S1-1 Bubble behaviors with different bubble-specific imaging

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Background

A combination of ultrasound pulsing sequences enables to extract nonlinear signals exclusively from microbubbles. Among several kinds of contrast pulsing sequences implemented on diagnostic ultrasound systems, amplitude modulation (AM) method is mainly used for Sonazoid contrast examination. It is because enhancement of Sonazoid's bubbles needs slightly larger mechanical power in ultrasound transmission pulse, where nonlinear signals from soft tissue are also generated. It is known that AM generates "nonlinear fundamental (NF)" components from microbubbles while less NF from soft tissue. It is major advantage of AM for applying to Sonazoid examination. However, the definition and mechanism of NF were not clear. It was also not evident that the phenomenon happens only in AM but not in phase inversion (PI) method. We in the phantom experiment compared nonlinear fundamental echo intensity between AM and PI with completely the same transmission frequency conditions.

Materials & methods

Ultrasound diagnosis system used was LOGIQ S8 and E9 (GE Healthcare). Transducer was C1-5 (3.5 MHz convex). An agar-graphite scattering phantom with a small water pool (4 cm in diameter) was used. Tissue-mimicking cellulose fibers were immersed into the pool to maintain echo intensity as same as in graphite region. Drop injection of Sonazoid was administrated into the pool at a concentration of 0.08 ml/L. The solution of Sonazoid perfuses in the pool and flows out. Ultrasound pulses were prepared experimentally at a frequency of 2.6 MHz, including an inverted pulse for PI and a half amplitude pulse for AM. Images of PI and AM obtained by the combination of ultrasound pulsing sequences respectively. Reception frequency was set to 2.6 MHz for both methods to extract only NF components. Mechanical index was set between 0.13-0.20.

Results

Echo intensity by AM showed much more intense signals than by PI. NF by PI was less but slightly visible (Fig.1). AM was found more robust against motion artifact than PI. Numerical simulation corresponded sufficiently to the result of the experiment (Fig.2). NF component by AM is +20 dB larger than by PI, although second harmonics components by PI are larger than AM's. A time-domain residual signal for AM shows very intuitive expression of the cycle of 2 fundamental frequency, also suggests the change of resonant frequency of the bubbles by different amplitude stimulation (Fig.3).

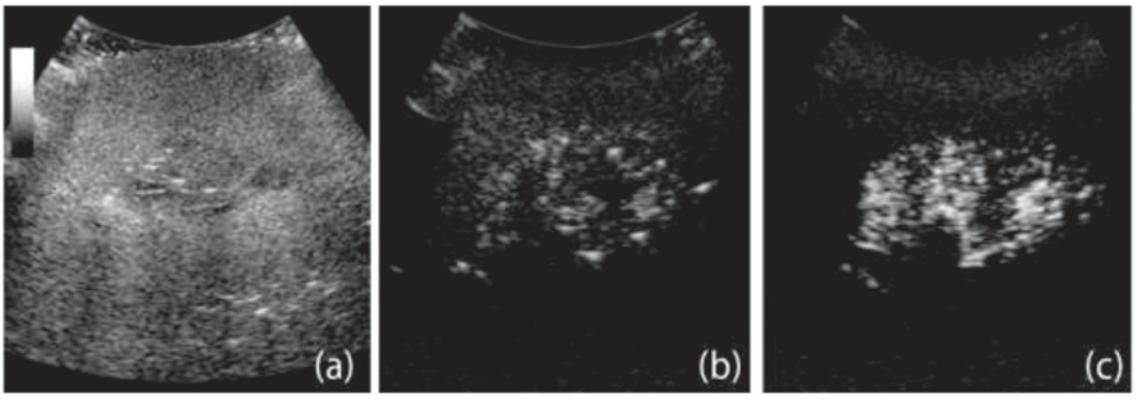

Fig.1 Image examples of the experimental phantom with Sonazoid solution imaged by (a) fundamental B-mode, (b) phase inversion and (c) amplitude modulation. Note that the images (b) and (c) are specialized to extract only NF components.

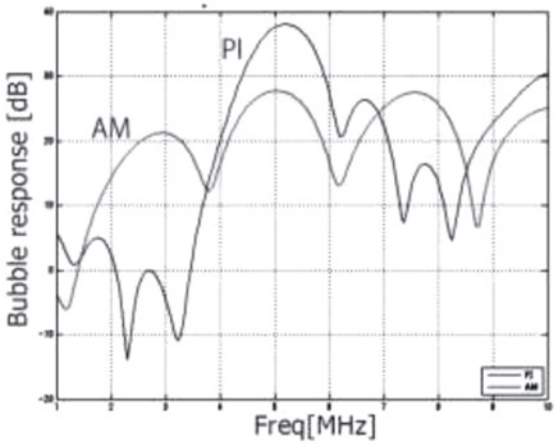

Fig.2

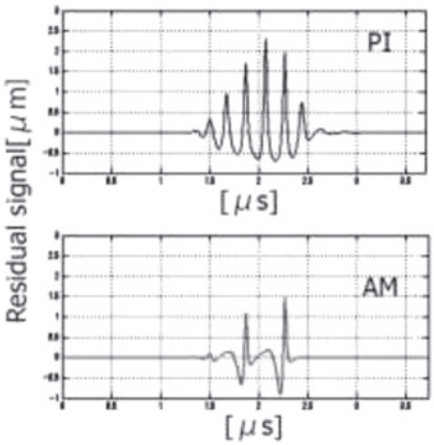

Fig.3

Fig.2 Results of numerical simulation of frequency response of the microbubble for PI and AM methods.
Model: Modified Rayleigh-Plesset, Solver: MATLAB ode15s, initial diameter of the bubble: 3.0 micron, shell thickness: 3.6 nm, pulse amplitude: 320 kPa.

Fig.3 Results of numerical simulation of time-domain response of the microbubble for PI and AM

S1-2 Enhancement of molecular targeting in CEUS using volumic acoustic radiation force

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CONTEXT: Ultrasound molecular imaging (using injection of targeted microbubbles) is expected to improve oncological outcomes in the future. Microbubbles are conjugated with a ligand to target a molecule (receptor) upregulated on tumor vasculature. The receptor-ligand interaction enables microbubbles to specifically adhere to endothelial cells at the location of the tumor. This makes tumor localization more conspicuous in ultrasound contrast images. Potential clinical applications range from early tumor detection to quantitative therapy monitoring, where determination of patient response to an anti-cancer drug treatment plays a crucial role in terms of survival.

OBJECTIVE: To increase probability of receptor-ligand interactions, application of acoustic radiation force to push targeted microbubbles on endothelium was proven effective [1-4]. As a result, molecular targeting is enhanced. Its development in 3D medical ultrasound is named volumic acoustic radiation force (VARF). The use of 3D makes sense for cancer detection since tumor location is a priori unknown. Detection in prostate gland is particularly relevant since the gland can be entirely scanned with a volume imaging transducer. Our objective was to implement VARF in a clinical ultrasound system and to quantify resulting molecular targeting enhancement at the pre-clinical stage.

METHODS: A clinical ultrasound scanner (Aplio™ XG; Toshiba Medical Systems, Otawara-Shi, Japan), equipped with a volume imaging transducer (PVT-681MV), was modified to generate VARF and to acquire 3D contrast data for quantification. To avoid microbubble destruction, optimal VARF parameters were defined using a suspension of agitated microbubbles in a glass beaker. First, an in vitro validation was performed with a targeted contrast agent: biotinylated microbubbles conjugated to anti-VCAM-1 antibody using streptavidin. A syringe pump (PHD 2000; Harvard Apparatus, Holliston) was used to create a laminar flow of agent in a parallel plates flow chamber (IBIDI, Martinsried, Germany) coated with murine VCAM-1 (3μg/ml). Flow was set to reflect physiological conditions in tumors (wall shear stress at 0.6 dyne/cm^2). Second, a pre-clinical validation in a small animal model was performed with non-xenograft mouse tumor. Targeted and control (no ligand) contrast agents were injected as bolus (~2×10^6 microbubbles). The 3D contrast data were acquired for both in vitro and in vivo experiments. The data were processed to quantify concentration of bound microbubbles in late phase (7 mins after contrast agent arrival at the target site). Improvement in molecular targeting was defined by the enhancement ratio: VARF-to-Native ratio of bound microbubble concentration; where Native means no application of VARF.

