



LIFE FROM INSIDE

Elaine Ferguson
FDA Advisors and
Consultants Staff (ACS)
HFD-21, Room 1076
5630 Fishers Lane
Rockville, MD 20857

May 22nd 2008

Re: Cardiovascular and Renal Drugs Advisory Committee Meeting
Safety Considerations in the Development of Ultrasound Contrast Agents

Dear Ms Ferguson,

Further to your request of April 16th 2008, I am pleased to enclose Bracco's briefing materials for the Advisory Committee of June 24th.

We are enclosing 15 paper copies and also 30 copies on CD.

On the day of the meeting, we will provide you with copies of the slides which will be presented by our experts.

Please feel free to contact me if there is anything additional we can provide for you.

With kind regards

Yours sincerely

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Introduction and Overview

This briefing document has been assembled at the request of the FDA to provide background information for the members of the Cardio-renal Advisory Committee, and the other invited experts, for the Advisory Committee meeting scheduled for 24th June, 2008. This document is intended to complement the presentations which will be made by Bracco on the day of the meeting.

Bracco was invited by the Division of Medical Imaging and Hematology Products of the FDA to make a formal presentation to the Advisory Committee of the safety experience, both non-clinical and clinical, which we have accumulated with the product SonoVue.

SonoVue is a phospholipid-stabilized microbubble preparation of sulfur hexafluoride (SF₆) which is used as an ultrasound contrast agent. It is supplied as a sterile, non-pyrogenic, lyophilized powder and it is intended for intravenous use after reconstitution with sodium chloride injection (0.9%).

One milliliter of SonoVue contains 1×10^8 to 5×10^8 microbubbles which have a median diameter of 2.3 μm . Each milliliter contains approximately 13 μL of SF₆ of which approximately 8 μL is encapsulated in the microbubbles and the remaining 5 μL is dissolved in the aqueous phase of the suspension.

These stabilized bubbles are used to enhance the reflectivity of blood-containing cavities and of perfused tissues and find applications in cardiology and in radiology. SonoVue does not diffuse into the extravascular compartment but remains within the blood vessels until the gas dissolves and is eliminated in expired air.

SonoVue was approved in the European Union (EU) in March 2001 under the centralised procedure. This procedure involves review by the Committee for Human Medicinal Products (CHMP) and the European Medicines Agency (EMA) and results in one approval, issued by the European Commission which is valid in all 27 countries of the EU.

The product is approved for the following indications, with the doses indicated in parentheses:

- Echocardiography - A transpulmonary echocardiographic contrast agent for use in patients with suspected or established cardiovascular disease to provide opacification of cardiac chambers and enhance left ventricular endocardial border delineation (2.0 mL for intravenous administration as a bolus).
- Doppler of macrovasculature - Increases the accuracy in detection or exclusion of abnormalities in cerebral arteries and extracranial carotid or peripheral arteries by improving the Doppler signal-to-noise ratio. Increases the quality of the Doppler flow image and the duration of clinically-useful signal enhancement in portal vein assessment (2.4 mL for intravenous administration as a bolus).
- Doppler of microvasculature - Improves display of the vascularity of liver and breast lesions during Doppler sonography, leading to more specific lesion characterization (2.4 mL for intravenous administration as a bolus).

SonoVue has also been approved in Norway, Iceland, Switzerland, China, Singapore, Hong Kong, South Korea, and Canada. The product is marketed in 21 countries as of May 2008.

No safety concerns were raised during the whole preclinical and clinical developments of the product but after the introduction of SonoVue in the markets, post market surveillance revealed some rare Adverse Reactions, three of which were associated with a fatal outcome.

The occurrence of three adverse reactions with fatal outcome in unstable cardiac patients raised the question of a possible direct cardiac toxic effect of SonoVue; even if these three events could not be clearly attributed to the administration of SonoVue.

In our non-clinical and clinical experience there were no signs suggesting a possible direct SonoVue cardiac bio-effect in humans. Four clinical studies which were conducted specifically to try and identify negative effects on cardiac repolarization caused by SonoVue with exposure to ultrasound at different acoustic energies, did not demonstrate any significant modification of QTc.

The analysis of the serious adverse events reported in post market surveillance showed that, in the majority of the cases, the nature of the reaction was allergy-like and the onset of the reaction happened quite typically a few minutes after administration.

The combination of symptoms reported suggested to Bracco that the type of reactivity caused by SonoVue was similar to what has been already reported in animals, particularly in pigs and in general in ungulates after the administration of particulates.

It is well known that pigs and ungulates are more sensitive than other species to particulates because of the presence of pulmonary macrophages which react with particulates producing release of vasoactive substances. In fact, knowing this reactivity occurs, in the non-clinical development and animal imaging studies the pigs were always pre-treated with indomethacine or aspirin.

As these adverse reactions are extremely rare in humans, it was necessary to try and find animal models to conduct research into possible mechanisms. Bracco decided to study more deeply the reactivity to microbubbles of pigs and some other species to understand mechanisms which might explain the pathogenesis of these allergy-like reactions in human.

The results of these studies in the pig demonstrate that SonoVue and the two ultrasound contrast agents currently marketed in the USA have similar actions upon hemodynamic parameters.

The symptoms observed in pigs show striking similarity to those reported in the allergy-like reactions which have occurred in humans.

Bracco believes that the pig model is a valuable test system for evaluating the possibility of the occurrence of allergic like reactions in humans.

SONOVUE® PRECLINICAL SAFETY DATA

1 Introduction

SonoVue® is a phospholipid-stabilized microbubble preparation of sulphur hexafluoride (SF₆) which is used as an ultrasound contrast agent (UCA). Supplied as a sterile, non-pyrogenic, lyophilized powder, it is intended for intravenous use after reconstitution with sodium chloride injection (0.9%). The diameter of SonoVue® microbubbles shows a median value of 2.3 µm. These stabilized bubbles are used to enhance the reflectivity of blood-containing cavities and of perfused tissues and find applications in cardiology and in radiology. SonoVue® does not diffuse into the extravascular compartment but remains within the blood vessels until the gas dissolves and is eliminated in expired air. The product was subject to a standard non-clinical development for a pharmaceutical product of this type. No major findings were observed during the preclinical and clinical developments of the product. Following approval in EU and some other markets of the world, post market surveillance revealed some rare SAEs, pseudo-allergic reactions. Bracco Research then went back to non-clinical studies, particularly in pigs, to try to understand the mechanisms which might underlie these reactions. In addition, a number of in vivo studies with concurrent insonation of the animals and in vitro studies using human whole blood or serum of healthy volunteers were performed on SonoVue® and other ultrasound contrast agents currently on the market in the USA. The detailed results of these mechanistic studies were sent to the FDA.

We describe below some relevant points of the SonoVue preclinical data which may help in the discussion for the Advisory Committee meeting on the "Safety Considerations in the Development of Ultrasound Contrast Agents" of June 24, 2008.

2 No mortality induced by SonoVue® in animal studies

In dogs, no mortality due to the administration of SonoVue® was noted in the following studies:

- A study in awake dogs administered intravenously with SonoVue® at dose levels of 0.3 and 1 mL/kg. No effects were observed at 0.3 mL/kg, 2 dogs out of 7 showed a reversible hypotension episode at 1 mL/kg. The dose level of 1 mL/kg corresponds to 33 multiples of the human dose of 0.03 mL/kg, based on body weight (MHD_{bw}) or 16 MHD based on body surface area (MHD_{bsa}) in dogs.
- A study in awake dogs treated at 0.1, 0.3 and 1 mL/kg of SonoVue® and receiving concurrent ultrasound exposure up to the maximum mechanical index of 1.2. There were no cardiovascular effects in 7 out of 8 animals, one dog showed a transient hypotension episode at the dose of 1 mL/kg, both in presence or in absence of ultrasound exposure. Details of the study are given below in Section 4.1.
- A study in anesthetized dogs in a model of pulmonary arterial hypertension induced by the injection of glass beads in anesthetized dogs. Cumulative doses of SonoVue® (0.1,

0.3 and 1 mL/kg) administered at 15 min intervals of time had no effects on arterial blood pressure, heart rate and on QT and QTc (Fridericia's formula) intervals of the ECG. Furthermore, increasing doses of SonoVue® did not modify the myocardial (LVP and myocardial contractility) or the pulmonary (tidal volume and respiratory rate) functions. At the highest dose (1 mL/kg) of SonoVue® only, a transient minimal increase (2.5 ± 1.3 mmHg) in pulmonary arterial pressure was observed between 5 to 7 min after the administration, this effect was more marked in 1 out of the 4 animals tested.

In rats, no mortality due to the administration of SonoVue® was noted in the following studies:

- A study with single dose intravenous administrations at the dose level of 20 mL/kg (600 MHDbw or 100 MHDbsa in rats) of SonoVue®.
- Two multiple-dose (daily for 28 days) toxicity studies in rats at dose levels of 0.2, 1 and 5 mL/kg (corresponding to 160 MHDbw or 25 MHDbsa in rats) for a duration of 28 days.

In monkeys,

- No mortality was observed during a multi-dose toxicity study in cynomolgus monkeys (*Macaca fascicularis*) receiving daily intravenous administration of SonoVue® at dose levels of 0.2, 1 and 5 mL/kg (i.e. up to 160 MHDbw, or 50 MHDbsa in monkeys) for a duration of 28 days.
- No mortality and no effects on the cardiovascular parameter in cynomolgus monkeys exposed to a single dose SonoVue® at dose levels of 1, 2 and 5 mL/kg and followed by telemetry.

3 Intra-arterial injections with SonoVue®

Since in patients right-to-left cardiac shunts can bypass the pulmonary filtration of large particles and produce microvascular occlusion and ischemia, the absence of ischemic effects of SonoVue® in the brain was demonstrated after intracarotid injection. In this experiment, SonoVue® was administered directly into the right carotid artery of anesthetized rats at the dose of 1 mL/kg (approximately 30 times the expected human dose). The ischemia-induced cerebral damages were evaluated by histology and by the assay of the neuron-specific enolase (NSE), a specific marker of neuronal degeneration, in the cerebrospinal fluid. In positive control groups, cerebral infarcts were detected after the injection of 1500, 2900 or 7500 polystyrene microspheres (size 50 μ m). In these groups the incidence of cerebral injuries, the infarct volume and the score of the lesion were dose dependent. Significant increases in cerebro-spinal fluid NSE were detected, showing also a dose dependency. No cerebral infarct was detected in the brain after intracarotid injections of saline or SonoVue® (1 mL/kg). NSE was not affected after injection of SonoVue®. The results of this study show that SonoVue® (1 mL/kg directly in a carotid artery) did not induce any cerebral ischemic lesion after intra-arterial injection.

Bracco also performed a study entitled "Microvascular behavior of SonoVue® microbubbles following intra-arterial administration in the rat (*spinotrapezius* microcirculation)". The microvascular behavior of SonoVue® microbubbles and the extent of microbubbles retention were evaluated by intravital microscopy immediately after intra-arterial injection at dose levels

of 1, 2.5 or 5 mL/kg, i.e. corresponding to large multiples of the imaging dose. A total of 67 intra-arterial injections of SonoVue® were performed in 38 rats and overall 1729 microscopical fields were observed. There were no substantial changes in arteriolar, venular and capillary blood flow (as subjectively assessed). In few microvessels some rare bubbles were transiently retained. There was no evidence of red blood cell modification, or of leukocyte and platelet adhesion at the site of retainment, nor of thrombosis. Retained microbubbles (defined as those retained at a single site for > 5 s) were primarily observed in capillaries (< 6 µm). No retained microbubbles were observed in post-capillary venules. Retained microbubbles were very flexible and could modify their shape. Elongation of microbubbles was seen in microvessels. Retained microbubbles moved along the microvessels intermittently while decreasing in size due to gas dissolution. No retained microbubbles were observed 20 min after intra-arterial injection of SonoVue® at all three doses.

4 Effects of ultrasound exposure at high MI after SonoVue® administration

Several studies were performed in animals to demonstrate the safety of SonoVue® with concurrent exposure to ultrasound at high power, expressed as mechanical index (MI). The mechanical indices (MI) used in animal studies to demonstrate the safety of SonoVue® were up to the levels of:

- MI 1.2 (emission frequency of 3 MHz) in a safety study in dogs
- MI 1.9 (emission frequency of 7 MHz) in a safety study in rats
- MI 1.6 (emission frequency of 3.5 MHz) in studies in pigs

In these studies, the ultrasound equipment and the transducer were the same as used in human clinical practice.

4.1 Study in dogs up to the maximum MI of 1.2

In seven out of eight non-anesthetized dogs no effect was observed during exposure of the heart to ultrasound at various levels (up to mechanical index of 1.2) with concurrent administration of SonoVue® (0.1, 0.3 and 1 mL/kg). In particular, no effect was seen on ECG parameters: RR, PR, QT, corrected QT and QRS complex duration, whatever the dose of SonoVue® and the intensity of the ultrasound exposure (Figure 1). These findings suggest that the SonoVue® -ultrasound exposure combination had no effect either on the atrioventricular, the intraventricular conduction or on the ventricular repolarization. Equally, the SonoVue® -ultrasound combination had no effect on arterial blood pressure. Therefore, SonoVue® at doses up to 1 mL/kg has no deleterious effect on the cardiovascular function in normal dogs.

In one dog, SonoVue® when administered at the highest dose induced hypotension and bradycardia during the first 10 minutes post-treatment, whatever the intensity of the ultrasound exposure. Then the animal recovered normal behavior before being returned to its pen. Such a reaction was also observed in this particular animal when SonoVue® was administered alone. It was not observed in the other seven dogs included in the study.

Histologically there were no heart lesions in any of the animals of this study.

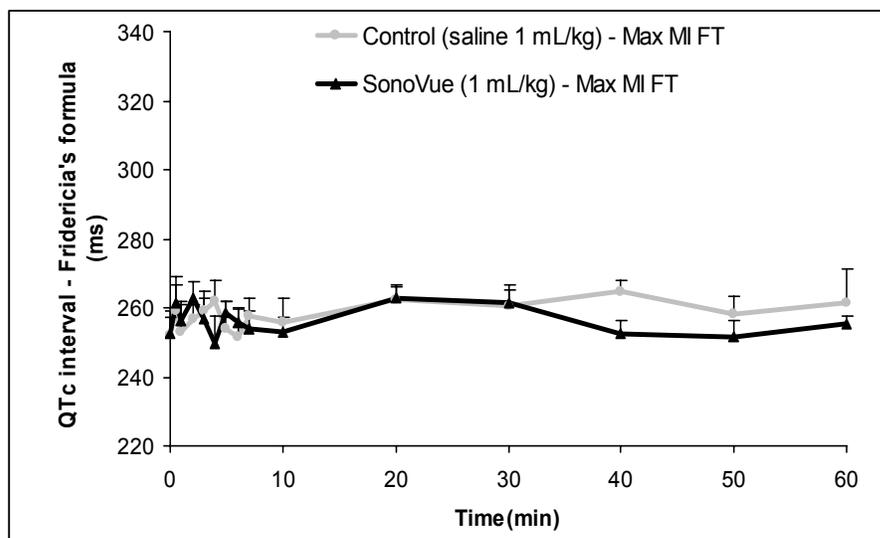


Figure 1. No changes in QTc interval (Fridericia's formula) in the conscious beagle dog administered with saline or SonoVue 1 mL/kg by intravenous route.

