the same 19 mm lesion showed iso- or already slightly hyperechogenic arterial phase contrast accumulation (Sonovue, Bracco, Italy), without any discernible washout. Lesion was diagnosed as a yet dysplastic nodule.

CEUS imaging after 3 months observed stronger unequivocal arterial enhancement, but again without any washout in delayed phase, i.e. change in characteristics comparing to first exam. CEUS and also a new MRI scan expressed concerns about possible early HCC, because now the arterial neovascularization became indisputable.

CEUS imaging after 6 months confirmed increase in tumour size (30 mm) and for the first time found also washout sign in late venous phase, thus was already conclusive for malignant transformation (HCC). Alpha-1-fetoprotein stayed within normal range. The patient was scheduled for RFA (radiofrequency ablation) and wait for transplantation.

These findings could provide support of the proposed step-wise carcinogenesis in HCC in vivo. Such observations are clinically significant and can be used for early detection of HCC, which may allow improved patient selection for further close follow-up and increased patient position on waiting list for liver transplantation.

Several studies have shown that the characteristic HCC profile includes the intense arterial uptake, which is followed by contrast washout in the delayed venous phase[1]. Diagnosis of HCC 20 mm or smaller can be even established without a positive biopsy if both CEUS and MRI are regarded as conclusive[2].

[1] Lencioni R, Cioni D, della Pina C, Crocetti L, Bartolozzi C, Imaging diagnosis. Semin Liver Dis, 2005;25(2):162-70. Review.

[2] Forner A, Vilana R, Ayuso C, Bianchi L, Solé M, Ayuso JR, Boix L, Sala M, Varela M, Llovet JM, Brú C, Bruix J. Diagnosis of hepatic nodules 20 mm or smaller in cirrhosis: Prospective validation of the noninvasive diagnostic criteria for hepatocellular carcinoma. Hepatology. 2007 Dec 10;

Adenosine contrast and fractional flow reserve

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Despite the fact that 34000 patients with stress echo were reported in literature no physiologic measurements to correlate the results were performed. Therefore, in the future echo perfusion should be correlated with physiologic parameters.

A 56 year old man was referred for cardiology screening because of atypical chest pain. Cardiac evaluation using nuclear scan and exercise test were not conclusive.
An adenosine contrast test was performed to measure quantitatively myocardial perfusion in the LAD area. Cardiac catheterisation showed a normal FFR (> 0.85). Adenosine contrast echo was normal in LAD perfusion but showed decreased perfusion inferiorly. This was confirmed by cardiac catheterisation with a severe RCA lesion which was treated by stent.

Conclusion

Adenosine contrast echo is feasible, quantitative and should be correlated with FFR for real physiologic correlation.