RESULTS: An enhancement ratio of 6.1 (SD of 2.1, n=3) was measured in vitro with the targeted contrast agent. For in vivo, an enhancement ratio of 4.1 (SD of 2.3, n=3) was measured. As expected, no relevant incidence of VARF was observed with the control agent for which the enhancement ratio was 0.8 (SD of 0.1, n=3). Furthermore, a dedicated volume rendering method was developed to display the enhancement by VARF as shown in Figure 1.

CONCLUSION: In this study, pre-clinical in vivo validation of VARF (enhancement ratio of 4.1) was demonstrated with a modified clinical ultrasound imaging system, offering potential implementation into the clinic. Further processing will include advanced 3D quantification to fully characterize the vascular network of tumoral tissue.

ACKNOWLEDGMENTS: The authors would like to thank Ms. Y. Kanayama, Mr. G. Guenette, Mr. R. Woods and Mr. C. Sanders from Toshiba for their technical support.

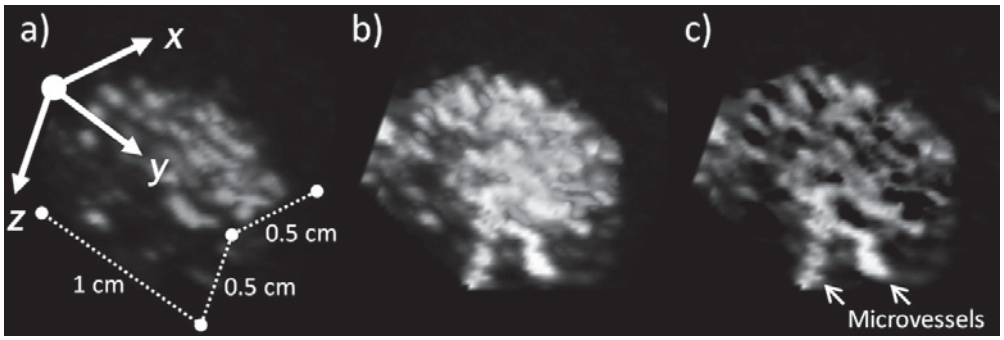

Figure 1. Volume rendering of contrast agent concentration in a tumor, (a) without VARF called Native, (b) with VARF, (c) enhancement defined as VARF volume subtracted from Native volume.

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S1-3 Microbubble-Enhanced focused ultrasound: Application for brain drug delivery

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Focused ultrasound (FUS) technology, which appears half century ago, has got more and more attention on its clinical therapeutic potential in recent years. But only within few years, focused ultrasound with the presence of microbubbles has been noticed to be capable of locally and reversibly disrupting blood-brain barrier (BBB) without causing neuronal damage and proof its potential in the delivery of macromolecules into CNS, including those therapeutic agents for CNS therapy which may not naturally cross the barrier into brain, and brings bright hopes for noninvasive brain drug delivery for treating brain cancers or neurodegenerative diseases. Since real-time image guidance is necessary, detecting and monitoring local drug release is one key step and a critical role to the success of FUS brain drug delivery to make this new technology to be clinically relevant. Current advanced nanotechnology brings opportunity to design novel nano-carriers to serve both as an image indicator and therapeutic agents to allow simultaneous diagnostic imaging and drug delivery monitoring in vivo in real time. In this presentation, we report the current progress on the in-vivo animal testing as the proof-of-concept. Novel nanoparticles (including diagnostic and therapeutic nanoparticles) will be introduced about their participation of the procedure (including safety, biodistribution, agent quantification, and treatment evaluation) among different imaging platforms (including MRI, ultrasound, nuclear imaging) and their efficacy will be discussed. We also review the current progress on the use of FUS-BBB opening technology for CNS disease treatment.

S1-4 The biological and sonochemical effects of microbubbles: Impact of formulation

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The use of ultrasound contrast agents has become indispensable in ultrasonography. Likewise, in therapeutic applications it is acknowledged that the effects of ultrasound are amplified when microbubbles are present in solution. However, the industry of acoustic contrast agents is still stumbling because of stability issues and the ambiguity of the dynamics of the interaction between microbubbles and cells. Solving the stability of microbubbles through the introduction of encapsulating shells and the use of different core materials has further complicated the situation. Shells and core gases have changed the physical and acoustical properties of the final formulations as well as their biological and sonochemical effects. On the other hand, the molecular machines inside the different cells of a human body are not equally programmed and in response to acoustic stimuli they behave differently. Despite the variability among the settings of each study, similar responses have been recorded at different occasions. These similarities may infer basic pathways governing the responses to ultrasound and should be taken seriously into consideration for validation or rebuttal as we proceed. In this presentation, we aim to provide a conclusive summary of available and recent findings and assumptions derived from thorough research on the biological and sonochemical effects of medical ultrasound, contrast agents and novel microbubbles. For instance, a comparative study of six microbubbles has shown that the shell elasticity and reactivity can influence the extent and onset of cell killing. Microbubbles with elastic shells were shown to be more capable of inducing delayed effects on cell viability whereas the combination between chemically reactive robust shells and high-density gases, such as perfluorocarbons, could exert a protective effect on cells.

S2-1 Quantification in ultrasound molecular imaging using VEGF targeting contrast agent, BR55

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Objectives: To quantitatively assess in mice the contrast kinetics of VEGFR2-targeted microbubbles (BR55) as compared with standard clinically used microbubbles (SonoVue) in both normal liver and human hepatocellular carcinoma (HCC) xenograft tumors.

Materials and Methods: Experiments on BR55 and SonoVue microbubbles were carried out using a clinical ultrasound imaging system. Microbubbles were injected intravenously into healthy mice (n=5) and mice bearing HCC xenograft tumors (n=10). Cine loops of contrast enhancement in normal liver and in the tumors were acquired for a period of 10 min in intermittent imaging mode with a low mechanical index (0.12). The cine loops were processed to objectively compare the differences in contrast kinetics. Quantitative perfusion parameters were derived by fitting time-intensity curves using a dedicated mathematical model combining a bolus function and a ramp function. The perfusion parameters were then statistically analyzed using the paired *t*-test. Immunohistochemical examination was also performed for normal liver and tumor specimens to determine the level of VEGFR2 expression.

Results: The peak contrast enhancement observed in normal liver with BR55 was comparable to that with SonoVue, whereas a significant difference was observed in late-phase enhancement at 10 min (ramp slope: P=0.0099). In the tumor model, SonoVue was rapidly cleared from the circulation, without any noticeable binding in the tumor, whereas BR55 showed a gradual decline, resulting in a longer washout period (mean transit time: P=0.0189). Immunohistochemical examination demonstrated that intratumoral vascular endothelial cells exhibited sparse and weak VEGFR2 expression, whereas the sinusoidal capillaries in normal liver exhibited much more diffuse and much stronger VEGFR2 expression.

Conclusions: Our results suggest that BR55 accurately reflects the VEGFR2 status in human HCC xenograft tumors. However, the mechanism of the uptake of VEGFR2-targeted microbubbles in the liver requires further investigation. Finally, we have demonstrated that quantification applied to ultrasound molecular imaging may provide an objective method for measuring the degree of binding of microbubbles.