4.2 Study in rats up to the maximum MI of 1.9

Histopathological examination of organs in rats after an intravenous injection up to 5 mL/kg and exposure to ultrasound at various mechanical index values was carried out in the Sprague-Dawley (SD) rat. In this study, 10 s after starting the ultrasound exposure the animals were injected with either SonoVue® (1 or 5 mL/kg) or saline. The organs (liver, spleen, kidneys, intestines and abdominal vessels, stomach, pancreas, heart, thymus, lung and carotid arteries) were exposed to ultrasound at various mechanical index values (MI of 0.4, 0.8 and 1.9) for a total duration of 5 min. The maximum dose level of SonoVue® corresponded to 17-20 times MHDbsa. The maximum MI value level of 1.9 is among the highest currently available on the market and, in addition, an effect on (destruction of) microbubbles was observed at the two highest MI levels of 0.8 and 1.9.

In rats sacrificed 1 h after exposure, some minimal areas of blood suffusion within the pulmonary alveoli or close to the mesenteric blood vessels were observed microscopically. The incidence and grade of these findings in the various experimental groups and their presence in the saline treated groups as well show that they are not related to the administration or the dose of SonoVue®. No other histopathological lesions, which could be attributed to the treatment of SonoVue® and ultrasound exposures, were observed.

4.3 Studies in pigs up to the maximum MI of 1.6

In a series of experiments on anesthetized pigs, non-pretreated with aspirin, the pigs were administered repeatedly with either SonoVue® or an ultrasound contrast agent (UCA) marketed in the USA, at the human imaging dose, at 40-min intervals. Hemodynamic parameters were measured in the absence of any ultrasound imaging (no insonation), with ultrasound imaging of the heart at a low MI (MI = 0.25) or at the maximum MI achievable with the equipment used in this experiment (MI = 1.6). The insonation procedure (i.e. the order of the different MI administered) for each animal was randomized. The same pigs were repeatedly tested with either SonoVue® (6 injections) or the other UCA (6 injections), and then received a 7th injection of the other contrast agent with ultrasound imaging performed at the maximal MI (MI = 1.6) to check that they responded to the first contrast agent.

The mechanical index of ultrasound exposure to which the pigs were exposed concurrently to the administration of the contrast agents did not influence the cardiovascular response of the animals as there was no modifications of the changes (PAP, AR, LC, SAP, HR, LVV_d, SpO₂) induced by the contrast agents alone. In particular, it should be noted that exposure to ultrasound at low (MI = 0.25) or high MI (MI = 1.6) did not modify the hemodynamic changes of the heart of pigs exposed to either SonoVue® (Figure 2) or the other UCA.

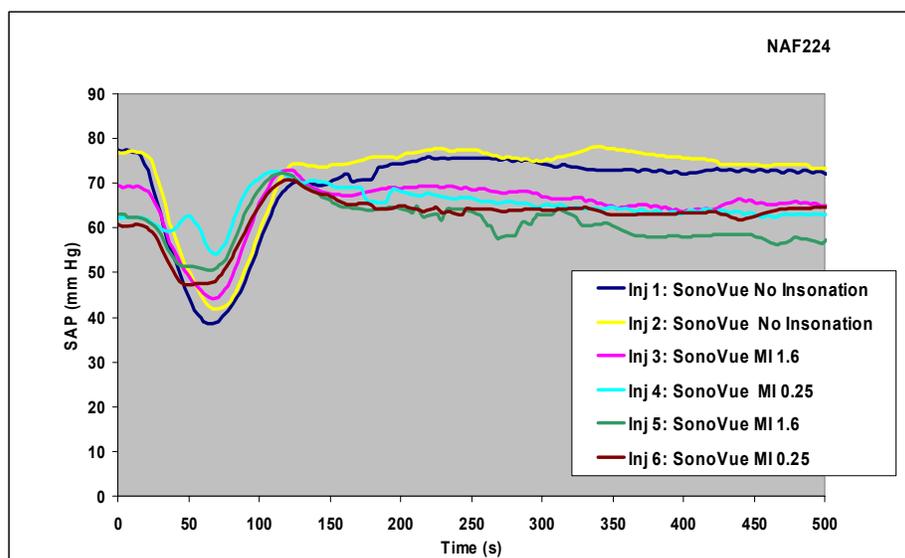


Figure 2. No effect of concurrent ultrasound exposure (mechanical index 0.25 or 1.6) on systemic arterial pressure changes after SonoVue® injections in pig at imaging dose

5 Carcinogenesis, Mutagenesis and Impairment of Fertility

No mutagenicity nor genotoxicity in the regulatory tests (Ames test, micronucleus test, chromosomal aberration), and no impairment of fertility in rats and in rabbits in the reproductive toxicology studies were observed in nonclinical studies with SonoVue®.

6 Use of pigs as an animal model for imaging studies by echocardiography and studies on the mechanism of anaphylactoid reactions in humans

The pig heart is close in morphology and size to the human heart. The pig is therefore routinely used as a model for echocardiographic studies. However since pigs are known to be particularly sensitive to the hemodynamic effects of microbubble contrast agents, as reported in the literature for Definity®¹ and Optison®^{2,3} as well as for other lipid-based nanoparticles⁴, all imaging experiments performed during the development of SonoVue® were done in pigs pretreated with indomethacin or aspirin which were found to suppress totally this reaction (Figure 3).

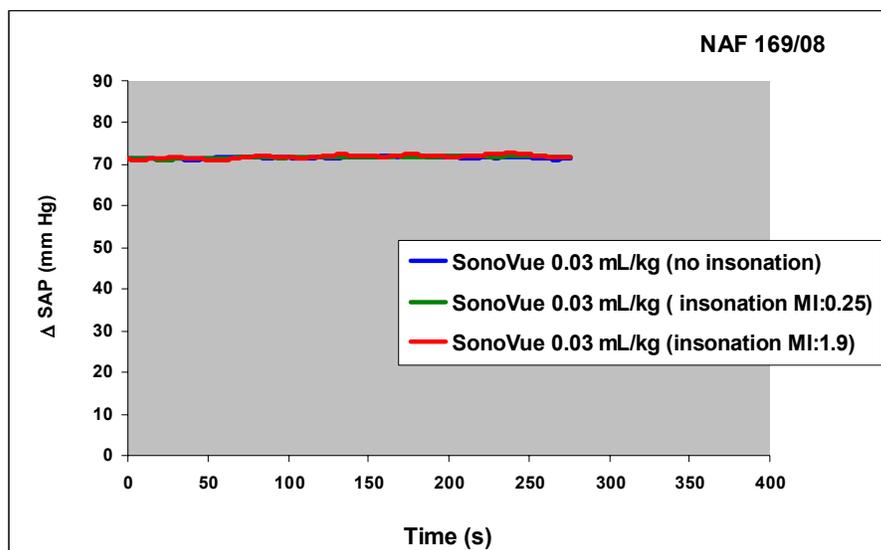


Figure 3. No effect of SonoVue® on systemic arterial pressure in pigs pretreated with aspirin (15 mg/kg) without or with ultrasound exposure (mechanical index 0.25 or 1.6)

Later on, after rare cases of anaphylactoid reactions were observed in humans during pharmacovigilance studies, it was hypothesized that the over-reaction in pigs could be used as a sensitive model to study the human findings since the symptoms shown in this species were analogous to the acute hypersensitivity reactions occasionally observed in humans. A series of experiments was performed in pigs, non-pretreated with aspirin, to try to understand the mechanism of the anaphylactoid reactions in humans.

When SonoVue® and the two other ultrasound contrast agents on the market in the USA are administered to non-pretreated pigs at the human imaging dose they elicit in most animals a similar reaction with an increase in pulmonary arterial pressure, a decrease in systemic arterial pressure and modifications of the cardiac dynamics (Figure 4, Figure 5, Figure 6). This reaction presents certain variability between individual pigs (Figure 7), is independent of the intensity (mechanical index) of the ultrasound exposure (Figure 2) and is related to the release of mediators in the blood stream, particularly of thromboxane (Figure 8). It is considered that the over-reaction in pigs, which occurs already at imaging doses, is related to the presence in this

animal species of pulmonary intravascular macrophages (PIM) present in high numbers in ungulates⁵.

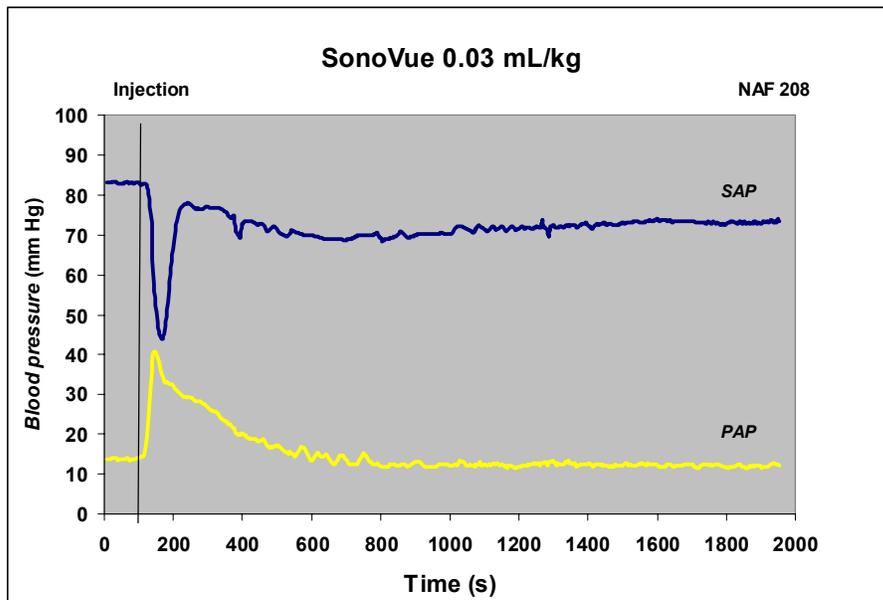


Figure 4. Example of reaction of non-pretreated pigs to administration of SonoVue

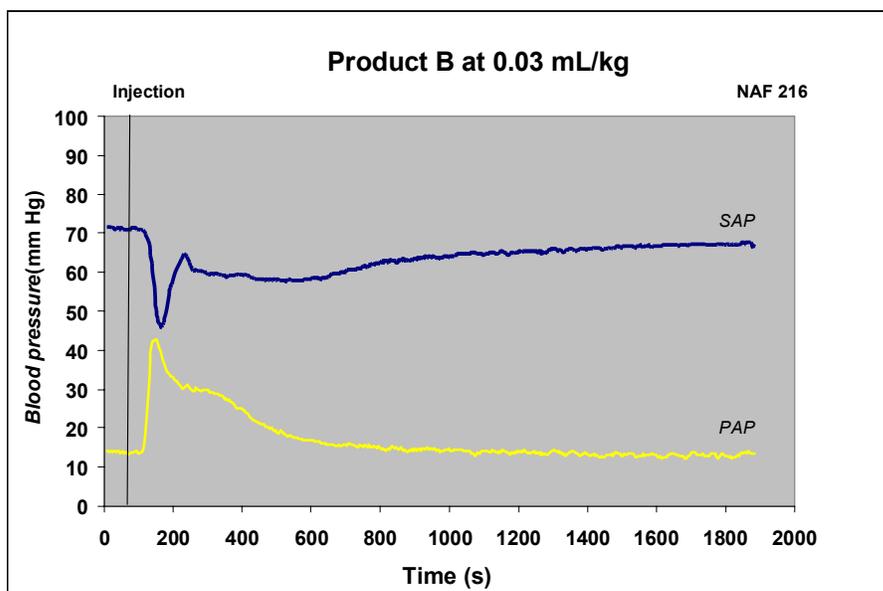


Figure 5. Example of reaction of non-pretreated pigs to administration of another UCA on the US market (Product B)

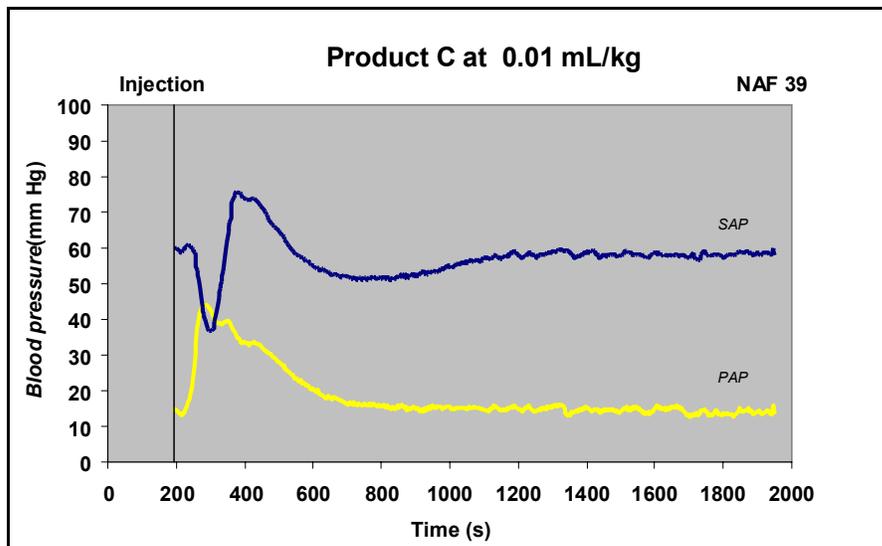


Figure 6. Example of reaction of non-pretreated pigs to administration of another UCA on the US market (Product C)

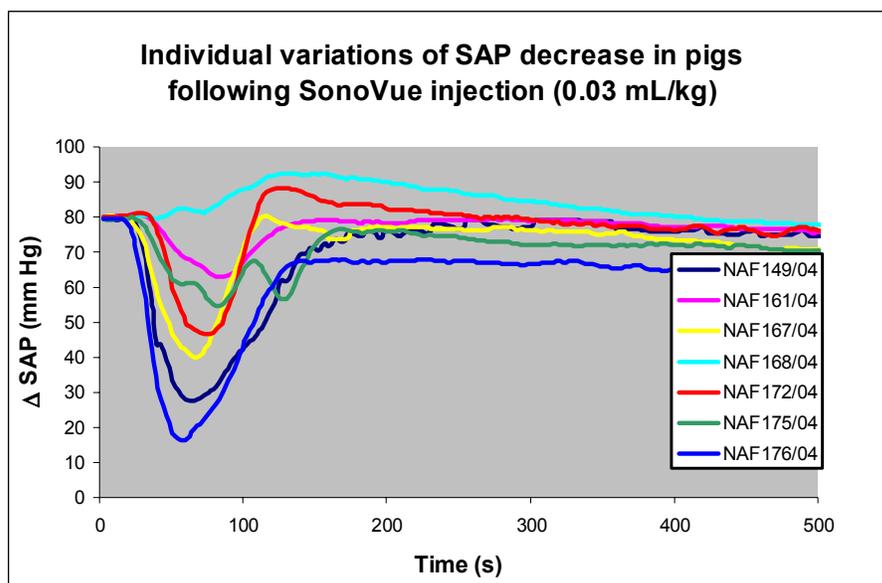


Figure 7. Large individual variations of the decrease in SAP in non-pretreated pigs after SonoVue injection at imaging dose

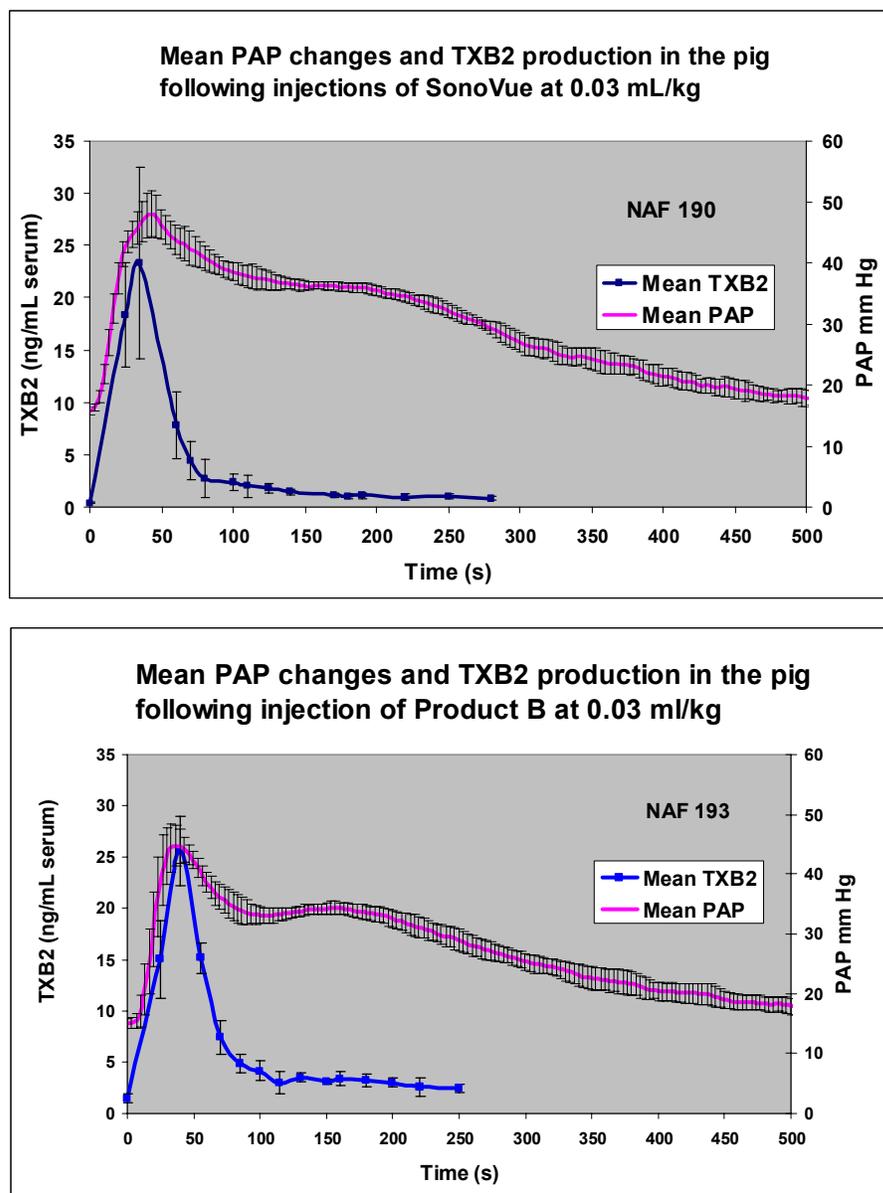


Figure 8. Kinetics of pulmonary arterial pressure and TXB2 changes in non-pretreated pigs following SonoVue® or another UCA (Product B) injections at human imaging doses (mean of 5 injections)

Rats which, like humans or monkeys, do not possess high numbers of PIM in normal conditions, did not show a decrease in systemic blood pressure until the dose of SonoVue® administered reached 25 or 50 fold the human imaging dose (Figure 9). In addition, the mediators appear to be different in rats since the reaction at these high doses of the UCA is not alleviated by pretreatment with aspirin but by anti-PAF or by depletion of the complement system with cobra venom factor. In the rats, the role of PIM in the mechanism involved in pigs is taken by the liver Kupffer cells.

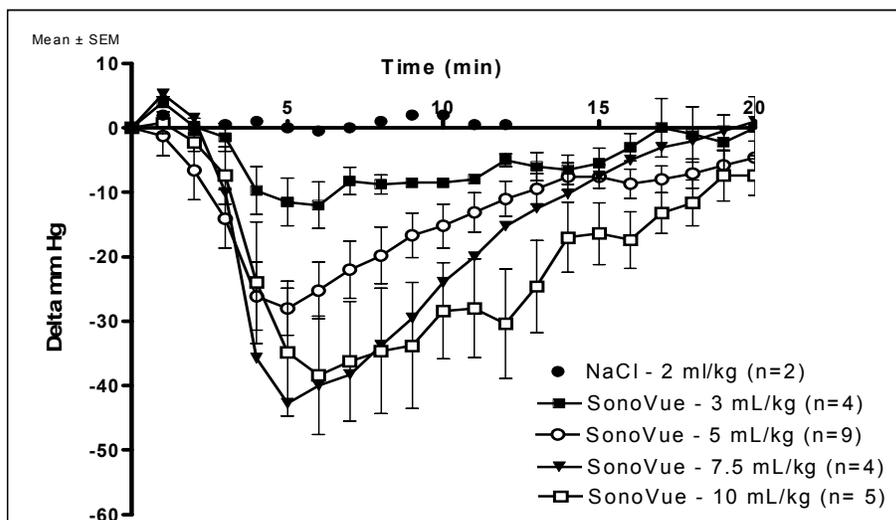


Figure 9. Only very high doses (25 - 50 x human dose) of SonoVue® induce systemic hypotension in rats

From the published data and our own studies, stimulation of macrophages by anaphylatoxins (with or without complement activation) could be the basic underlying mechanism of SonoVue® reactions.

7 Conclusion

In conclusion, Bracco has shown and considers that:

- SonoVue® is a very safe agent with no significant effects observed in several animal species (with the exception of pigs) at very large multiple of the human imaging dose.
- The very rare SAR observed during pharmacovigilance show some analogy with the reaction observed in pigs – a reaction related to the presence of PIMs which has been broadly discussed following the administration of different particles as well as other ultrasound contrast agents.
- Since the symptoms observed in pigs are strikingly similar to ones observed in most very rare cases of SARs in humans, the pig could be a valuable test system for the evaluation of novel ultrasound contrast agents.

8 Appendix: List of preclinical studies on SonoVue®

Table A: Nonclinical Pharmacology Studies

Type of Study	GLP Status	Animal Species	Dose	Major Findings
Cardiovascular/ Respiratory System	Yes	Rat Rabbit	0.3 and 1.0 mL/kg	No modifications in heart rate, mean arterial pressure respiratory resistance, pulmonary compliance.
Arterial Blood Gases	Yes	Rat	0.3 and 1.0 mL/kg	No modifications in arterial blood gases and pH.
Arterial Pressure/ Hematology	No	Dog	0.3 and 1.0 mL/kg	No effects following 0.3 mL/kg. Reversible hypotension (2 out of 7 dogs) and thrombocytopenia and leukopenia (5 out of 7 dogs).
Cardiovascular System	Yes	Monkey	20 mL/kg, single injection; 0.2, 1.0 and 5.0 mL/kg, 28 days repeat injections	No effects on arterial blood pressure and heart rate (single injection) and on ECG (repeated injections).
Cardiovascular System	Yes	Monkey	1.0, 2.0 and 5.0 mL/kg	No effects on blood pressure, heart rate, RR, PR, QRS, QT and QTc intervals in cynomolgus monkeys monitored by telemetry.
Cardiovascular System	Yes	Dog	0.1, 0.3 and 1.0 mL/kg	No effects on ECG parameters: RR, PR, QT, QTc and QRS complex duration in dogs treated with SonoVue and examined in echocardiography up to MI=1.2.
Cardiovascular and Pulmonary Systems	Yes	Dog	0.1, 0.3 and 1.0 mL/kg	No effects on blood pressure, heart rate, QT and QTC of ECG, and no effects on myocardial and pulmonary functions in dogs with experimentally induced pulmonary hypertension. Transient (5 to 7 minutes) increased PAP at 1 mL/kg.
Effect on internal organ	Yes	Rat	1.0 and 5.0 mL/kg	No effects of SonoVue on the organs of rats exposed to ultrasound at MI=0.4, 0.8 and 1.9 as determined by histopathological examination of the organs.
Microcirculation	No	Rat	1.0, 2.5 and 5.0 mL/kg	No effects in <i>spinotrapezius</i> muscle microcirculation after intra-arterial injections.
Microcirculation	No	Hamster	3.8 and 7.6 mL/kg	No effect in hamster cheek pouch microcirculation after i.v. injections.
Cerebral Circulation	Yes	Rat	1.0 mL/kg	No effect.
Blood	Yes	Humans	In vitro	No hemolytic effect (10:1 ratio); no effect on hematocrit (up to 20% final concentration); no effect on coagulation (up to 40% final concentration); no effect on erythrocyte morphology (up to 20% final concentration). No platelet aggregating effect (up to 0.02 mg/mL).
Spontaneous Locomotor Activity	Yes	Mouse	0.3 and 1.0 mL/kg	No effect.
PTZ-induced convulsions	Yes	Mouse	0.3 and 1.0 mL/kg	No effect.
Intestinal Motility	Yes	Rat	0.3 and 1.0 mL/kg	No effect.

Table A: Nonclinical Pharmacology Studies

Type of Study	GLP Status	Animal Species	Dose	Major Findings
Urine volume and electrolytes	Yes	Rat	0.3 and 1.0 mL/kg	No effect.
Pentobarbital-induced anesthesia	Yes	Mouse	0.3 and 1.0 mL/kg	No effect.
Body temperature	Yes	Mouse	0.3 and 1.0 mL/kg	No effect.

Table B: Fertility and Embryotoxicity Studies

Type of Study	Animal Species	Dose (mL/kg) and Schedule of Treatment	Major Findings
Preliminary fertility and embryo-toxicity	Rats (6 males, 6 females/group)	0 (control), 0.2, 1.0, 5.0; i.v. bolus; for 15 days before mating until littering of the females	No adverse effects.
Fertility and embryo-toxicity	Rats (22 males, 22 females/group)	0 (control), 0.2, 1.0, 5.0; i.v. bolus; males treated from Day 29 before mating until termination, females from Day 15 before mating to Day 17 after mating. Autopsy on Day 20.	No effects on estrous cycle, mating performance and fertility. No effects on fetal development.
Preliminary embryo-fetal toxicity	Rabbits (4 females/group)	0.2, 1.0, 5.0; i.v. bolus; from Day 6 to 19 after mating; autopsy at Day 29 after mating	No necropsy finding in the dams. No effects on the litters.
Embryo-fetal toxicity	Rabbits (22 females/group)	0 (control), 0.2, 1.0, 5.0; i.v. bolus; from Day 6 to 19 after mating; autopsy at Day 29 after mating	One control and one at 1.0 mL/kg/day aborted or gave birth prematurely. No evidence of systemic toxicity to the dams. No effects on fetal survival and placental weights. No effects on the litters.
Pre- and post-natal toxicity	Rats (25 females/group)	0 (control), 0.2, 1.0, 5.0; i.v. bolus; from Day 6 of gestation until the end of lactation; selected F ₁ offspring necropsied on Day 13 post-coitum	No effects on dam weight. Pups were delivered regularly. Their weight at birth was normal. Their physical and functional development was not affected by treatment. Three dams from the 0.2 mL/kg dose group were sacrificed on Days 14, 15 or 16 of lactation. Necropsy revealed marked distension of the cecum, ileum and jejunum. At necropsy one surviving low dose group and 3 intermediate dose dams had distended intestines. Histopathology revealed ulceration of the cecum (1 low dose and 2 intermediate dose) and inflammation of the mesentery adjacent to the intestine (4 low dose and 4 intermediate dose). No lesions were detected at high dose. No abnormality observed in F ₁ generation.

Table C: Genetic Toxicity Studies

Test	Test system	Results
Ames test (on SF ₆)	<i>S. typhimurium</i> (5 strains, 3 plates per dose in an atmosphere of 50% SF ₆ in air, with and without metabolic activation)	No mutagenicity after exposure to 48% to 50% SF ₆ in air for 48 hours
Ames test (on SonoVue)	<i>S. typhimurium</i> (5 strains, 3 plates per dose with and without metabolic activation) <i>S. typhimurium</i> (TA102 strain, 3 plates per dose with and without metabolic activation)	No mutagenicity up to the maximum level of 10,000 µg SonoVue dry matter/plate No mutagenicity up to the maximum level of 5,000 µg SonoVue dry matter/plate
Micronucleus test	Mouse bone marrow cells	Not mutagenic at 4 x 20 mL SonoVue/kg i.v.
Chromosomal aberration assay	Cultured human lymphocytes (3-hour incubation with and without metabolic activation) Cultured human lymphocytes (20-hour incubation without metabolic activation)	No chromosome aberrations No chromosome aberrations

Table D: Local Tolerance and Immunological Studies

Type of Study	Animal Species	Dose	Major Findings
Local Tolerance	Rabbit	10 mL or 0.5 mL per animal	No effects after 10 mL/animal (i.v. injection) or 0.5 mL/animal (paravenous injection).
Immunological Studies	Rat Monkey	Up to 5 mL/kg (from repeated dose toxicity studies)	No signs of immunological reactions from histopathology of thymus, spleen, mandibular and mesenteric lymph nodes.