S2-2 Microbubble mediated gene and drug delivery

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Ultrasound contrast agents, in the form of gas-filled microbubbles, are becoming popular in perfusion monitoring as intravascular contrast agent, besides, they are also employed as carrier of drug substances or genes.
Microbubbles have great potential to improve the delivery of therapeutic materials into cells and to modify the vascular permeability causing increased extravasation of drugs and drug carriers.
Ultrasound energy can be applied for releasing local drug and genetic materials from microbubbles circulating in the blood, enhancing the extravasation out of vessels, and increasing delivery into the cells. The combination of nanotechnologies and imaging technology is providing novel approaches to achieve spatio-temporal control of drug delivery.
Drug substances, including genetic therapeutics, can be attached to or incorporated in the microbubble particles for ultrasound-triggered release in the insonated organs and tissues.
The targeting ligands including antibodies, peptide or aptamers also can be attached to the surface of microbubbles. For the molecular imaging and specific delivery of drug substances, the use of specifically modified microbubbles would be needed.
Several researches regarding drug and gene delivery strategies using nanotechnologies and microbubbles will be introduced as well as targeting strategies.
Microbubbles and ultrasound also show great potential in delivering materials into central nervous system by transient disruption of BBB (blood brain barrier) for treatment of brain tumor or brain disease including Alzheimer's disease. Ultrasound and microbubbles can induce transient disruption of BBB and recovery after several hours in animal studies.
The use of microbubbles as a tool for increased local gene and drug delivery has an enormous clinical potential, especially in oncology, vascular diseases and CNS diseases.
In this talk, we will present our experience regarding synthesis of microbubbles with targeting ligands on surface of microubbles. Besides, we will present several experiments regarding delivery *in vitro* as well as *in vivo* conditions of breast cancer, prostate cancer and VX2 tumor models. We could conclude that we could enhance the delivery of anticancer drugs (Doxorubicin) and/or siRNA into the tumor cells targeting HER2. Our product would be helpful in delivering new drugs or genetic therapeutics into several organs such as breast, prostate, pancreas or liver, where ultrasound can reach.

S2-3 Enhancement of ultrasound-facilitated gene transfer using nanoparticles

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Gene therapy is becoming increasingly important in the treatment of certain inherited and acquired disorders, such as congenital immunodeficiency, atherosclerosis, and cancer. Comparing with viral vectors, gene transfer mediated by non-viral vectors exhibits many of the desired characteristics for future human gene therapy, including low immunogenicity, treatment convenience, and site specificity Ultrasound-facilitated gene therapy is a newly developed biomedical technique that can target gene expression at specific sites, leading to expression of therapeutic proteins locally, thus providing great potentials for the treatment of various diseases. However, ultrasound-facilitated gene transfer suffers from low transfection efficiency and short expression duration. The purpose of this presentation is to show our recent work on evaluating the synergic effect of combining nanoparticle carriers (such as polyethylenimine or PEI) and cavitation on gene transfer to enhance and prolong the therapeutic effect of gene transfer. Strong expression of reporter gene expression could be found at least 45 days after the treatment of DNA-nanoparticle complex with US exposure in vivo, in contrast to the low efficiency (more than 100 folds less) and short duration (within a week) of gene expression by traditional sonoporation. Mechanisms responsible for the internalization of gene materials into cells, the PKC-delta pathway of endocytosis, will also be addressed.

Keywords: Gene therapy, Ultrasound, Cavitation, Antiangiogenesis, Sonoporation, Endocytosis

S2-4 Cavitation-assisted delivery of gold nanoparticles for photothermal therapy

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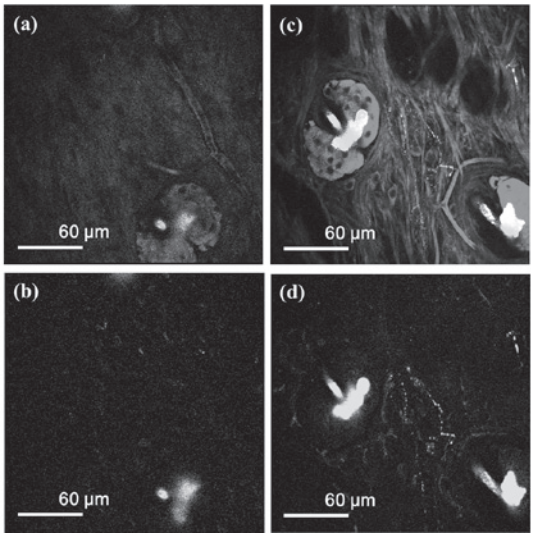
Ultrasonic imaging and photoacoustic imaging provide unique imaging capabilities but also face certain limitations. Ultrasonic imaging, on one hand, is based on mechanical properties of the image object and many unique applications have been developed. With the aid of superior spatial resolution, high frequency ultrasound imaging has also evolved from clinical anatomical imaging to probing of molecular processes on small animals for pre-clinical research. Photoacoustic imaging, on the other hand, combines advantages of both optics and acoustics. New developments in imaging physics and the combination with other disciplines have found promising biomedical applications. For example, microbubbles typically used in ultrasonic imaging as the contrast agent present unique mechanical properties and the associated acoustic cavitation has been exploited for therapeutic purposes. Similarly, gold nanoparticles (AuNPs) are found to be a good contrast agent for photoacoustic imaging for its bioconjugation capabilities. The efficient light absorption of AuNPs and abilities to tune their optical properties have also led to new photothermal therapy methods. In the past, we have developed instrumentations that were used for applications of ultrasonic and photoacoustic imaging. New development in combined diagnosis with therapy for both modalities have also been introduced. AuNPs encapsulated with microbubbles (AuMBs) have been introduced as a photoacoustic and ultrasound dual-modality contrast agent. Applications can be extended to theranosis purpose. In this study, an enhanced delivery method of AuNPs is proposed by using microbubbles as a targeted carrier and by inducing acoustic cavitation to enhance permeability. The hypothesis was confirmed with *in vivo* and *in vitro* examinations. First, these AuMBs are modified with anti-VEGFR2 to bind to angiogenesis. The targeting efficiency was observed by an ultrasound system and the extended retention was recorded for 30 minutes in a CT-26 tumor bearing mouse. Second, cavitation induced by time-varying acoustic field is also applied to disrupt the microbubbles and induce increased transient cellular permeability (a.k.a., sonoporation). Photoacoustic and phototehrmal experiments were conducted and results were correlated with the cavitation dose. At least 10 times improvement in AuNP delivery and twenty degrees of temperature elevation were achieved. An optical microscope which collects the two photon fluorescence emitted by AuNPs further confirms the enhanced delivery. Finally, *in vivo* delivery of AuNPs was demonstrated with laser-induced thermotherapy that showed hyperthermia (>45℃) with sonoporation. Therefore, controlled release of AuNPs is feasible with acoustic cavitation and the procedure can further improve therapeutic effects of the AuNPs.



Nonlinear optical microscopy of mouse ears (magenta: THG for microbubble shell, green: 2PF for AuNP). Panel (a) and (b) are THG-2PF and 2PF-only images without AuMB injection, respectively. After injection and sonoporation, images of a normal mouse's ear are shown in (c) and (d).

S3-1 Contrast-enhanced ultrasonography using Sonazoid® for breast cancer

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3) Nara Medical University Department of Surgery

Since August 2012, Sonazoid®, which is the only ultrasound contrast medium approved for clinical use in Japan, has been covered by insurance for focal breast lesions.

In 2009, we began clinical trials for contrast-enhanced ultrasonography (CEUS) at Nara Medical University. From March 2009 to March 2014, we used Sonazoid® to examine 138 patients, with 142 breast lesions among them. Of these, 104 patients (with 107 lesions) were diagnosed with breast cancer, while the remaining lesions were found to be benign. 26 of the 104 patients diagnosed with breast cancer received neoadjuvant chemotherapy.

At Mie University, we conducted CEUS trials from September 2012 to March 2014, for 36 patients, with 37 breast lesions among them. Of these, 28 patients (29 lesions) were diagnosed with breast cancer, while 8 patients only showed benign lesions. Among those diagnosed with breast cancer, 9 patients received neoadjuvant chemotherapy.

At this conference, I will present a few case examples as well as some of our findings regarding CEUS using Sonazoid® and the application value of CEUS using Sonazoid®, for evaluating the response of breast cancer to neoadjuvant chemotherapy.

S3-2 Real-time shear wave elastography on the application for distinguishing inflammatory from fibrotic stenosis in Crohn's disease

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Background and aim: Differentiation between inflammatory and fibrotic strictures in Crohn's disease (CD) is difficult but crucial for therapeutic decisions. Prior work has demonstrated that ultrasound elastography imaging (UEI) can identify intestinal fibrosis in animal models of CD. The purpose of our study was to determine if real-time shear wave elastography (SWE), a new technique of UEI can be used to distinguish inflammatory from fibrotic strictures in CD patients.