Toxicology Studies: Details of single dose and repeated doses toxicology studies performed on SonoVue® in rats and monkeys are given below

Name of company BRACCO RESEARCH S.A. Name of finished product Sonovue (BR1) Name of active ingredient Sulphur Hexafluoride	<input type="checkbox"/> TABULATED STUDY REPORT ref. to III.A.1.1 1 / 8 Page/Number							
SINGLE DOSE TOXICITY								
Ref. to Document. Report date: 22 Oct. 1993	Volume: 7 Number: TOX-3-93	Page: 1 Study Period (years): 1993	Addendum No: 0					
Species/strain: rat/Sprague Dawley			Number of animals: 40					
Administration route: intravenous, via tail vein			Dose (mL/kg): max. non lethal: > 20 min. lethal: >20					
Treatment of controls: saline			Observation period (Appl. day = Day 1): sacrifices after 24 h, 7 d, 14 d					
Study group	(1) contr.		(2)		(3)		(4)	
Dosage (mL/kg)	0		20		20		20	
Sex (m/f)	m	f	m	f	m	f	m	f
Animals per dosage	5	5	5	5	5	5	5	5
Sacrifice at	14 d	14 d	24 h	24 h	7 d	7 d	14 d	14 d
DEATHS	Within 6 hours							
	7-24 hours							
	days 2-7							
	day 8- end of observ.							
	total		0	0	0	0	0	0

Summary of salient findings:

Rate of injection: 1 mL/min

Limit test: no mortality at 20 mL/kg.

Symptoms: no abnormal clinical signs throughout the study, either during or after administration of the test article.

Necropsy: no abnormalities detected.

Histopathology: no microscopic findings suggestive of systemic or organ toxicity on kidneys, adrenals, liver, lung and spleen.

Name of company BRACCO RESEARCH S.A.	<input type="checkbox"/> TABULATED STUDY REPORT ref. to III.A.1.2 2 / 8 Page/Number
Name of finished product Sonovue (BR1)	
Name of active ingredient Sulphur Hexafluoride	

SINGLE DOSE TOXICITY

Ref. to Document.	Volume: 7	Page: 34	Addendum No: 0			
Report date: January 1994	Number: CHE1183/1	Study Period (years): 1993				
Species/strain: monkey/purpose bred Macaca fascicularis (cynomolgus)	Number of animals: 4					
Administration route: intravenous, via cephalic vein	Dose (mL/kg): max. non lethal: > 20 min. lethal: >20					
Treatment of controls: saline (20 mL/kg)	Observation period (Appl. day = Day 1): 14 d (group 2), 7 d repeat dose (group 3)					
Study group	(1) contr.		(2)		(3)	
Dosage (mL/kg)	0		20		2*	
Sex (m/f)	m	f	m	f	m	f
Animals per dosage	1	1	1	1	1	1
DEATHS	Within 6 hours					
	7-24 hours					
	days 2-7					
	day 8- end of observ.					
	total		0	0	0	0

* = doses of BR1 repeated daily for 7 d on animals used as controls previously

Summary of salient findings:

Rate of injection: 10 mL/min

Limit test: no mortality at 20 mL/kg

Symptoms: no abnormal clinical findings, no change in body weight, no effect on blood pressure, transient increase in neutrophils counts, no change in clinical chemistry or organ weights.

Necropsy: no abnormalities detected.

Histopathology: no microscopic findings suggestive of systemic or organ toxicity in organs examined.

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Non Clinical Safety Experience with SonoVue

SonoVue

Name of company BRACCO RESEARCH S.A. Name of finished product Sonovue (BR1) Name of active ingredient Sulphur Hexafluoride	<input type="checkbox"/> TABULATED STUDY REPORT ref. to III.A.2.1 3/ 8 Page/Number
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REPEATED DOSE TOXICITY Subacute toxicity (up to 3 months)

Ref. to Document.	Volume: 8	Page: 1	Addendum No: 0
Report date:	Number:	Study Period (years):	
November, 1994	CHE1183/2	1994	

Species/strain: rat/Crl:CD(SD)BR

Number of animals: 100	Duration of treatment: 28 days
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Observation period after the end of dosing: 14 day treatment free period in controls and group 4

Administration route: intravenously by bolus in a caudal vein

Treatment of controls: 0.9% NaCl saline (5 mL/kg)	Age: more than 6 and less than 8 weeks
	Body weight (g): M 269, F 193 at study initiation
Treatment days per week: 7	

Study group	(1) Contr.		(2)		(3)		(4)	
Dosage (mL/kg/day)	0		0.2		1		5	
Sex (m/f)	m	f	m	f	m	f	m	f
Number of test animals (R = recovery animals)	10 + 5R	10 + 5R	10	10	10	10	10 + 5R	10 + 5R
Number of animals died or sacrificed in extremis	1*	0	0	0	0	0	1*	1*

Clinical observation:	<input checked="" type="checkbox"/> yes	<input type="checkbox"/> no	Clin. chemistry:	<input checked="" type="checkbox"/> yes	<input type="checkbox"/> no
Food consumption:	<input checked="" type="checkbox"/> yes	<input type="checkbox"/> no	Urinalysis:	<input checked="" type="checkbox"/> yes	<input type="checkbox"/> no
Water consumption:	<input type="checkbox"/> yes	<input checked="" type="checkbox"/> no	Organ weights:	<input checked="" type="checkbox"/> yes	<input type="checkbox"/> no
Body weight:	<input checked="" type="checkbox"/> yes	<input type="checkbox"/> no	Necropsy:	<input checked="" type="checkbox"/> yes	<input type="checkbox"/> no
Haematology:	<input checked="" type="checkbox"/> yes	<input type="checkbox"/> no	Histology:	<input checked="" type="checkbox"/> yes	<input type="checkbox"/> no

Additional examinations: ophthalmoscopy, histological testicular staging for male fertility evaluation

Additional information: none

*: In the report it is mentioned that these deaths are procedural, due to global warming of the animals under an infrared lamp for inducing vasodilatation of the tail for the injection and are not due to the product. No more deaths were observed after the vasodilatation was changed by heating the tails only.

Name of company BRACCO RESEARCH S.A. Name of finished product Sonovue (BR1) Name of active ingredient Sulphur Hexafluoride	<input type="checkbox"/> TABULATED STUDY REPORT ref. to III.A.2.1 4/ 8 Page/Number	
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REPEATED DOSE TOXICITY Subacute toxicity (up to 3 months)

Ref. to Document. Report date: November, 1994	Volume: 2 Number: CHE1183/2	Page: 1 Study Period (years): 1994	Addendum No: 0
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Important findings	Group 2		Group 3		Group 4	
	m	f	m	f	m	f
Haematology						
Red blood cell counts					D/REC	
Haemoglobin					D/REC	
Urine analysis						
Volume	D/T		D/T		D/T	
Necropsy						
Caecum findings at terminal kill (n=10/group)	4	2	3	5	4	9
Caecum findings at recovery group (n=5)					0	0
Histopathology						
Caecitis	4	3	3	5	4	9
Caecum erosion/ulcer	4	2	2	5	1	7
Colitis	3	2	2	4	5	4
Colon erosion/ulcer	0	0	0	0	0	1
Caecum/colon lesions at recovery kill					0	0

Explanations: D = decrease I = increase P = permanent T = transitory
 ns = not significant * = p < 0.05 ** = p < 0.01 REC = recovery
 + = mild ++ = moderate +++ = severe n = no. of animals
 [] = due to abnormal control values

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Non Clinical Safety Experience with SonoVue

SonoVue

Name of company BRACCO RESEARCH S.A. Name of finished product Sonovue (BR1) Name of active ingredient Sulphur Hexafluoride	<input type="checkbox"/> TABULATED STUDY REPORT ref. to III.A.2.2 5 / 8 Page/Number
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REPEATED DOSE TOXICITY Subacute toxicity (up to 3 months)

Ref. to Document.	Volume: 9	Page: 1	Addendum No: 0
Report date: November, 1994	Number: CHE1183/6	Study Period (years): 1994	

Species/strain: monkey/ purpose bred *Macaca fascicularis* (cynomolgus)

Number of animals: 24	Duration of treatment: 28 days
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Observation period after the end of dosing: none

Administration route: intravenously, in cephalic or saphenous veins

Treatment of controls: 0.9% NaCl saline at 5 mL/kg	Age: young animals	at study initiation
	Body weight (kg): 2.3 -2.4	
Treatment days per week: 7		

Study group	(1) Contr.		(2)		(3)		(4)	
Dosage (mL/kg/day)	0		0.2		1		5	
Sex (m/f)	m	f	m	f	m	f	m	f
Number of test animals	3	3	3	3	3	3	3	3
Number of animals died or sacrificed in extremis	0	0	0	0	0	0	0	0

Clinical observations:	<input checked="" type="checkbox"/> yes	<input type="checkbox"/> no	Clin. chemistry:	<input checked="" type="checkbox"/> yes	<input type="checkbox"/> no
Food consumption:	<input checked="" type="checkbox"/> yes	<input type="checkbox"/> no	Urinalysis:	<input checked="" type="checkbox"/> yes	<input type="checkbox"/> no
Water consumption:	<input type="checkbox"/> yes	<input checked="" type="checkbox"/> no	Organ weights:	<input checked="" type="checkbox"/> yes	<input type="checkbox"/> no
Body weight:	<input checked="" type="checkbox"/> yes	<input type="checkbox"/> no	Necropsy:	<input checked="" type="checkbox"/> yes	<input type="checkbox"/> no
Haematology:	<input checked="" type="checkbox"/> yes	<input type="checkbox"/> no	Histology:	<input checked="" type="checkbox"/> yes	<input type="checkbox"/> no

Additional examinations: ophthalmoscopy, electrocardiography, blood pressure

Additional information: --

Name of company BRACCO RESEARCH S.A. Name of finished product Sonovue (BR1) Name of active ingredient Sulphur Hexafluoride	<input type="checkbox"/> TABULATED STUDY REPORT ref. to III.A.2.2 6 / 8 Page/Number	
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REPEATED DOSE TOXICITY Subacute toxicity (up to 3 months)

Ref. to Document.	Volume: 9	Page: 1	Addendum No: 0
Report date:	Number:	Study Period (years):	
November, 1994	CHE1183/6	1994	

Important findings	Group 2		Group 3		Group 4	
	m	f	m	f	m	f
Clinical						
Food consumption					D/T	D/T
Haematology						
Neutrophils count				I*/T		
Eosinophils count		I*		D*/T		
Blood chemistry						
Glucose	I*/T					

Explanations: D = decrease I = increase P = permanent T = transitory

ns = not significant * = p < 0.05 ** = p < 0.01 REC = recovery
 + = mild ++ = moderate +++ = severe n = no. of animals
 [] = due to abnormal control values

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Non Clinical Safety Experience with SonoVue

SonoVue

Name of company BRACCO RESEARCH S.A. Name of finished product Sonovue (BR1) Name of active ingredient Sulphur Hexafluoride	<input type="checkbox"/> TABULATED STUDY REPORT ref. to III.A.2.3 7/ 8 Page/Number	
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REPEATED DOSE TOXICITY Subacute toxicity (up to 3 months)

Ref. to Document.	Volume: 9	Page: 290	Addendum No: 0
Report date:	Number:	Study Period (years):	
May, 1995	TOX-1-95	1995	

Species/strain: rat/Sprague-Dawley

Number of animals: 32	Duration of treatment: 7, 14, 21 and 28 d according to group
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Observation period after the end of dosing: none

Administration route: intravenously by bolus in a caudal vein

Treatment of controls:	Age: young adult females
0.9% NaCl saline at 5 mL/kg (group 1)	Body weight (g): 180 ± 20% at study initiation
0.5% PEG 4000 at 5 mL/kg (group 2)	Treatment days per week: 7

Study group	(1) Contr.		(2) Contr.		(3)		(4)		(5)		(6)	
	m	f	m	f	m	f	m	f	m	f	m	f
Dosage (mL/kg/day)	0		0		5		5		5		5	
Sex (m/f)												
Number of test animals		5		5		5		5		5		5
Duration of treatment		28d		28d		7d		14d		21d		28d
Number of animals died or sacrificed in extremis												

Clinical observation:	<input checked="" type="checkbox"/> yes	<input type="checkbox"/> no	Clin. chemistry:	<input type="checkbox"/> yes	<input checked="" type="checkbox"/> no
Food consumption:	<input type="checkbox"/> yes	<input checked="" type="checkbox"/> no	Urinalysis:	<input type="checkbox"/> yes	<input checked="" type="checkbox"/> no
Water consumption:	<input type="checkbox"/> yes	<input checked="" type="checkbox"/> no	Organ weights:	<input checked="" type="checkbox"/> yes	<input type="checkbox"/> no
Body weight:	<input checked="" type="checkbox"/> yes	<input type="checkbox"/> no	Necropsy:	<input checked="" type="checkbox"/> yes	<input type="checkbox"/> no
Haematology:	<input type="checkbox"/> yes	<input checked="" type="checkbox"/> no	Histology:	<input checked="" type="checkbox"/> yes	<input type="checkbox"/> no

Additional examinations: none

Additional information: none

Name of company BRACCO RESEARCH S.A. Name of finished product Sonovue (BR1) Name of active ingredient Sulphur Hexafluoride	<input type="checkbox"/> TABULATED STUDY REPORT ref. to III.A.2.3 8 / 8 Page/Number	
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REPEATED DOSE TOXICITY Subacute toxicity (up to 3 months)

Ref. to Document.	Volume: 9	Page: 290	Addendum No: 0
Report date:	Number:	Study Period (years):	
May, 1995	TOX-1-95	1995	

Important findings	Group 3	Group 4	Group 5	Group 6
	f	f	f	f
Sacrifice at	7 d	14 d	21 d	28 d
Necropsy				
Kidney to body weight ratio vs control same age				I*/ns
Histopathology				
Caecum lesion	0	0	0	0
Colon lesion	0	0	0	0

Explanations: **D** = decrease **I** = increase **P** = permanent **T** = transitory
 ns = not significant ***** = p < 0.05 ****** = p < 0.01 **REC** = recovery
 + = mild **++** = moderate **+++** = severe **n** = no. of animals
 [] = due to abnormal control values

9 References with Abstracts

- ¹ Grauer SE, Pantely GA, Xu J, Ge S, Giraud GD, Shiota T, Sahn DJ. Myocardial imaging with a new transpulmonary lipid-fluorocarbon echo contrast agent: experimental studies in pigs. *Am Heart J.* 1996 Nov;132(5):938-45.

Abstract: The development of echo contrast agents that can provide reliable opacification of the myocardium after intravenous injection is an important advancement for the clinical application of contrast echocardiography. In this study, the hemodynamic effects and echocardiographic characteristics of a new lipid-fluorocarbon echo contrast agent, Aerosomes MRX 115 (ImaRx Pharmaceutical Corp., Tucson, Ariz.) were studied in six anesthetized ventilated pigs. Intravenous injection of this new agent in doses ranging from 0.0005 to 0.01 ml/kg produced significant measurable and visible myocardial opacification without any effect on heart rate, systemic pressure, partial pressure of oxygen, or left ventricular systolic function. The two largest doses (0.005 and 0.01 ml/kg), however, resulted in mild reversible increases in mean pulmonary artery pressure of 12 and 16 mm Hg, respectively. In four animals, epicardial images were obtained before and during coronary artery occlusion. Intravenous contrast injection during coronary occlusion permitted delineation of the hypoperfused myocardial segment. This capability may further expand the utility of contrast echocardiography. PMID: 8892764 [PubMed - indexed for MEDLINE]

- ² Ostensen J, Hede R, Myreng Y, Ege T, Holtz E. Intravenous injection of Albunex microspheres causes thromboxane mediated pulmonary hypertension in pigs, but not in monkeys or rabbits. *Acta Physiol Scand.* 1992 Mar;144(3):307-15.

Abstract: Intravenous injection of the ultrasound contrast agent Albunex (manufactured by Nycomed AS, Oslo, Norway; 400 million air-filled albumin microspheres per ml, mean diameter 4 +/- 1 microns) caused a dose-dependent increase of mean pulmonary arterial pressure in nine pigs. The highest dose (0.014 +/- 0.002 ml kg⁻¹) increased mean pulmonary arterial pressure from 17 +/- 1 mmHg to 42 +/- 3 mmHg and decreased mean systemic arterial pressure from 111 +/- 9 to 93 +/- 12 mmHg. The pressure responses began 22 +/- 1 s after particle injection, and reached maximum after 51 +/- 3 s. No changes in mean pulmonary arterial pressure or mean systemic arterial pressure were observed after Albunex injections during treatment with indomethacin (10 mg kg⁻¹ + 5 mg kg⁻¹ h⁻¹ i.v., n = 6) or the thromboxane A2 receptor antagonist HN-11500 (10 mg kg⁻¹ + 5 mg kg⁻¹ h⁻¹ i.v., n = 3). No Doppler enhancement could be detected in a carotid artery following injection of 0.12 ml kg⁻¹ Albunex during indomethacin treatment. In five rabbits, Albunex caused Doppler enhancement in a carotid artery, and 0.48 ml kg⁻¹ did not affect mean pulmonary arterial pressure or other haemodynamic parameters in five rabbits or in three cynomolgus monkeys. The pressure response in pigs may be explained by release of thromboxane A2 from the pulmonary intravascular macrophages during phagocytosis of the microspheres. This response to Albunex was totally absent in rabbits and monkeys. PMID: 1533987 [PubMed - indexed for MEDLINE]

- ³ Luo W, Zderic V, Carter S, Crum L, Vaezy S. Detection of bleeding in injured femoral arteries with contrast-enhanced sonography. *J Ultrasound Med.* 2006 Sep;25(9):1169-77.

Abstract: OBJECTIVE: The purpose of this study was to investigate the feasibility of detecting acute arterial bleeding by means of contrast-enhanced sonography. METHODS: Puncture injury was produced transcatheterously with an 18-gauge needle in 26 femoral arteries (13 in the control group and 13 in the contrast-enhanced group) of rabbits. A sonographic contrast agent (Optison; Mallinckrodt Inc, St Louis, MO) was administered intravenously at a dose of 0.06 to 0.07 mL/kg. Sonography of the femoral arteries was performed before and after injury, both before and after injection of Optison, with B-mode imaging, color Doppler imaging,

and pulse inversion harmonic imaging (PIHI). RESULTS: The specific location of active bleeding could not be visualized in B-mode and PIHI scans in the control group (no Optison injection). After administration of Optison, the bleeding site was visualized because of the increased echogenicity of the extravasated blood at the puncture site in both B-mode imaging and PIHI. In color Doppler images, bleeding sites were localized successfully in 84.6% of the cases in the presence of Optison and in 30.8% of the cases without Optison. Histologic examination (light microscopy) of the hematoma confirmed the presence of contrast agent microbubbles in the extravascular space surrounding the artery. CONCLUSIONS: Contrast-enhanced sonography may provide an effective method for detecting arterial bleeding. PMID: 16929018 [PubMed - indexed for MEDLINE]

- ⁴ Szebeni J, Alving CR, Rosivall L, Bünger R, Baranyi L, Bedöcs P, Tóth M, Barenholz Y. Animal models of complement-mediated hypersensitivity reactions to liposomes and other lipid-based nanoparticles. *J Liposome Res.* 2007;17(2):107-17.

Abstract: Intravenous injection of some liposomal drugs, diagnostic agents, micelles and other lipid-based nanoparticles can cause acute hypersensitivity reactions (HSRs) in a high percentage (up to 45%) of patients, with hemodynamic, respiratory and cutaneous manifestations. The phenomenon can be explained with activation of the complement (C) system on the surface of lipid particles, leading to anaphylatoxin (C5a and C3a) liberation and subsequent release reactions of mast cells, basophils and possibly other inflammatory cells in blood. These reactions can be reproduced and studied in pigs, dogs and rats, animal models which differ from each other in sensitivity and spectrum of symptoms. In the most sensitive pig model, a few milligrams of liposome (phospholipid) can cause anaphylactoid shock, characterized by pulmonary hypertension, systemic hypotension, decreased cardiac output and major cardiac arrhythmias. Pigs also display cutaneous symptoms, such as flushing and rash. The sensitivity of dogs to hemodynamic changes is close to that of pigs, but unlike pigs, dogs also react to micellar lipids (such as Cremophor EL) and their response includes pronounced blood cell and vegetative neural changes (e.g., leukopenia followed by leukocytosis, thrombocytopenia, fluid excretions). Rats are relatively insensitive inasmuch as hypotension, their most prominent response to liposomes, is induced only by one or two orders of magnitude higher phospholipid doses (based on body weight) compared to the reactogenic dose in pigs and dogs. It is suggested that the porcine and dog models are applicable for measuring and predicting the (pseudo)allergic activity of particulate "nanodrugs". PMID: 17613700 [PubMed - indexed for MEDLINE]

- ⁵ Winkler GC. Pulmonary intravascular macrophages in domestic animal species: review of structural and functional properties. *Am J Anat.* 1988;181(3):217-34.

Abstract: In dogs, laboratory animals, and man, the clearance of bacteria and particulates from blood occurs predominantly in hepatic Kupffer cells and splenic macrophages. In contrast, removal of blood-borne particulates in calves, sheep, goats, cats, and pigs occurs predominantly in pulmonary intravascular macrophages (PIMs). Review of recent studies indicates that PIMs are a resident cell population, junctionally adherent to the capillary endothelium of lungs and morphologically similar to hepatic Kupffer cells. PIMs are a pulmonary constituent of the mononuclear phagocyte system with respect to secretory, endocytic, and functional properties. Differentiated PIMs are rare in newborn pigs, and the majority of cells closely apposed to capillary endothelium consists of monocytes, which are occasionally in mitosis. In 7-day-old and older pigs, most cells apposed to capillary endothelium have characteristics of differentiated PIMs. This suggests a monocytic origin of PIMs in pigs. Perinatal colonization of lung capillaries by monocytes and their subsequent differentiation into PIMs represent a component of postnatal lung development. Estimates of relative PIM numbers in ovine and porcine lung parenchyma suggest cell densities similar to that of rat hepatic Kupffer cells. Apart from phagocytic properties, PIMs participate in the removal and disintegration of aged and impaired blood cells. After phagocytic

stimulation, isolated PIMs secrete oxygen radicals, which are essential for microbicidal function. Similarly, by secreting bioactive lipids, stimulated PIMs may contribute to regulation of pulmonary hemodynamics. After receiving minute amounts of bacterial endotoxin, pulmonary injury is pronounced in sheep, calves, pigs, and cats, but not in laboratory animals and dogs. This presumably is related to the secretion of bioactive lipids by PIMs. PMID: 3284325 [PubMed - indexed for MEDLINE]



SonoVue®
Reports Integrated USMD

Clinical Safety Experience with SonoVue

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1 Executive Summary

SonoVue® is an ultrasound contrast agent, originally developed by Bracco that is commercialized in Europe and Asia since 2001. The compound is not approved for use in any indication in the United States of America (USA).

The present document summarizes the safety and tolerability of SonoVue based on data from Bracco-sponsored clinical studies and post-marketing surveillance (PMS) data. A risk benefit analysis of the use of ultrasound contrast agents in different applications, with particular reference to SonoVue, is also provided.

Available data indicate that SonoVue is a very safe and well tolerated ultrasound contrast agent. In clinical trials the incidence of adverse events was low (all: 11.9%, 6.1% drug related; serious) 0.3%, 0.2% drug related). The most frequent drug-related events were headache (1.2%) and nausea (0.7%) that resolved in a short time.

Based on PMS data, the serious adverse event rate after SonoVue administration is 0.014% (1.4:10,000). The majority of serious events are allergy-like or cardiac. Their estimated rate is in the order of 0.8-1: 10,000 and 0.4:10,000, respectively. The death rate after SonoVue administration is of 7: 1 million subjects exposed. These figures compare favorably with the risk for serious or fatal events reported for Computer Tomography or Magnetic Resonance Imaging contrast agents and other imaging procedures.

Most of the serious cardiac events occurred within few minutes from SonoVue injection in connection with serious allergy-like reactions and they probably represent manifestations of allergy-like reactions rather than reflecting a cardiotoxic effect. The low prevalence and the temporal relationship of cardiac events to SonoVue administration render continuous ECG and oxygen saturation monitoring not useful to either prevent or precociously treat these events.

Contrast ultrasound today is the only imaging modality to assess hemodynamic microcirculation in real time reducing the need for more costly and invasive diagnostic procedures for cardiac function evaluation, focal liver lesions characterization, and evaluation of intracranial circulation.

Besides currently indications approved, contrast-enhanced ultrasound imaging promise to be an effective tool for assessment of myocardial perfusion, early assessment of response of malignant liver lesions to the application of local treatments and early assessment of response of several solid tumors to systemic treatment with targeted therapies. Bracco is planning to expand SonoVue in these and other indications.

Bracco believes that benefits deriving from SonoVue-enhanced US in the different approved indications and those expected from investigational applications by far outweigh related risks.

2 Introduction

SonoVue® is an ultrasound contrast agent, originally developed by Bracco that has been commercialized in Europe since 2001. It is characterized by a microbubble structure consisting

of a low solubility gas (sulfur hexafluoride) stabilized by a phospholipids shell. It remains in the intravascular space and strongly increases the ultrasound backscatter. Therefore, SonoVue is useful in the enhancement of blood echogenicity for the assessment of blood flow in the vasculature.

SonoVue has been approved in the European Union (EU) under the centralised procedure since 26 March 2001. It has been also registered in Norway, Iceland, Switzerland, China, Singapore, Hong Kong, South Korea, and Canada. The product is marketed in 21 countries in the following indications:

- Echocardiography - A transpulmonary echocardiographic contrast agent for use in patients with suspected or established cardiovascular disease to provide opacification of cardiac chambers and enhance left ventricular endocardial border delineation (2.0 mL for intravenous administration in bolus).
- Doppler of macrovasculature - Increases the accuracy in detection or exclusion of abnormalities in cerebral arteries and extracranial carotid or peripheral arteries by improving the Doppler signal-to-noise ratio. Increases the quality of the Doppler flow image and the duration of clinically-useful signal enhancement in portal vein assessment (2.4 mL for intravenous administration in bolus).
- Doppler of microvasculature - Improves display of the vascularity of liver and breast lesions during Doppler sonography, leading to more specific lesion characterization (2.4 mL for intravenous administration in bolus).

3 Safety of SonoVue in Clinical Studies

As of 30 September 2007, clinical experience with SonoVue encompassed 65 Bracco-sponsored completed studies including 158 healthy subjects and 4572 patients, 4440 of whom received SonoVue (5 mg/mL; mean cumulative volume 10.60 mL; maximum volume 161.3 mL) mostly as bolus injection (Table A).

Most of these studies were conducted in cardiologic indications (including delineation of endocardial borders and evaluation of myocardial perfusion in patients with ischemic heart disease) and for assessment of microvasculature for characterization of parenchymal lesions (including 15 studies in characterization of focal liver lesions [FLLs] and 3 in monitoring of response to local treatment of FLLs) (Table A). Special population studies were conducted in patients with congestive heart failure, chronic obstructive pulmonary diseases (COPD), and sarcoidosis.

Table A: Summary of Completed SonoVue Studies

Population	No. of Studies	No. of Treated Subjects		
		SonoVue ^a	Control Only	Total
All Completed Studies ^b	65	4568	162	4730
Healthy Volunteers ^c	8	128	30 ^d	158
All Patients	57	4440	132	4572
Cardiac Population	23	1714	126 ^e	1840
Macrovascular Population	9	555	0	555
Microvascular Population	22	2133	0	2133
Special Patient Populations	3	38	6 ^d	44

^a Received SonoVue only or SonoVue plus control in crossover studies; included as SonoVue subjects in all summary data displays.
^b Completed studies are those for which a final clinical trial report was available as of 30 September 2007.
^c Clinical PK and pilot efficacy studies in non-patient volunteers.
^d Saline.
^e Albunex/saline.

Table Source: Derived from Investigator Brochure (December 2007).

In addition to the above studies there are 6 ongoing Bracco-sponsored clinical studies including 766 subjects.

Safety information from completed Bracco-sponsored clinical trials indicates that SonoVue is very well tolerated (Table B).

Table B: Summary of Adverse Events in Completed Bracco-Sponsored Clinical Studies – All Patients

Category	SonoVue		Placebo/Comparator	
	No. of Patients Dosed: 4440		No. of Patients Dosed: 132	
	Related ^a	All	Related ^a	All
No. of adverse events ^b	441	866	29	53
No. (%) of patients with at least 1 adverse event	269 (6.1)	529 (11.9)	17 (12.9)	34 (25.8)
No. (%) of patients with at least 1 serious adverse event	3 (0.1)	21 (0.5)	0	1 (0.8)
No. (%) of patients who discontinued due to adverse events	6 (0.2)	15 (0.3)	0	0
No. (%) of deaths	0	7 (0.2)	0	0
No. of patients with at least 1 non-serious adverse events by intensity ^c	266 (6.0)	512 (11.5)	17 (12.9)	33 (25.0)
- No. (%) with mild adverse events	228 (5.1)	408 (9.2)	15 (11.4)	27 (20.5)
- No. (%) with moderate adverse events	37 (0.8)	96 (2.2)	2 (1.5)	6 (4.5)
- No. (%) with severe adverse events	1 (<0.1)	8 (0.2)	0	0

^a Includes definite, probable, possible, doubtful, unknown, and missing relationship to investigational product.
^b Includes all adverse events reported after the first administration of investigational product. Multiple occurrences of the same adverse event in a patient are counted individually.
^c If a patient experienced more than 1 non-serious adverse event, the patient was counted only once at the maximum intensity.

Table Source: Derived from IB (Appendix 3).