Methods: A total of one hundred and twenty established CD patients underwent transcutaneous UEI by the technique of real-time SWE using the ultrasound system, Aixplorer (SuperSonic Imagine S.A., Aix-en-Provence, France). Thicken bowel wall and proximal normal bowel were analyzed by measuring Young's Modulus (YM) of the tissue. According to a formalized endoscopic, CT/MR enterography and histologic protocol, strictures were classified as inflammatory and fibrotic stenotic. One-way ANOVA and Bonferroni were used for statistical analysis, and receiver operating characteristic (ROC) curves were created to assess diagnostic performance.

Results: One hundred and ten CD patients had been measuring YM successfully (male n=40, female n=70; mean age 40y, range 13y-65y,). Elastography for 10 patients had not been performed successfully because of being fat and/or deep lesions. 110 patients were divided into three groups: acutely inflamed non-stenotic (n=46), inflammatory stenosis (n=44) and fibrotic stenosis cases (n=20), with measuring YM 15.5 ± 6.7 KPa, 16.7 ± 5.7 KPa, and 25.8 ± 8.0 KPa respectively. Transcutaneous UEI demonstrated YM was higher in fibrotic stenosis than inflammatory stenosis (P=0.016) and acutely inflamed non-stenotic (P=0.015). YM was higher in diseased bowel than adjacent normal bowel (6.5 ± 2.1 KPa) in 3 groups (each group P<0.05). No significant difference existed between acutely inflamed stenosis and acutely inflamed non-stenotic bowel (P>0.05). The most accurate cut-off value for distinguishing inflammatory from fibrotic stenosis was 17.5 KPa, achieved 98% sensitivity and 71.4% specificity. The area under the receiver operating characteristic curve (AUC) was 0.883 (95% CI: 0.84-0.93).

Conclusion: UEI provides a noninvasive new method in distinguishing inflammatory from fibrotic strictures in CD patients, and may thereby be helpful guide herapeutic decisions.

Keywords: Ultrasound, Crohn's Disease, Elastography, Stricture

S3-3 Contrast-enhanced endoscopic ultrasound in pancreatobiliary diseases

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1. Utility of contrast-enhanced harmonic EUS in diagnosis of pancreatobiliary diseases

Endoscopic ultrasonography (EUS) is widely used to diagnose pancreatic diseases because its spatial resolution is superior to that of other modalities. Recent innovation of ultrasound technology allowed characterization of a lesion depicted by EUS on the basis of their vascularity.

Particularly, EUS system specific for contrast harmonic imaging has been developed to evaluation of vascularity of the lesions using ultrasound contrast agents[1-8].

The use of this EUS system enabled us to observe images of microcirculation and parenchymal perfusion without Doppler-related artefacts in the pancreatobiliary system[1,7,8]. Contrast-enhanced harmonic EUS can differentiate diagnose pancreatic carcinomas as hypovascular masses with a high sensitivity (89-96%) and specificity (64-88%)[6-8]. Contrast-enhanced harmonic EUS is also useful for differentiating malignant from benign lymphadenopathy. The sensitivity and specificity in identifying malignant lymphadenopathy as heterogeneous enhancement in contrast-enhanced harmonic EUS are 90% and 95%, respectively. CH-EUS has a significantly higher diagnostic accuracy than conventional EUS.

2. Relationship between contrast-enhanced EUS and EUS-FNA

EUS-guided fine needle aspiration (EUS-FNA) is also a tool for characterizing a solid mass that is detected by EUS, with a high sensitivity and specificity. However, EUS-FNA has limitations in obtaining samples from some subtle solid lesions depicted by EUS. Improved depiction of the lesions by contrast-enhanced harmonic EUS facilitates the yield of EUS-FNA[6,8].

In addition, we sometimes hesitate making decisions between surgery and follow-up in patients whose EUS-FNA findings are negative, because false negative EUS-FNA cannot be excluded. Therefore, when contrast-enhanced EUS shows a typical pattern of carcinomas, even when the EUS-FNA findings are negative, surgical resection or pathological re-evaluation by EUS-FNA of the tumor should be recommended[7,8].

Conclusion

Contrast-enhanced EUS improves the ability of EUS in diagnosing pancreatobiliary diseases, and help making decision of their treatment.

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S3-4 Real-time ultrasound contrast imaging as guidance for target biopsy of prostate cancer

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Although increasing number of studies supports the use of contrast-enhanced ultrasound (CEUS), ultrasonic elastography as well as multi-parametric MRI (mpMRI) for targeting prostate cancer (PCa), the optimal biopsy method using the imaging techniques remains undefined. From 2011 to 2013, we performed prospective studies to evaluate which method using them is more helpful in improving prostate cancer (PCa) detection to guide a target biopsy (TB) when additionally performed before conventional systematic biopsy (SB). We performed re-biopsy study to evaluate mpMRI-CEUS correlated, imaging-guided target biopsy (TB) method for the repeat prostate biopsy of patients with suspected PCa. Patient- and core-based overall cancer detection rates (CaDR) of TB and SB were compared. Within the same patient, core-based CaDR was compared between SB and TB. In conclusion, Not only could the number of biopsy cores be reduced, but CEUS correlated, imaging-guided TB technique for the repeat prostate biopsy of patients with suspected PCa can improve CaDR based on the number of cores taken.

S4-1 Fusion imaging and CEUS-guided RFA of HCCs

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With the technical development of US, electromagnetic tracking based fusion imaging techniques of real-time US and CT/MR images have been introduced for radiofrequency ablation (RFA) (1-3). It is a technique that fuses real-time US with CT or MR images. Therefore, while we are performing real time US, the fused CT or MR image shows the same plane and moves synchronously. With this information, fusion imaging can enhance lesion detectability and reduce false positive detection of hepatocellular carcinomas (HCCs) during percutaneous RFA of HCCs (2). 3D US can be fused with real-time US for larger HCCs requiring multiple overlapping treatments.

However, liver shape can be deformed and its location can be displaced by breathing motion of patients. Therefore, mistargeting can occur even though fusion imaging is used for guidance of percutaneous RFA of small HCCs located in the periphery of the liver (4). This is because large vessels as anatomic landmarks are frequently not available for image fusion in the periphery of the liver.

Like fusion imaging, contrast-enhanced ultrasonography (CEUS) is also useful for guiding percutaneous RFA of small HCCs (5-8). Among various contrast agents, Sonazoid (perflubutane microbubbles; GE Healthcare), which offers both vascular and post-vascular phases, is known to be very effective in localizing small HCCs inconspicuous on B-mode US (9-11). If fusion imaging and CEUS are coupled simultaneously, they can help localize small inconspicuous HCCs.

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S4-2 Irreversible electroporation (IRE, NanoKnife®) and CEUS

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Recently, IRE has been focused on as a new technology for local ablation therapy comparing with RFA (radio frequency ablation) and MWA (microwave ablation). IRE is unique comparing with hitherto known RFA and MWA. RFA and MWA are thermal ablation and on the other hand IRE is non-thermal ablation treatment.

IRE makes nano-sized pores in the cell membrane and causes apoptosis of the cell by using high voltage electric currency with a short pulse length (100 μ sec or less). Therefore, IRE does injure only cells but not fibrous structures such as blood vessels, bile ducts and nerves.

However, it has been cited as a disadvantage in the clinical settings that there is a difficulty to evaluate the ablation area by using imaging modalities such as ultrasound, CT and MRI, especially just after IRE procedures, although high echogenic changes can be seen in RFA and MWA by gas production while vaporing water in the ablation area.

We have investigated which modality is most reliable to evaluate efficacy of IRE ablation during and after the ablation procedures. B-mode echogenicity has not yet changed just after IRE and it is getting echogenic in one to two hours.

Ultrasound elastography is useful to evaluate the IRE responses after treatment, which suggests that visco-elasticity is increased in the ablated area. Cut off value for margin demarcation of the ablated area is 15 kPa or more. However, increase in visco-elasticity has been elevated taking time, ten minutes to half an hour.

Contrast ultrasound is also useful to evaluate IRE ablation area. Being different from RFA and MWA, vascular enhancement of the ablated area is maintained in the vascular phase of CEUS. This is explained by the fact that blood vessels in which the vascular diameter is 1 mm or more does not loose blood flow in IRE. However, it is impressive that blood flow in the tumor blood vessels is lost completely.

Most useful imaging to evaluate IRE effects is Kupffer phase imaging with Sonazoid. There is a discrepancy between contrast effect of Kupffer phase and vascular phase imaging. Defect of contrast enhancement in vascular phase is much smaller than that of Kupffer phase imaging. This is explained by the fact that IRE does not affect blood vessels but induces dysfunction of phagocytosis and apoptosis of the Kupffer cell.

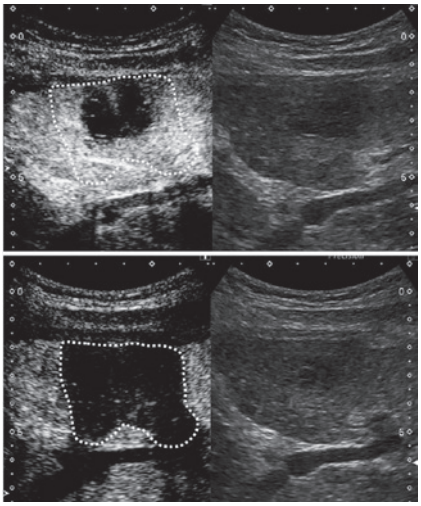

Figure 1. Contrast ultrasound after IRE. In the vascular phase (upper) only the tumor area indicates a perfusion defect. However, Kupffer phase imaging (lower) indicates that the contrast defect area is much wider than that in the vascular phase. B-mode imaging (right) informs us nothing regarding the ablation area.

Puncturing the liver cancer using NanoKnife needle is guided using ultrasound imaging rather than CT guidance. Not only guidance but also simulation is assisted by “Fusion imaging”, which is composed of ultrasound and CT/MRI. Contrast ultrasound, especially Kupffer phase imaging is used for fusion imaging. This is because that Kupffer phase imaging indicates that border delineation is much clearer than that of non-contrast B-mode imaging.

In conclusion, contrast ultrasound is useful in fusion imaging and related technologies in local ablation therapy, especially in IRE.

S4-3 Simulation and navigation with CEUS for detection and ablation therapy of liver cancer

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Current clinical studies have demonstrated the excellent diagnostic performance of contrast-enhanced ultrasound (CEUS) in the detection and characterization of benign and malignant hepatic tumors in correlation with histology and standard imaging techniques such as contrast-enhanced computed tomography (ceCT), contrast-enhanced magnetic resonance imaging (ceMRI), as well nuclear medicine modalities such as positron-emission tomography (PET) and PET-CT.

Hepatocellular carcinoma (HCC) is the sixth most common type of cancer and the third most frequent cause of cancer death worldwide. Patients with chronic liver disease related to hepatitis B or C virus constitute a high-risk group for the development of HCC.

The most successful treatment modality of HCC is surgery, although most patients with HCC are not considered good candidates for a surgical resection at diagnosis because of a limited hepatic functional reserve and the multiplicity of HCC.

Several non-surgical procedures have been established for the treatment of unresectable HCC, including percutaneous ethanol injection (PEI), cryoablation, radiofrequency (RF) and microwave (MW) ablation, and transcatheter arterial chemoembolization (TACE).

A treatment option for non-resectable localized primary and secondary liver tumors, the use of thermal – predominantly radiofrequency ablation has rapidly expanded in the last decade. It is considered as curative treatment method for HCC Stage I according to the Barcelona Clinic Liver Cancer (BCLC) Group, with reported local tumor control rates higher than 80%.

Currently real time image guided biopsy is performed either based on conventional B-scan ultrasound (US) or CT fluoroscopy.

Optimal detection and assessment of liver lesion is possible through the simultaneous use of CEUS and image fusion. For image fusion a magnetic field generator and a corresponding probe sensor are required hardware components. Additionally, dedicated software must be installed on the ultrasound system.

The probe sensor is detected by a magnetic positioning system, which calculates the exact position of the sensor in the room. Standard DICOM data sets of all crosssectional modalities (CT, MRI, PET-CT/MRI) may be used for image fusion. The DICOM data are loaded in the ultrasound system, following which a registration of the datasets takes place. This registration can be performed based on a number of fixed reference points or by plane.

After successful image fusion, the registered MRI or CT image are seen to move simultaneously with the examined ultrasound imaging plane. There is also the option of viewing the registered images either in overlay mode or side-to-side mode. Standard ultrasound options like colour Doppler and CEUS may be integrated easily into the fused images. Thus, the simultaneous assessment of tumor detection and characterisation using CEUS in parallel with information from ceCT and ceMRI images is possible.

With the latest development in contrast enhanced ultrasound and new techniques like image fusion percutaneous biopsy and thermal ablation could be monitored.

In this course new ultrasound techniques like CEUS, image fusion and navigation will be discussed in terms of clinical settings.

S4-4 Therapeutic response evaluation in hepatic tumor model using Dynamic Contrast-Enhanced US (DCE-US)

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Monitoring the therapeutic efficacy of variable anti-cancer therapy as well as the early prediction of tumor response is of great importance, as it may quicken making a decision for each condition, which will maximize the benefits and minimize the drawbacks of treatment. Although tumor size change has traditionally been used to assess the cancer treatment effects, size measurement may be insensitive or delayed chronologically during the monitoring of anti-cancer treatment and thus cannot be relied upon to accurately and promptly indicate the therapeutic effect.
Current studies have reported the usefulness of quantitative imaging methods including dynamic contrast-enhanced (DCE) MRI, DCE-CT, and DCE ultrasound for monitoring the therapeutic effect of anti-cancer treatment, which can demonstrate hemodynamic changes noninvasively and longitudinally. Among those imaging methods, DCE-US has several advantages over DCE-MRI and DCE-CT, as it can be easily performed repeatedly at low cost and without patient exposure to ionizing radiation, and the ultrasound contrast agent is a purely intravascular marker of blood flow and perfusion that is not confounded by extravascular diffusion.
This course will review the recent update of DCE-US in hepatic tumor model and introduce my experience of DCE-US for the early quantification of hemodynamic change following administration of the vascular disrupting agent using a rabbit VX2 liver tumor model, the therapeutic efficacy after gemcitabine and HIFU for mouse model of hepatic metastasis, and the therapeutic efficacy after targeted chemotherapeutic agent using a rabbit VX2 liver tumor model. Finally, I will introduce dynamic contrast-enhanced 3D US (4D-volume analysis).

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S5-1 Parametric imaging for diffuse liver diseases

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For taking care of the patient with liver disease, it is important to understand both liver histology and the liver test data. Liver biopsy is a most reliable way (Gold standard) to know the histology, but invasive. It is also well accepted that the peripheral blood flow within the liver parenchyma is easily influenced by histological changes, such as liver fibrosis, necrosis, edema, collapse, and so on. On the other hand, the liver function is regulated by 4 main factors, up-take, metabolism, secretion of the liver cells, and liver blood flow. Therefore, analyzing the hemo-dynamics in peripheral area of the liver parenchyma may become a relevant way for estimating the liver histology and the function.

The aims of this study were to assess the significance of the arrival-time parametric imaging and the perfusion parametric imaging obtained by Sonazoid® enhanced ultrasound.

Materials and Methods: During a 2-year period, 157 consecutive patients with biopsy proven CH-C, 46 with LC-C, 17 with acute liver injury, and 11 with alcoholic hepatitis and/or alcoholic LC were studied by Sonazoid® enhanced ultrasound. All ultrasound were performed by using Aplio XG (Toshiba, Japan) with 3.75 MHz probe. Obtained movie images were analyzed by using an on board application soft "Parametric MFI (Toshiba, Japan)".