3.1 Non Serious Adverse Events

Following SonoVue administration the incidence of adverse events in patients was low (11.9%, 6.1% drug related) and not higher than that reported with comparator agents (11.9%, 6.1% drug related) (Table B). The most frequent drug-related events were general disorders, central nervous

systems and gastrointestinal systems and occurred with a frequency of 2.0%, 1.9%, and 1.0%, respectively (Table C).

Table C: Summary of Adverse Events Reported by $\geq 0.5\%$ of Patients in Completed Bracco-Sponsored Clinical Studies – All Patients

MedDRA System Organ Class ^a Preferred Term ^b	No. of Patients Dosed with SonoVue: 4440	
	N (%) Related ^a	N (%) All ^b
Number of Subjects with Adverse Events ^c	269 (6.1)	529 (11.9)
Gastrointestinal Disorders	45 (1.0)	97 (2.2)
Nausea	29 (0.7)	46 (1.0)
General Disorders and Administration Site Conditions	90 (2.0)	166 (3.7)
Chest Discomfort	15 (0.3)	29 (0.7)
Chest Pain	9 (0.2)	33 (0.7)
Feeling Hot	16 (0.4)	22 (0.5)
Injection Site Pain	16 (0.4)	22 (0.5)
Nervous System Disorders	86 (1.9)	162 (3.6)
Headache	52 (1.2)	101 (2.3)

^a Includes definite, probable, possible, doubtful, unknown, and missing relationship to investigational product.
^b Includes all adverse events reported after the first administration of investigational product. Multiple occurrences of the same adverse event in a patient are counted individually.

Table Source: Derived from Integrated Safety Database(as of September 30, 2007).

3.2 Serious Adverse Events

No serious adverse events or deaths were reported in the healthy volunteer population.

In patients, serious adverse events whose relationship with SonoVue could not be ruled out occurred in 3 out of 4440 patients (0.067%) including 1 case of sensory motor paresis (drug-relationship unknown), 1 case of chest pain associated with elevation of ST segment at electrocardiogram (ECG) and hypotension (drug-relationship unknown), and 1 case of skin rash associated with vasovagal syndrome (drug-relationship probable).

In addition, 1 drug-related serious adverse event (anaphylactic shock) has been reported in 1 out of 766 patients exposed to SonoVue in the 6 Bracco-sponsored studies ongoing as of the cut-off date.

3.3 Cardiovascular and pulmonary Effects

Two retrospective clinical studies assessing retrospectively validated 12-lead ECG data and of 2-lead Holter ECG data collected in different clinical studies did not show any detrimental modification of QTc in patients with CAD.

One study¹ analysed of 2-lead Holter ECG data obtained from 156 patients with suspect or known ischemic heart disease enrolled in 2 SonoVue pivotal clinical trials. This study indicated that SonoVue was not associated with an increased risk of prolonged cardiac repolarization as shown by no effect on the QTc interval during the first 15 minutes after injection as compared to saline or Albunex¹.

A second study² analysed retrospectively the findings from validated 12-lead ECG data obtained from 477 patients in 9 cardiac studies have not shown that SonoVue has any effect on QTc interval.

Two prospective, placebo-controlled safety studies did not show any detrimental effects on cardiac electrophysiology in patients with CAD.

In one prospective study³ 49 patients with diagnosis of coronary artery disease were administered with two dose regimen of SonoVue 0.1 or 0.5 mL/kg and placebo. The study demonstrated that SonoVue did not cause prolonged cardiac repolarization in patients with CAD undergoing B-mode echocardiography with a high mechanical index of 0.7 to 0.8.

- There was no statistically significant difference between SonoVue and placebo in the maximum increase from baseline in corrected QT interval following administration of study agent and no evidence of a dose-response relationship.
- Administration of SonoVue was not associated with any increased incidence of cardiac abnormalities as evaluated by quantitative and qualitative ECG parameters.

In the second prospective study⁴ 53 patients with diagnosis of coronary artery disease were administered with SonoVue 0.1 mL/kg or placebo while undergoing echocardiography using a low Mechanical Index (MI): 0.4 and high MI:1.5

Each subject underwent 4 echocardiographic examination according to a cross over design.. The results of the study support the following conclusions:

- Administration of SonoVue 0.1 mL/kg does not appear to cause prolonged cardiac repolarization in patients with CAD undergoing B-mode echocardiography using either low (0.4-0.5) or high (1.5-1.6) MI settings.
- There was no statistically significant difference between SonoVue and placebo in the maximum increase from baseline in corrected QT interval following administration of study agent during both low MI and high MI echocardiography.
- Administration of SonoVue during low MI and high MI echocardiography is not associated with any increased incidence of cardiac abnormalities including Premature Ventricular Contractions as evaluated by quantitative and qualitative ECG parameters.

Pulmonary circulation and cardiac function parameters were assessed during right heart catheterization in 19 patients with congestive heart failure (13 treated with 2.0 mL and 4.0 mL SonoVue and 6 with placebo)⁵ in a special population study. Measurements of pulmonary arterial pressure indicated comparable changes from baseline for SonoVue when compared with placebo and for the 2 SonoVue doses. There were no clinically significant changes from baseline in pulmonary circulation (mean Pulmonary arterial pressure - MPAP, pulmonary vascular resistance - PVR, Pulmonary capillary wedge pressure- PCWP) cardiac function parameters (left ventricular Ejection Fraction, Left ventricle end-systolic and end-diastolic volumes). One patient in the placebo group had a clinically significant worsening of systemic systolic arterial pressure. Small mean increases from baseline in systolic and diastolic blood pressure were observed in both the SonoVue and control groups. No clinically significant changes from baseline in ECG (12-lead and 3-lead) were observed in either the SonoVue group or the control group.

Two studies were performed in patients with pulmonary impairment.

In one study⁶, 12 patients with moderate or severe Chronic Obstructive Lung disease were administered with 4 mL single bolus injection of SonoVue and placebo. No effects were seen after SonoVue in pulmonary function(Mean FEV1(Forced Expiratory Volume in 1 Second),

FVC (Forced Expiratory Vital Capacity) and FEF25-75%(Forced mid expiratory flow rate), oxygen saturation, cardiovascular function and general safety in both moderate and severe Chronic Obstructive Lung disease patients which would raise safety concerns.

The second study⁷ was performed to assess the pharmacokinetic of 0.3 mL/kg of SonoVue in 13 patients with diffuse interstitial pulmonary fibrosis. The results of the pharmacokinetic analyses were consistent with those previously observed in healthy subjects. No serious event was reported.

3.4 Fatal Events

No drug-related deaths were reported within Bracco-sponsored trials.

4 Safety of SonoVue based on Post-Marketing Surveillance

Spontaneously reported serious and non-serious adverse events, regardless of the causal relationship, received in the period of **01 April 2001 - 30 September 2007** from the countries where SonoVue is marketed, were evaluated.

The adverse event terminology provided reflects the diagnosis or terminology used by the reporters.

Patient exposure was calculated on the basis of the number of 5-mL dose vials distributed from 01 April 2001 to 30 September 2007. Each unit distributed was considered to correspond to 1 patient exposed to the agent.

Based on the above assumptions, the estimated adverse event rate (serious and non serious) with SonoVue is 0.02% (2 patients out of 10,000 exposed) based on PMS data. The incidence of reported non-serious events is of 0.006% (0.6:10,000) and of serious events 0.014% (1.4:10,000). Clearly, non serious adverse events are underreported and their incidence underestimated based on PMS data. On the other hand, the actual incidence of serious adverse events might be lower than estimated in patients treated for liver imaging considering that frequently 2 patients per vial are treated rather than 1 as assumed.

The total number of subjects with adverse events over the last 6 years of marketing of SonoVue is described in Figure 1. The reporting rate ranged from 0.0130% (1.3:10,000) in the second year of marketing to 0.0254% (2.5:10,000) at the end of the fourth year of marketing. During year 2003-2004 there was an increased reporting of adverse events. As shown in Figure 1, this was related to an increased reporting of non-serious events, probably elicited by public discussions ongoing at that time with the European Health Authorities as regards 3 fatal events occurred after SonoVue administration (see Section 3.4). Actually, the reporting rate per year for serious cases remained low (ranging from 0.0130% to 0.0156%, or 1.3 - 1.56: 10,000) and has not changed in the last 6 years of Pharmacovigilance surveillance.

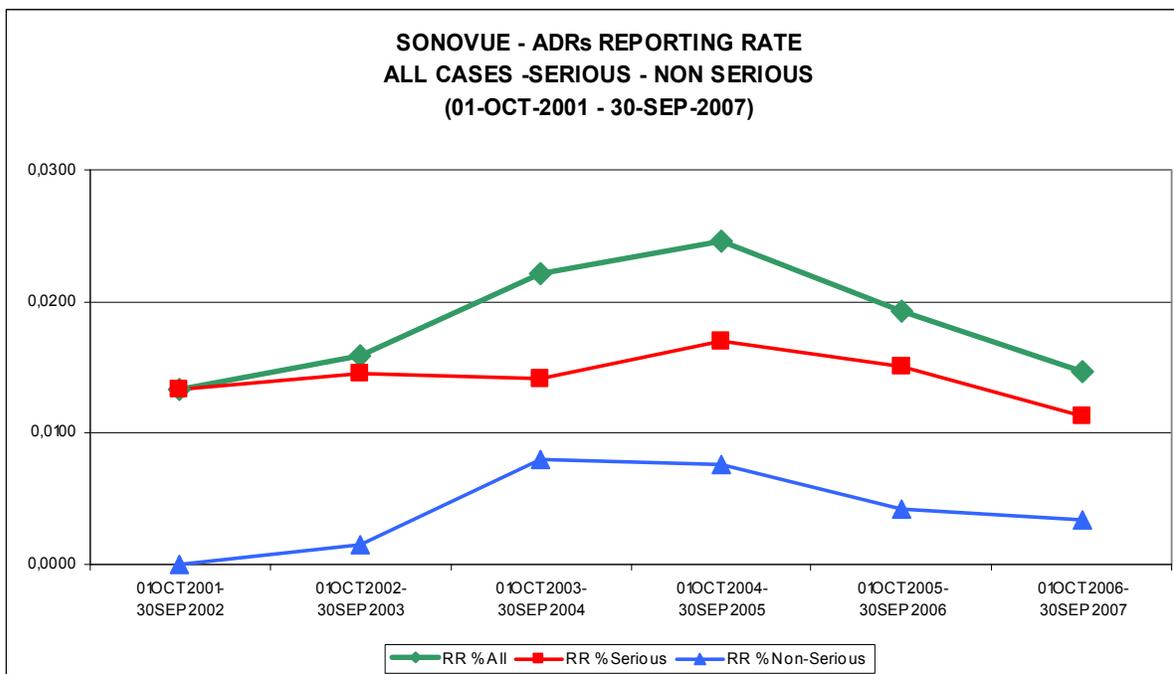


Figure 1. Reporting Rate of Adverse Events (Serious and Non-serious) in the Last 6 years of SonoVue Marketing

Reported events are summarized by MedDRA System Organ Class (SOC) in Table D.

Combining both serious and non-serious events, the most frequently reported events (incidence \geq 0.0050% or 1:20,000) were related to immune system disorders, and general disorders and administration site conditions.

Table D: PMS: Spontaneously Reported Adverse Reactions By SOC*

MedDRA System Organ Class	Percentage of Subjects with Events		
	Serious	Non-Serious	Total
Total No. of Subjects with Adverse Events	0.0141	0.0062	0.0203
Cardiac disorders	0.0043	0.0001	0.0045
Ear and labyrinth disorders	0	0.0001	0.0001
Eye disorders	0.0001	0.0002	0.0004
Gastrointestinal disorders	0.0006	0.0026	0.0032
General disorders and administration site conditions	0.0013	0.0045	0.0058
Immune system disorders	0.0078	0.0009	0.0087
Injury, poisoning and procedural complications	0.0001	0	0.0001
Investigations	0.0027	0.0007	0.0035
Musculoskeletal and connective tissue disorders	0.0003	0.0009	0.0012
Nervous system disorders	0.0033	0.0019	0.0048
Renal and urinary disorders	0.0001	0.0003	0.0004
Reproductive system and breast disorders		0.0001	0.0001
Respiratory, thoracic and mediastinal disorders	0.0020	0.0006	0.0026
Skin and subcutaneous tissue disorders	0.0019	0.0030	0.0049
Vascular disorders	0.0029	0.0009	0.0037

*Patients are counted once for each SOC; the same patient may be reported for more than one SOC

Table data derived from PMS database.

Serious adverse events were spontaneously reported in 0.0141% of exposed subjects (approximately 1.4:10,000). The most frequently reported serious events were related to immune system disorders and cardiac disorders.

4.1.1 Serious Allergy-like Reactions

All reported serious events under the immune system disorders SOC were allergic reactions including anaphylactic reaction/shock, anaphylactoid reaction/shock and hypersensitivity terms. Their incidence was estimated in the order of 0.0078% or approximately 0.8:10,000. In addition, according to Bracco's review of reported serious events, there were 19 additional patients with reactions classified under other SOCs (such as severe hypotension, clouded/loss of consciousness, dyspnea, bradycardia, tachycardia, sweating, cutaneous rash, skin redness) that could be as well considered allergic-like reactions. Adding these events, the estimated incidence of allergic-like reaction was in the order 0.0105% (1:10,000).

Allergy-like reactions represented 76% of the total serious adverse events cases reported with SonoVue; 13.7% of allergy-like reactions were diagnosed as anaphylactic shocks and one had fatal outcome.

In most cases (65 % of allergy-like reaction) the symptoms started within 5 minutes from the injection of the product.

No bronchospasm has been described thus far as a clinical component of initial symptoms, even if few cases of dyspnea were reported.

In 42.7% of allergy-like reactions, hypotension/drop of blood pressure was reported as first symptom. The severity of hypotension could range from a few mmHg reduction in systolic and diastolic blood pressure to unmeasurable values. Moreover, 60% of cardiac symptoms reported as serious events were associated to serious allergy-like reactions. The occurrence of cardiac events is described as part of anaphylactic reaction symptomatology and their manifestation

could be related to the acute hemodynamic misbalance caused by the allergy-like reaction or by the interaction of inflammatory mediators directly on the heart.

4.1.2 Serious Cardiac Reactions

Overall 0.0043% (0.4:10,000) of subjects exposed to SonoVue reported serious cardiac events.

Cardiac reactions represented 18.5 % of serious adverse events reactions reported with SonoVue.

Reported cardiac events included in decreasing frequency bradycardia, cardiac arrest, tachycardia, ventricular fibrillation, myocardial infarction, angina pectoris, complete atrio-ventricular block, cardio-respiratory arrest, cyanosis, arrhythmia, coronary arteriospasm, cardiac failure, and cardiac disorder not otherwise specified.