Results are as followings:

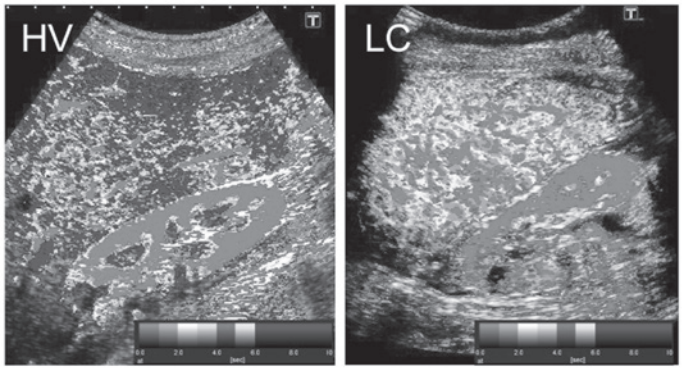

Fig.1: Original "Arrival-time Parametric image" of a healthy volunteer and a liver cirrhosis.

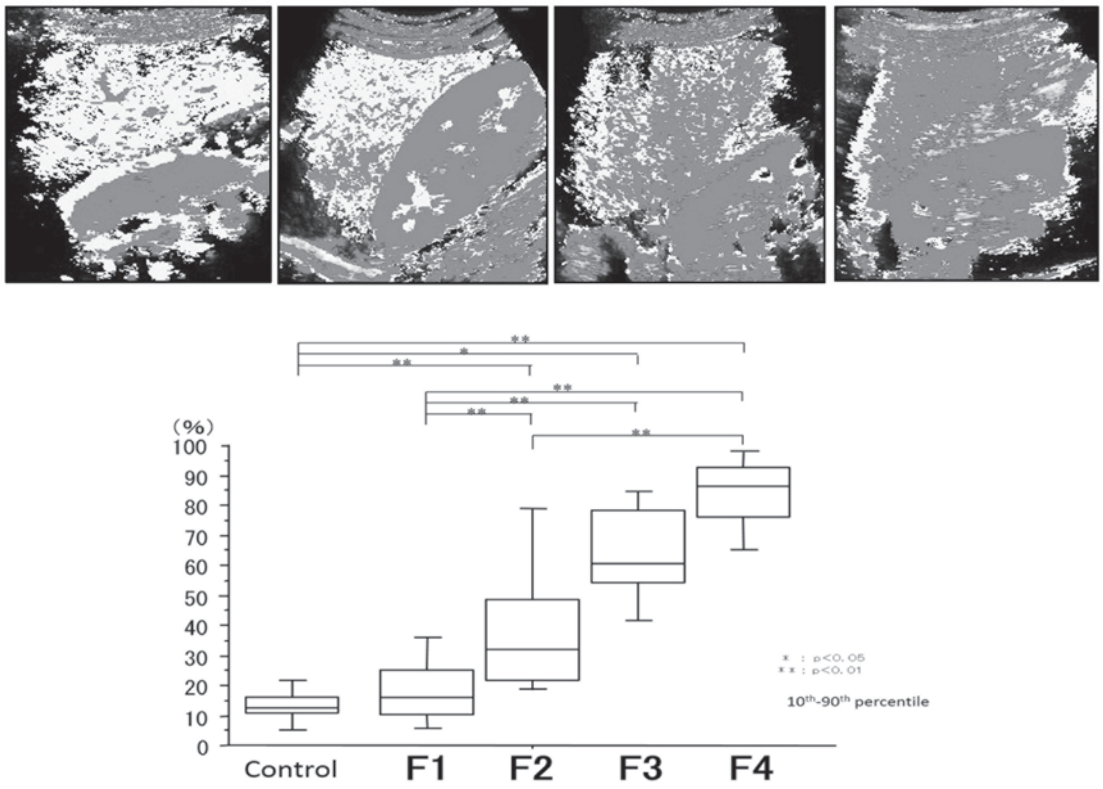

Fig.2: Arrival-time Parametric image arranged for estimating the grade of liver fibrosis. We divided the arrival time into two groups, before 5 sec (red) and after 5 sec (yellow). The proportion of the red colored pixel became significantly higher with progression of the liver fibrosis.

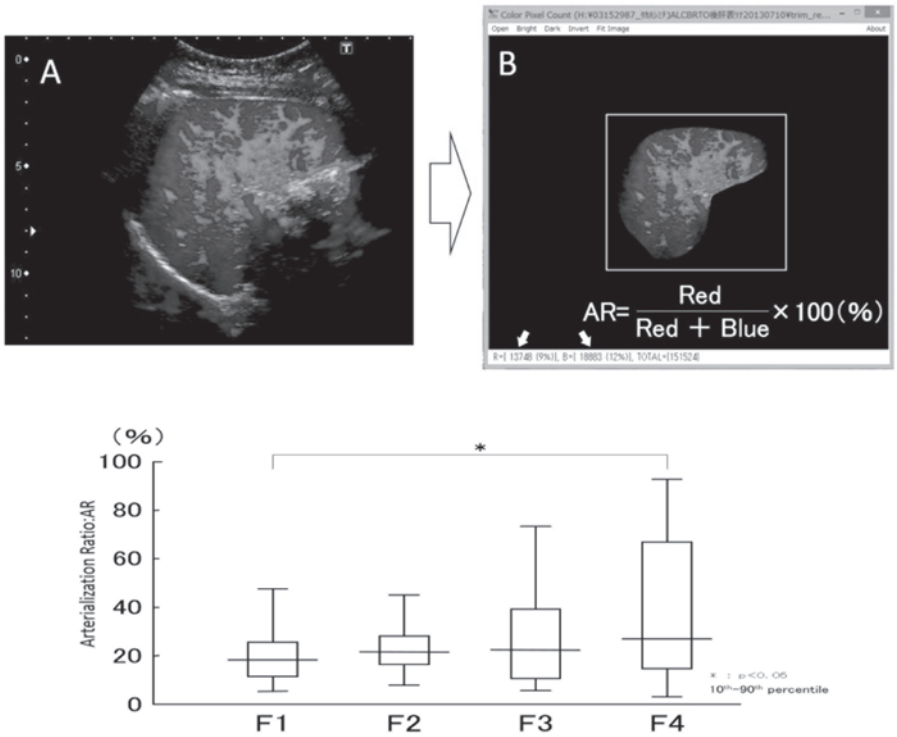

Fig.3: Arrival-time Parametric image arranged for evaluating the blood perfusion into the liver parenchyma. Red pixel means blood perfusion from the arterial flow and blue pixel means perfusion from the portal flow. We further analyzed a proportion of the red colored pixel (arterialization ratio) by pixel count application software, and the results strongly suggest that most of cirrhotic liver shows markedly arterialized perfusion within liver parenchyma.

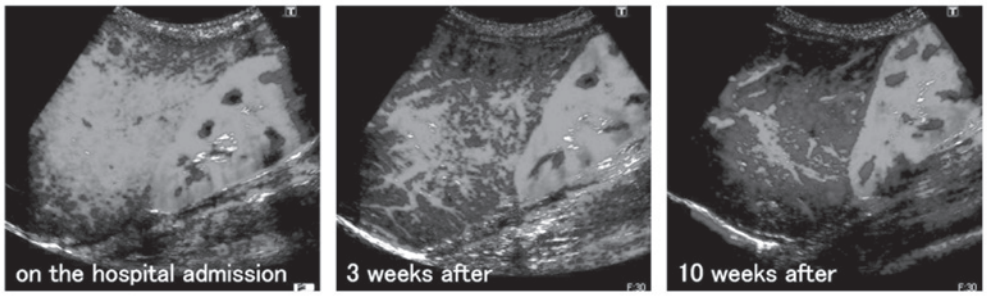

Fig.4: 37yo, gentleman, acute hepatitis A. Perfusion parametric image showed marked arterialization of the liver parenchymal blood perfusion just after hospitalization. Then, the arterialization ratio gradually decreased according to the recovery of the hepatitis, and became portal flow dominant 10 weeks after hospitalization.