Overall, 22.5%, of cardiac reactions were diagnosed as cardiac or cardio-respiratory arrest and 10% as ventricular fibrillation. Four cardiac events had fatal outcome (see Section 4.1.4).

As shown in the Table E, 60% of serious cardiac events were associated to serious allergy-like reactions suggesting that cardiac events in these cases were themselves part of an allergic syndrome. In some of the remaining 40% of cases, a symptomatology that might be associated to allergy-like reactions was also reported though a definite diagnosis of anaphylactic reaction could not be established either by the reported nor according to Bracco’s criteria for diagnosis of anaphylaxis.

In no case vascular events such as thrombosis were reported.

Table E: PMS: Cardiac Adverse Reactions*

Events classified as Cardiac Disorders by MedDRA SOC	Non Cardiac Events Associated		
	NO	YES	
		Allergy-like Reactions According to the Reporter and/or Bracco	Other
Bradycardia (n= 9)		6 : anaphylactic shock (2) anaphylactoid reaction (2) hypersensitivity (2)	3: 1: hypotension, paresthesia, loss of consciousness, myocardial infarction, ventricular fibrillation (fatal event) ^o 1: hypotension 1: hypotension, chest pain, nausea
Cardiac arrest (n=7)	1 (fatal event)	4: anaphylactic shock (2) anaphylactic shock (fatal event) anaphylactic reaction	2: 1: loss of consciousness (fatal event) 1: angina, flushing, blood pressure decrease §
Tachycardia (n=5)		4: anaphylactoid reaction (2) hypersensitivity anaphylactic shock	1: nausea, heart rate decreased, cardiac disorder nos, visual disturbance, asthenia, hyperidrosis, hypotension #
Ventricular fibrillation (n=3)		2: anaphylactic shock anaphylactic reaction	1 ^o
Myocardial infarction (n=3)		2: anaphylactic shock (2)	1 ^o

Events classified as Cardiac Disorders by MedDRA SOC	Non Cardiac Events Associated		
	NO	YES	
		Allergy-like Reactions According to the Reporter and/or Bracco	Other
Angina pectoris (n=3)	1	1: anaphylactic shock	1§
Complete atrio-ventricular block (n=2)		1: hypersensitivity	1: hypotension and syncope
Cardio-respiratory arrest (n=2)		2: anaphylactic reaction anaphylactic shock	
Cyanosis (n=2)		1: hypersensitivity	1: feeling hot malaise, blood pressure decreased, cough
Arrhythmia (n=1)		1: hypersensitivity	
Coronary arteriospasm (n=1)			1: chest pain, nausea, abdominal pain, peripheral coldness
Cardiac failure (n=1)			1: acute pulmonary edema post anthracycline (classified as respiratory)
Cardiac disorder, nos (n=1)			1: #

**Patients are counted once for each event; the same patient may be reported for more than one event
 °, #, §: same symbol correspond to same patient
 Table data derived from PMS database.*

4.1.3 Other Serious Reactions

Apart from patients who developed allergic and/or cardiac reactions there were few cases of vagal syncope/syncope classified under nervous system disorders SOC and a single case of blood pressure reduction of 40-50 mmHg not associated to other symptoms.

4.1.4 Fatal Events

Fatal outcome was reported in 5 events (0.0007% or 7: 1 million exposed cases). A causal relationship between SonoVue administration and the fatal event could be reasonably established only for 1 case (Case BRO-011933), for an other case BRO-008552 the reporting physician excluded a causal relationship with SonoVue while the other 3 cases with fatal outcome were temporally related but not clearly casually attributable to SonoVue administration. Thus, the rate of drug-related deaths is estimated at 0.00029% or 3: 1 million exposed cases.

A brief summary of all deaths occurred after SonoVue administration is provided below.

1. A 61-year-old (Case BRO-008552) female suffering from lung cancer with cerebral metastases 9 hours after an uneventful SonoVue-enhanced ultrasound of the liver received morphine (10 mg/mL Subcutaneously) for abdominal pain and dyspnea. A few minutes after, she became unconscious and died. Though autopsy was not performed, the reporting physician excluded a causal relationship with SonoVue and considered

myocardial infarction or pulmonary embolism as possible causes based on patient's history of hypercholesterolemia and myocardial infarction.

2. A 65-year-old female cancer patient (Case BRO-011933) who was being investigated for liver metastases from colon cancer died due to a possibly drug-related anaphylactic reaction that occurred immediately after SonoVue injection.
3. A 69-year-old male (Case BRO-005943) with history of myocardial infarction died due to cardiogenic shock. On the second day after a stent implantation to the Left anterior descending coronary artery (LAD) and the diagonal branch he underwent a SonoVue-enhanced echocardiography. Five minutes after the second SonoVue administration he slowly developed bradycardia and hypotension, and then had ventricular fibrillation requiring defibrillation and cardiopulmonary resuscitation (CPR). He recovered and underwent a rescue angioplasty due to acute occlusion of the recently treated LAD stent. The patient was then transferred to intensive coronary unit with an intra-aortic pump and on the same day developed again bradycardia and a ventricular escape rhythm. The patient died despite specific treatment and CPR. All the clinical and autoptic elements indicated that the fatal outcome was not related with SonoVue but was attributable to an underlying LAD stent occlusion with ongoing myocardial infarction (as indicated by elevated troponin levels before SonoVue injection), which subsequently led to cardiogenic shock.
4. A 49-year-old male (Case BRO-006772) with history of multiple myocardial infarctions and left ventricular ejection fraction (LVEF) of 31% 1 month after an incomplete multiple percutaneous coronary interventions (PCI) underwent a SonoVue-enhanced echocardiography. When standing up from the examination table he lost consciousness and did not respond to immediate resuscitation maneuvers. Asystole was recorded by ECG followed by electromechanical dissociation and ventricular fibrillation. Though drug-relationship cannot be definitely ruled out, available data suggest that the patient was in unstable conditions and at high risk for a sudden coronary event, since he had severe coronary lesions still present, severe reduction in the LVEF, required oxygen saturation monitoring during echocardiography, had documented hypoxemia (Oxygen saturation: 92%), had high baseline heart rate (92 bpm) indicative for insufficient beta-blocker treatment.
5. A 51-year-old male (Case BCM-000767) with history of recent anterior myocardial infarction and primary PCI of the LAD, 40% stenosis of the left main coronary artery, moderate left ventricular dysfunction (LVEF 41%) with akinesia in the anterior apex died due to cardiac arrest. The patient approximately 2 minutes after SonoVue administration had a cardiac arrest preceded by throat burning and back pain. The cardiopulmonary resuscitation measures failed and the patient died. Though there is a strict temporal relationship between the event and SonoVue injection, the autopsy findings (showing 95% stenosis of the main stem of the LAD and no sign of allergic reaction) and the clinical picture of unstable cardiac disease (rapid ECG deterioration with development of bundle branch block) indicate that the patient was at increased risk for spontaneous death.

5 Discussion

Available data indicate that SonoVue is a very safe and well tolerated contrast agent.

In clinical trials the incidence of adverse events was low (all:11.9%, 6.1% drug related; serious) 0.3%, 0.2% drug related). The most frequent drug-related events were headache (1.2%) and nausea (0.7%) that resolved in a short time.

The estimated rate of serious events for SonoVue based on PMS data is 0.014% (1.4:10,000) and the incidence of serious allergy-like reactions was in the order of 0.078% (0.8:10000). Thus, SonoVue can be classified as a substance at low risk for inducing anaphylaxis according to The International Collaborative Study of Severe Anaphylaxis⁸ (Table F)

Table F: Incidence of Severe Anaphylaxis by Substance Class as Defined by the International Collaborative Study of Severe Anaphylaxis

Risk Category	Incidence	Substance Class
Low	0.005% - 0.015%	Analgesics Antibiotics
Medium	0.03% - 0.1%	Penicillin IV Blood Dextrane Pentoxifylline Iodine-Contrast Media
High	> 0.1%	Plasma Streptokinase

The risk for serious events after SonoVue administration is lower than that reported in medical imaging for iodinated contrast media (ionic agents: 0.16% or 1.6:1,000; non ionic agents: 0.03% or 3:10,000)⁹ and similar to that reported for MRI contrast agents (0.01 % or 1:10,000).^{10,11}

Most of serious events reported with SonoVue (76%) are allergy-like, the second most frequent serious events being cardiac (18.5 %). Their estimated rate is in the order of 0.8-1: 10,000 and 0.4:10,000, respectively.

The estimated death rate with SonoVue is 7: 1 million subjects exposed, a figure that compares favorably with the risk for fatal events reported for iodinated contrast agents that is approximately 1-3:100,000 contrast administrations.⁹

Available data suggest that most of the cardiac events reported after SonoVue administration are not related to SonoVue direct cardiotoxicity but rather may represent manifestations of allergy-like reactions similar to those described in literature after intravenous administration of liposomes and other lipid-based nanoparticles such as some liposomal drugs, diagnostic agents, and micelles.¹² These substances can cause acute hypersensitivity reactions in a high percentage (up to 45%) of patients, with hemodynamic, respiratory and cutaneous manifestations. The phenomenon has been explained with activation of the complement (C) system on the surface of lipid particles leading to anaphylatoxin (C5a and C3a) liberation and subsequent release reaction of mast cells, basophiles, and possibly other inflammatory cells in blood. This has been reproduced and studied in animal models including pigs, dogs, and rats. In the most sensitive pig model, a few mg of liposome (phospholipids) can cause anaphylactoid shock, characterized by

pulmonary hypertension, systemic hypotension, decreased cardiac out-put and major cardiac arrhythmia. Pigs also display cutaneous symptoms such as flushing and rash.

The hypothesis that a similar mechanism is responsible for cardiac reactions to SonoVue seems supported by preclinical studies carried out by Bracco in the intent of understanding the pathophysiology of this phenomenon (they are detailed in a separate document).

The clinical picture observed in humans developing allergy-like reactions is quite similar to that observed in the pig model. In this animal species a sudden rise of pulmonary resistance, pulmonary hypertension and consequent increased after-load of the right ventricle is observed within 1-2 minutes after SonoVue administration causing reduction of the pre-load of the left ventricle eventually producing a left ventricle failure. The systemic blood pressure reduction, observed in all cases, might be related to the left ventricle failure as well to a direct effect of the vasoactive substances released by pulmonary or cardiac macrophages. These hemodynamic reactions are associated with cardiac symptoms such as ventricular fibrillation, cardiac arrest, acute myocardial ischemia/infarction and/or severe arrhythmias and conduction abnormalities, all symptoms that have been reported in humans in association with allergy-like reactions to SonoVue.

Currently no experimental data are available to document in humans the allergic nature of cardiac reactions after SonoVue. Considering the extremely low incidence of the phenomenon in the clinical setting it is very difficult to plan a clinical study aimed at clarifying the underlying pathophysiologic mechanism. In 2004 a clinical trial was initiated aimed at identification of predictive factors for allergy-like reactions to SonoVue in subjects who had reported serious reactions to SonoVue compared to matching subjects. The organization of such a study was extremely complicated due to the fact that many of the clinical sites where the SonoVue reaction had occurred were not organized for research activity. Bracco was able to collect blood samples for biological measures only in a few subjects, the results were inconclusive, and ultimately the study had to be abandoned. *In vitro* studies are currently planned to investigate whether SonoVue activates serum complement and/or triggers activation of basophiles.

The low rate of serious and fatal events with SonoVue testifies to the safety of this agent, considering that SonoVue is frequently used in critically ill patients.¹³ A retrospective study recently published¹⁴ on 751 patients undergoing Dobutamine stress echocardiogram using either SonoVue (229 patients), Optison (120 patients) as compared to unenhanced stress echo (332 patients) has demonstrated that contrast ultrasound was not associated to an increased risk of side effects. In addition, recently a large retrospective analyses of hospitalized patients undergoing echocardiography with or without ultrasound contrast agents other than SonoVue has shown that contrast-enhanced examinations are not associated with an increased mortality risk, despite evidence for higher clinical acuity and more co-morbidity in patients undergoing contrast studies.¹⁵

It should be noted that the alternative imaging methods for cardiologic indications of ultrasound contrast agents are not without risks. The reported mortality rate for diagnostic coronary angiography ranges from 1:1,000 to 3: 10,000,^{16,17,18,19,20,21} and for transesophageal echocardiography is about 1:10,000.²² The risk for myocardial infarction or death for

echocardiography with exercise treadmill testing is approximately 1:2,500.²³ In cardiology, transesophageal echocardiography is performed to assess wall motion when a trans-thoracic examination is not feasible or convincing. With this technique the risk of upper gastrointestinal hemorrhage is in the range of 0.03% and the risk of esophageal perforation about 0.01%.^{22, 24} Stress-echocardiography (without contrast enhancement) may be inconclusive due to limited image quality in 15-30% of patients; but a non-diagnostic stress echocardiography poses the patient at a 1:1000 risk for a serious events including cardiac death.²⁵ In addition, a non-diagnostic stress-echocardiography is in many cases followed by further downstream diagnostic testing with stress-SPECT, which not only exposes the patient to a relevant amount of X-ray radiation but has a rate of life-threatening adverse events varying from 0.26% to 0.48%.^{26,27}

Bracco believes that the safety of SonoVue and of the other ultrasound contrast agents should be considered in view of the clinical benefit that the use of CE-US can provide in the every day practice. Contrast ultrasound today is the only imaging method to assess hemodynamic microcirculation in real time reducing the need for more costly and invasive diagnostic procedures for cardiac function evaluation, focal liver lesions characterization, and evaluation of intracranial circulation.

Contrast-enhanced echocardiography through the evaluation of the left ventricle opacification allows an accurate assessment of left ventricle endocardial border and constitutes a primary non-invasive method for the evaluation of regional and global left ventricular function. Measurement of left ventricular volumes and of left ventricular ejection fraction (LVEF), as well as the observation of left ventricular wall motion, either at rest or during stress, provides important diagnostic clues about the condition of the cardiac muscle and of the coronary circulation.²⁸

Since the introduction of ultrasound contrast media, various technical improvements have been introduced in ultrasound cardiac imaging to optimize the assessment of cardiac function, from B-mode to tissue harmonic imaging. Despite these improvements, contrast enhancement is still required in 10-15% of patients undergoing echocardiography to achieve a definite diagnosis.^{29,30} Moreover, using harmonic imaging, beside cardiac function information, it is possible to reliably assess the status of myocardial perfusion.³¹

The accurate assessment of regional and global cardiac function is an important prognostic factor in patients with chronic ischemic disease, heart failure, cardiomyopathy and valvulopathies. These cardiac diseases require a precise cardiac function evaluation for selection of the most appropriate therapeutic approach and for assessing the impact of the different treatments on the progression of the disease, allowing the cardiologist to tailor the treatment specifically on the patient need.