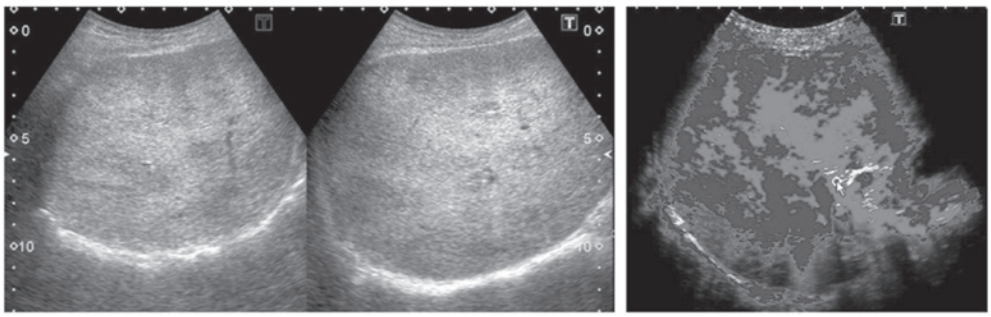

Fig.5: 40yo, gentleman, acute alcoholic hepatitis. One year after recovering from the severe alcoholic hepatitis, his liver parenchyma became showing markedly irregular pattern on the B mode ultrasound. At the same time, perfusion parametric imaging was performed, and disproportional perfusion was observed within the liver parenchyma. Arterialization was observed at the high echoic area on B mode image, and low echoic area showed portal flow dominant (blue).

Conclusions: We found analyzing the liver parenchymal micro-circulation and the portal vein blood flow by contrast-enhanced ultrasound may be of great help for understanding what is happening within the liver parenchyma in patients with liver disease.

S5-2 Contrast-enhanced US quantitatively detects changes of tumor perfusion in a breast cancer model after treatment with adriamycin

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Background: Early evaluation of tumor response to chemotherapy in cancer patients may help to avoid unnecessary treatment and enable the use of alternative therapies that may be more helpful. The purpose of this study was to quantitatively detect the changes of tumor perfusion during chemotherapy with contrast-enhanced ultrasound.

Materials and methods: MCF-7 breast cancer bearing nude mice were received either adriamycin or a control substance were imaged before and after treatment with an ultrasound scanner after bolus injection of SonoVue. Dynamic contrast enhanced ultrasound was performed at pretreatment, Day 2, Day 4 and Day 6 after treatment. Regions of interest within the tumor were analyzed off-line to determine perfusion parameters including peak enhancement (PE), area under the curve of wash-in (WIAUC), wash-in rate (WIR), and wash-in perfusion index (WIPI). The section equivalent to ultrasound imaging plane was stained with Hematoxylin and Eosin to allow for assessment of tumor cell density. The Ki-67 protein as a proliferation marker is used in the research to number the tumor cell in both groups. By using region of interest (ROI) analyzing, the perfusion parameters measured by contrast enhanced ultrasound was correlated with quantitative measures of existing (CD34-expressing) vessels.

Results: There were no significant differences in tumor volumes and tumors perfusion before treatment. Treatment with adriamycin resulted into a significant decrease in PE, WIAUC, WIR and WIPI when compared with control group ($P < 0.05$) at Day 2, when there was no significant difference in tumor volume. The volume of treated tumors was significant smaller than that of control tumors ($P < 0.05$) at day 6, later than perfusion parameters change. The tumor cell density estimated by pathology slice was significantly lower in treated tumors than in control tumors at day 6 ($P < 0.05$). The number of positive Ki67 cells and MVD were significantly lower in treated tumors than in control tumors.

Conclusions: This study indicates that quantification of tumor perfusion with contrast-enhanced ultrasound can be used for early detection of tumor response to chemotherapy prior to detectable tumor shrinkage.

S5-3 Qualitative and quantitative analysis of contrast-enhanced ultrasound (CEUS) in Crohn's disease to discriminate patients with different severity of lesions

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Objective: To discriminate mild activity from severe activity of Crohn's disease using qualitative and quantitative analysis of CEUS.

Methods: In this study, thirty-seven patients (20 male and 17 female; mean age ± SD=35.27 ± 12.96 years, range 18-66 years) with initial diagnosis of active Crohn's disease were recruited. Patients underwent endoscopy, CDAI assessment and unenhanced ultrasound examination, by which the severity of the disease in each patient was identified. The terminal ileal loops were scanned by CEUS after SonoVue contrast agent injection, and the DICOM files registered during the first dynamic enhancement were analyzed qualitatively and quantitatively by an experienced radiologist.
In qualitative analysis, enhancement of terminal ileal loops in patients with active Crohn's disease is divided into 4 patterns: pattern 1 corresponds to transmural hyper-enhancement; pattern 2 corresponds to hyper-enhancing inner bowel layers and iso-enhancing outer bowel layers; pattern 3 depicts iso-enhancement of both inner and outer layers; pattern 4 shows iso-enhancing inner layers and hypo-enhancing outer layers. 4 patterns of enhancement of terminal ileum wall were divided into two groups to determine the difference between mild activity and severe activity of Crohn's disease. Kinetic parameters of time-intensity curves including Imax, RT, TTP, mTT and QOF were compared using t test in patients with mild disease and severe disease by means of the software Sonoliver.

Results: Endoscopy grading system and CDAI scores identified 19 patients with mild disease (10 male, 8 female) and 18 patients with severe disease (9 male, 9 female). In qualitative analysis, pattern 1 and pattern 2 (18 patients) were considered as severe activity (sensitivity 69% and specificity 86%) while pattern 3 and pattern 4 (14 patients) were considered as mild activity (sensitivity 73% and specificity 89%).
As for quantitative analysis, 15 patients (7 male and 8 female; mean age ± SD=36.40+15.58 years, range 19-66 years) were included while the rest were excluded due to evident peristalsis-related movements of small bowels. Quantitative comparison showed that patients with mild disease and patients with severe disease differed significantly in Imax that represents inner bowel wall enhancement (2746.89 ± 911.09 vs. 12814.53 ± 9802.37; $P<0.05$) of all parameters of time-intensity curves.

Conclusions: CEUS qualitative and quantitative analysis provides the perfusion information of inflammatory bowel walls that was useful to determine the severity of the disease.

S6-1 Current status of US contrast agents and relevant researches in Taiwan

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Ultrasound (US) provides a valuable tool for medical diagnosis offering real-time imaging with excellent spatial resolution and low cost. Microbubbles are used for contrast-enhanced ultrasound (CEUS) imaging as blood-pool agents or organ-specific enhancing agents in cardiology and radiology. The advent of microbubble contrast agents has provided the additional ability to obtain essential quantitative information related to tissue vascularity, tissue perfusion and even endothelial wall function. Their promise as targeted agents for molecular imaging is now being recognized. Microbubbles can also be functionalized with ligand molecules that bind to molecular markers of disease. Potential clinical applications of molecular imaging with microbubble-based ultrasound contrast agents (USCA) are in the monitoring of the biomarker status of vascular endothelium, visualizing tumor vasculature, and imaging inflammation and ischemia-reperfusion injury zones and thrombi. The early attempts to produce USCA at Taipei Veterans General Hospital in 1985 were not successful although the enhancing effect had been documented. During the past three decades, exciting advances have been made in CEUS imaging, particularly in developing better agents, understanding the complex interaction between the agents, the US and the *in vivo* environment, developing techniques to generate images of remarkable quality and demonstrating a wide and continuously expanding range of clinical applications. Before the currently available second-generation USCA, transarterial infusion of carbon dioxide microbubbles had been used to enhance US examination of hepatic tumors as early as mid-1990s. Levovist (Schering AG, Germany), the most important first-generation intravenous USCA is the first agent introduced to Taiwan ultrasound community in 1996 by Dr. Yi-Hong Chou. The clinical experience of Levovist was promising in characterization of focal liver lesions and in assessing treatment efficacy of malignant hepatic tumors. In the year 2003, Schering decided to stop marketing of Levovist in Asia including Taiwan. Clinical studies using Levovist generally stopped and only animal studies remained for another 2-3 years. Sonovue (Bracco, Italy) was introduced to many laboratories in Taiwan since 2008 mainly for animal studies. Some important studies in Taiwan were accomplished using Sonovue. “Homemade” USCAs were produced in some of the leading laboratories such as Tsing Hua University (Prof. Chih-Kuang Yeh) and National Taiwan University (Prof. Jen-Ho Tsao). One of the homemade USCAs is now registered in Taiwan with pending approval of Taiwan Food and Drug Administration (TFDA). Now, there are totally 12 medical centers and 8 teaching hospitals joining the USCA Working Group and a total of more than 40 research members are invited to form a subcommittee in Committee of Research and Development in Ultrasound Medicine under the umbrella of Society of Ultrasound in Medicine of the Republic of China (SUMROC, also known as CTSUM). The initial plan for USCA application in human body will be mainly abdominal studies including liver, pancreas, gastrointestinal tracts, female internal genital organs, intraabdominal vessels, followed by neurological, pulmonary, and musculoskeletal systems. The most important contributors in the molecular imaging part of USCA include Pai-Chi Li, Win-Li Lin, Shuo-Hui Hung, Jen-Ho Tsao, Chih-Kuang Yeh, Hao-Li Liu. The total publications in the recent 10 years are more than 100. The clinical researches on CEUS are limited now due to lack of TFDA approved USCA. However, there are some researchers such as Chiung-Nien Chen, Ran-Chou Chen, Ko-Jen Li, Shi-Ming Lin, Ke-Vin Chang, Chien-Hua Chen, Pei-Ming Yang, and Yi-Hong Chou, who are contributing to the clinical part of CEUS. The clinical publications in this aspect are about 24. We expect TFDA will approve at least one commercially available USCA to be marketed in Taiwan within 1 year. A total annual USCA administration will be more than 10,000 vials in the initial stage according to the expectation of USCA subcommittee.

S6-3

Diagnostic accuracy of contrast-enhanced ultrasonography with perfluorobutane in macroscopic classification and histological differentiation of nodular hepatocellular carcinoma

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Aims

It is important to identify the macroscopic type and degree of differentiation in hepatocellular carcinoma (HCC) using imaging because there is a strong correlation between these pathological findings and the prognosis after diagnosis.

Materials and Methods

A total of 105 surgically resected nodular type HCCs with a maximum diameter of 3 cm or less were investigated. HCCs were evaluated preoperatively on the basis of contrast-enhanced ultrasonography (CEUS) with perfluorobutane. HCCs were pathologically classified as simple nodular (SN) or non-SN, which included simple nodular with extranodular growth (SN-EG) and confluent multinodular (CMN). CEUS findings were evaluated with arterial, portal, and post-vascular phase imaging. The arterial phase was classified as follows: finely homogeneous, and dendritic, and chaotic in the vascular image; homogeneous and heterogeneous in the perfusion image; and regular and irregular in the shape of perfusion image. The portal phase was classified as follows: presence and absence in the washout image. The post-vascular phase was classified as follows: defect, heterogeneous, and iso in the echo intensity level; and regular, irregular, and unclear in the shape image.

Results

According to receiver operating characteristic (ROC) curve analysis, the area under the ROC curve (AUC) for the diagnosis of non-SN HCC were 0.824 using the perfusion image in the arterial phase and 0.874 using the shape image in the post-vascular phase. The AUC for the diagnosis of non-SN HCC was 0.933, of high diagnostic value, with images combined with perfusion image in the arterial phase, washout image in the portal phase, and the shape image in the post vascular phase. The AUC for the diagnosis of moderately and poorly differentiated HCC was 0.800 using the vascular image in the arterial phase, 0.760 using the washout image in the portal phase, and 0.724 using the echo intensity level in the post-vascular phase. Using the combined images with the vascular image in the arterial phase, the washout image in the portal phase, and the echo intensity level in the post-vascular phase, the AUC for the diagnosis of moderately and poorly differentiated HCC was 0.919, of high diagnostic value.

Conclusions

CEUS can provide high quality imaging assessment of macroscopic findings and histological differentiation in nodular HCC.

S6-4 The World and European Federations of Societies for ultrasound in medicine and biology: Guidelines and good clinical practice recommendations for contrast enhanced ultrasound

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The document commonly referred to as 'the Guidelines' started as the views of an ad hoc committee of experts under the chairmanship of Prof. Michel Claudon in 2003/4. Its intention was to assist users of CEUS in the liver to obtain reliable and repeatable clinical information from the use of microbubbles in diagnosis. At the time of formulation of the first edition, Levovist was the predominant agent available in Europe and the guidelines reflected this, recommending the use of microbubble-destructive high mechanical index (MI) techniques based on colour Doppler. The process of achieving consensus was long and hard and consisted of literature reviews, emphasising meta-analyses when available, supplemented by authors' opinions when needed. The eventual report [1] dealt with the detection and characterisation of focal liver lesions. Despite its limitations, it was rapidly accepted as a useful document not only to guide clinical practice but also to inform purchasing decisions.

However, with the replacement of Levovist by SonoVue and the development of low MI non-destructive real-time scanning techniques, a revision was obviously needed and so the board was reconvened with a view to writing a second edition. The process of consensus was the same and the intent to stick to clinically established applications rather than research applications was continued, but as experience with applications outside the liver had developed, the Guidelines were extended to include other abdominal organs as well as the brain (each with one or more lead investigators) and references to Levovist and high MI imaging were removed [2].

Again, the major content was the liver, with an emphasis on detection and characterisation of focal lesions, the former based on the fact that metastases wash out more rapidly than normal liver and so appear as clear-cut filling defects in scans performed from 1-5 minutes after injection. Characterisation is based mainly on the haemodynamics of the arterial phase from around 20 seconds after injection to about one minute, and the characteristic filling patterns are condensed into a table. This also details the exceptions that need to be kept in mind, particularly the variable behaviour of hepatocellular carcinomas (HCCs) (which depends on their degree of differentiation). Shortly before the end of the deliberations, the Barcelona agreement on screening for HCCs in patients with cirrhosis was published ([3]): this added CEUS as one of the accepted dynamic imaging modalities, alongside CE-CT and CE-MR. This is incorporated into the Guidelines and many felt it represented a strong endorsement of the value of CEUS in the liver. A third liver section is devoted to the monitoring of the response to interstitial ablation of liver malignancies, an important topic because of its clinical value and the resulting cost savings.

The renal tract forms a major new section, with emphasis on the value of CEUS in determining the risk of complex cysts being cystic renal cell carcinomas, so that cysts classified as possibly malignant on the ultrasound Bosniak grading system can be regraded, as well as its value in identifying developmental anomalies, with their normal haemodynamics as compared with the abnormal arterial patterns of carcinomas and angiomyolipomas. Differentiating these two common pathologies does not seem to be possible at present and focal sepsis also produces confusing patterns. Another section covers the use of CEUS in assessing vesico-ureteric reflux which in some countries is preferred to x-ray micturating cystography because of its lack of ionising radiation.

The pancreas is included; here a main benefit of CEUS lies in distinguishing complex pseudocysts from solid and cystic masses. The different arterial patterns of carcinomas and focal pancreatitis, the latter being more vascular,

was included.

A section deals with the value of CEUS in blunt abdominal trauma where good evidence was emerging about its value, especially in minor injuries to the liver, spleen and kidneys that require monitoring where CEUS can replace CT, with attendant reduction in ionising radiation.

Another new section covers the use of CEUS in transcranial applications, especially in repeated assessment of the vasospasm associated with stroke.

In 2010 the World Federation started work on a set of global Guidelines, but, in view of the magnitude of the task with several types of microbubble to be considered, a decision was made to cover only the liver and this appeared as the WFUMB Guidelines [4]. It includes the use of Sonazoid with its low dosages and extended retention both in normal liver and in benign solid masses, which was afforded a new phase the "post-vascular phase" to recognise the Kupffer cell incorporation of this microbubble.

Simultaneously the European Federation worked on Guidelines for non-liver uses with wide ranging content, including some that were not considered as well established clinically, such as its use for detecting early response of tumours to anti-angiogenic therapy [5]. A distinctive feature of these Guidelines is their evidence weighting so that readers can form an impression of the available strength of evidence.

Bothe the current Guidelines are freely available on the WFUMB and EFSUMB websites.

Any such document has limitations. An important one is the reliance on consensus; the selection of the advisory board might be considered arbitrary and the process of agreement, as with any committee, tended to depend on their personalities as well as the evidence base. Importantly, Guidelines are just that and they should not be used prescriptively since that could stifle innovation.

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