LVEF is one of the main determinants for decisions on specific pharmaceutical treatment (e.g. for initiation or dose adjustments of ACE-inhibitor therapy, beta blockers, anticoagulation or for assessment of cardiotoxicity of cancer treatments) or for deciding on prophylactic implantation of an Implantable Cardioverter Defibrillator in post-myocardial infarction patients.

SonoVue has shown to improve the delineation of the endocardial border in patients with “endocardial drop-out”, i.e. with impaired trans-thoracic image quality and failure to visualize the endocardial border in one or more segments of the left ventricle.^{32,33,34} In controlled clinical studies, 59% to 97% conversion rates from non-diagnostic to diagnostic images were achieved with SonoVue administration, depending on the offsite reader.³⁵

In a recent European multicenter-study^{36,37} in 120 stable ischemic patients the assessment of LVEF and cardiac volumes on unenhanced and SonoVue-enhanced echocardiography were compared to invasive cardioangiography (in all patients) and cardiac MRI (in a subset of 55 patients). Unenhanced echocardiography resulted in an underestimation of LVEF and cardiac volumes with only moderate correlation to the data obtained by cineventriculography or magnetic resonance imaging, while SonoVue-enhanced echocardiography resulted in a more accurate measurement of cardiac volumes and LVEF and improved correlation with the reference methods. SonoVue enhanced echocardiography also significantly improved inter-observer agreement between two blinded off-site readers as compared to unenhanced echocardiography.

Apart from the improved assessment of LVEF, the contrast opacification of the left ventricle leads also to an improved detection and delineation of space-occupying masses within cardiac chambers, such as thrombi and/or tumor. Up to 20% of all ischemic strokes are considered to be the result of emboli from the heart. Transthoracic and transesophageal echocardiography have been the principal diagnostic tools for detecting associated cardiac abnormalities and for guiding medical and surgical approaches to these patients. In case of good echocardiographic window, transthoracic echocardiography may have a sensitivity and specificity around 95% in the detection of intracardiac thrombi.^{38,39} However, in case of suboptimal imaging conditions (near-field clutter or reverberation artifacts) no reliable diagnosis can be obtained unless ultrasound contrast agents are used.⁴⁰ In patients with left ventricle thrombus the use of contrast not only improves the diagnostic accuracy of echocardiography but also allows monitoring the efficacy of anticoagulant therapy.

Use of transthoracic echocardiography in critically ill patients is often limited by suboptimal images resulting from mechanical ventilation, use of bandage, lung disease, subcutaneous emphysema, chest tubes, inadequate cooperation and inability to position the patients in left lateral decubitus position. The benefit of using harmonic imaging combined with intravenous contrast media was assessed in 50 mechanically ventilated patients, where conversion of non-diagnostic to diagnostic imaging occurred in 85% of patients with contrast echocardiography versus 15% of patients with tissue harmonic alone⁴¹.

In addition, the extent of microvascular damage as assessed with myocardial contrast echocardiography in patients with acute ST elevation myocardial infarction has been identified as the most powerful independent predictor of left ventricle remodelling.^{42,43,44}

Stress-echocardiography is indicated in clinically stable patients to investigate the presence of a hemodynamically relevant coronary artery stenosis. The accuracy of stress echocardiography rests on the correct interpretation of wall motion abnormalities. Excessive chest wall motion during hyperventilation and cardiac translational movement during tachycardia alter image quality during stress, and non diagnostic echocardiograms have been reported in up to 30% of patients.^{45,46,47}

SonoVue has been demonstrated to significantly improve the endocardial border delineation during stress echocardiography in patients with suspected or known coronary heart disease.^{48,49,50}

Beside cardiological indications, the clinical utility of ultrasound contrast agents and particularly of SonoVue has been established in different applications including visualization of

microvasculature for characterization of focal liver lesions (FLL) and visualization of macrovasculature (intracranial circulation, carotid, aorta, peripheral vessels).

Imaging plays a vital role in the diagnosis of liver cancer and of liver metastases/recurrences. The recognition of a liver lesion as a metastatic focus or local recurrence may significantly influence the patient's treatment and prognosis. Unenhanced US has variable and often low sensitivity (28-78%) and specificity (23%-92%) for FLL characterization.

As no ultrasound contrast agent is approved for this indication in the U.S.A., liver masses are usually further investigated in the U.S.A. using CE-CT and/or CE_MRI or biopsy.

In Europe and Asia, where SonoVue is registered for this indication, contrast-enhanced ultrasonography (CE-US) is extensively used for FLL characterization. Data from ex-USA experience indicate that when used for liver imaging CE-US has a sensitivity and specificity superior to that of unenhanced-US and at least similar to that of CE-CT and MRI imaging representing a reliable alternative to CE-CT /MRI for identification of HCC lesions in patients with chronic liver diseases ^{51, 52, 53, 54}

Indeed the European Federation Society for Ultrasound in Medicine and Biology (EFSUMB) guidelines have provided since 2004 Ultrasound contrast agents imaging patterns that characterize the different benign and malignant lesions which mainly superimpose to the patterns used by CE-CT or CE-MRI. ^{55, 56} In addition, the American Association for Study of Liver Diseases (AASLD) in 2005 has indicated CE-US as a key imaging modality for diagnosis of HCC. ⁵⁷

Several publications (more than 35) provide evidence for the diagnostic value of SonoVue-enhanced US in the characterization of FLL. ^{58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68} including incidental findings on routine unenhanced ultrasound, ^{69, 70} lesions or suspected lesion in patients with chronic hepatitis or liver cirrhosis ^{71, 72, 73, 60} or with known history of malignancy. ^{74, 75,}

In the 7 studies comparing SonoVue enhanced Vs. unenhanced US ^{59, 76, 52, 77, 66, 68, 78} CE-US demonstrated a higher accuracy and a much lower variability than unenhanced imaging: reported sensitivity and specificity ranges were of 81%-100% and 78% -96%, respectively, for SonoVue as compared to 28%-78.4% and 22%-92%, respectively, for unenhanced US. In the 6 studies comparing CE-US to CE-CT, ^{79, 80, 81, 82, 83, 84} CE-US demonstrated a sensitivity and specificity comparable to those of CE-CT in characterizing FLL, with concordance rates higher than 85%.

In addition, two large, prospective, multicenter trials have been presented at the European Congress of Radiology 2008 providing further evidence in favor of use of SonoVue-enhanced ultrasound in characterization of FLLs. The STIC study ⁸⁵ is a multicenter protocol that enrolled 878 subjects and that was sponsored by the French Ministry of Health mainly to assess the pharmaco-economic aspects of contrast-enhanced ultrasound use. It compared contrast-enhanced ultrasound versus unenhanced ultrasound using as final diagnosis i.e. a combination of histology, CT/MRI imaging, and clinical data as truth standard. Based on a blinded off-site review, contrast-enhanced ultrasound had an overall sensitivity of 79.5% and a specificity of 88.1%. The second study ⁸⁶ was conducted by the German Society for Ultrasound in Medicine (DEGUM) and included 1349 patients of whom 1006 had histology as the reference true standard. In this study contrast-enhanced ultrasound sensitivity was 95.8% and specificity 83.1%.

Furthermore, a recent retrospective analysis including 23,188 abdominal SonoVue-enhanced US provides further evidence of the safety of the compound in clinical practice.⁸⁷ No fatal event occurred. AEs were reported in 29 cases, of which only two were classified as serious (dyspnea, bronchospasm, hypotension and bradycardia in a patient with prostate cancer; clouding of consciousness, dorso-lumbar pain, non measurable pulse, severe hypotension, and skin rash in a patient with occlusion of a renal artery stent due to thrombosis). Thus, the rate of subjects with serious adverse events in this study in abdominal applications was 0.0086%.

CE-US has several advantages over other imaging modalities when used for FLL characterization. It is a real-time imaging technique, readily and widely available that allows immediate differential diagnosis between benign and malignant lesions avoiding second patient referral for diagnosis and patient's psychological discomfort related to diagnostic uncertainties, particularly when malignant disease is suspected. CE-US examinations can be repeated without concerns related to radiation exposure or to toxicity from CT iodinated and MRI contrast agents (e.g. nephrotoxicity) and, as previously discussed, the risk for allergic reactions is lower as compared to CE-CT. CE-US also represents an alternative modality for patients with contraindication for other imaging modalities, e.g. with contraindications to iodinated contrast agents (renal insufficiency, thyroid disorders) or suffering from claustrophobia. Costs related to the procedure are relatively low as compared to other imaging techniques.

Doppler sonography is the most widely used modality for the investigation of macrovasculature.^{88,89,90} When compared with angiography, still the gold standard for vasculature investigation, Doppler sonography has many advantages: it is non-invasive, it lacks ionizing radiation, it is well accepted by the patient and it is relatively low-cost. However, a number of factors may preclude a full Doppler US examination of a vessel; heavily calcified plaque causes acoustic shadowing, overlying bowel gas obscures the artery under investigation, a deep-seated artery returns a poor echo-signal and vessel tortuosity precludes a satisfactory Doppler angle for accurate velocity measurements.^{91,92,93,94} In case of suboptimal image quality, there is no alternative but to seek confirmatory evidence of arterial disease with conventional angiography and increasingly with CT angiography and MR angiography. All these modalities are less available and not devoid of risk of serious ADRs, as previously discussed^{95, 23,22}

The use of SonoVue in this indication facilitates visualization of difficult arteries, with remarkable improvements in the diagnostic agreement with conventional angiography, MR angiography or CT angiography, thus overcoming inherent problems associated with ultrasound, and ultimately reducing unnecessary invasive and expensive diagnostic procedures.^{96,97}

SonoVue enhanced ultrasound has been proved to be clinically useful in transcranial Doppler analysis of intracerebral vessels⁹⁸. This assessment is particularly useful in patients with acute stroke to decide on administration of thrombolytic therapy and in monitoring the effect of the thrombolytic therapy.^{99,100,101} In this application no serious adverse event has been reported thus far during PMS and the data have been reported indicating no blood brain barrier damage.¹⁰²

Published experience also supports the use of CE-US to assess changes of FLL microcirculation following the application of either physical or pharmacological local treatments with accuracy comparable to that of CE-CT or CE-MRI.^{103,104,105,106,107,108,109,110,111}

More recent data suggest that CE-US may also be a safe and effective tool for monitoring tumor response to systemic anticancer treatment with targeted therapies, including antiangiogenic

agents, in several tumor types including sarcomas, melanoma, hepatocellular carcinoma (HCC), gastrointestinal soft tissue sarcoma (GIST), and renal cell cancer (RCC). This imaging technique has been reported to predict tumor response to targeted therapy as early as 1 week after therapy initiation, before any change in tumor size, and to allow early detection of secondary resistance. Correlation of response as evaluated by CE-US with progression free survival has been reported in selected tumor types (HCC, GIST, and RCC).^{112,113,114,115,116}

Bracco is planning to expand SonoVue indications beyond the one currently approved in several countries by conducting studies in additional applications including liver imaging for monitoring response to local and systemic anticancer treatment, assessment of myocardial perfusion, and guidance of prostate biopsy for prostate cancer diagnosis. Bracco expects that CE-US can provide an important clinical benefit in each of these indications, including precocious assessment of the therapeutic response to local and systemic antitumor therapies based on vascular changes in tumor lesions, improved accuracy in the assessment of patients with ischemic heart disease, improved prostate cancer detection rate by guided biopsy.

6 Conclusions

In conclusion, available data indicate that SonoVue is a very safe and well tolerated ultrasound contrast agent. The risk for allergy-like reactions to SonoVue is not higher than that reported with CT or MRI contrast agents and the mortality rate is much lower than that reported for CE-CT.

The rate of cardiac events reported with SonoVue is extremely low. Serious cardiac events mostly occurred in connection with serious allergy-like reactions and they probably represent one of the manifestations of the latter. The low prevalence and the temporal relationship of cardiac events to SonoVue administration (onset within few minutes from injection) render continuous ECG and oxygen saturation monitoring not useful to either prevent or precociously treat these events.

Bracco believes that benefits deriving from SonoVue-enhanced US in the different applications by far outweigh related risks.

7 References

1 Final Clinical Trial Report BR1-111 Blinded validation of 12-Leads ECG Tracing for QTc from patients participating in SonoVue™ Studies. An extended summary of this study is provided in attachment 1

2 Final Clinical Trial Report BR1-109. Blinded validation of Holter Tracing for QTc measurements from patients participating in SonoVue™ Studies 19A/B. An extended summary of this study is provided in attachment 1.

3 Final Clinical Trial Report BR1-112 A single-blind, placebo controlled, randomized, three way crossover, phase III clinical study to evaluate the effect of single injections of 0.1 mL/kg and 0.5 mL/kg of SonoVue™ on cardiac electrophysiology in coronary artery disease patients volunteers An extended summary of this study is provided in attachment 1.

4. Final Clinical Trial Report BR1-113 A single-blind, placebo controlled, randomized, four-way crossover, phase III clinical study to evaluate the effect of ultrasound exposure and SonoVue on cardiac electrophysiology in coronary disease patient volunteers An extended summary of this study is provided in attachment 1.

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- 5 Final Clinical Trial Report BR1-016. evaluation of the effects of the intravenous administration of SonoVue (BR1) on pulmonary and cardiac haemodynamic in patients with congestive heart failure and pulmonary hypertension. An extended summary of this study is provided in attachment 1.
- 6 Final Clinical Trial Report BR1-022 A phase II, single blind, fixed single intravenous dose study to evaluate the safety and tolerability of SonoVue™ (BR1), a contrast enhancing agent for ultrasonography in patients with chronic obstructive pulmonary disease and <70% of predicted forced expiratory volume in one second, or FEV1. An extended summary of this study is provided in attachment 1.
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EVALUATION OF THE EFFECTS OF THE INTRAVENOUS ADMINISTRATION OF SONOVUE™ (BR1) ON PULMONARY AND CARDIAC HAEMODYNAMICS IN PATIENTS WITH CONGESTIVE HEART FAILURE AND PULMONARY HYPERTENSION

Summary and Conclusions:

Demographics: The study population was mainly either white (47.4%) or Asian (36.8%) and male (94.7%); mean age was 62.9 years (SD = 9.68). The most common cardiovascular abnormalities at screening were congestive heart failure (100%), ejection fraction <45% (94.7%) and previous myocardial infarction (52.6%).

Safety Results: The mean maximum changes from baseline in MPAP as well as in the other haemodynamic parameters were of no clinical significance and the magnitude of changes following administration of SONOVUE and placebo were comparable. In general, there were slightly larger decreases in the placebo group which could possibly be explained by the higher mean baseline values, reflecting the higher percentage of patients (50.0% versus 30.8%) with congestive heart failure NYHA functional class III in the placebo group. No dose-dependent effect of SONOVUE on haemodynamic parameters was apparent. Derived values of PVR confirmed the concern that this method may not have been adequate in assessing the primary variable, due to technical limitations preventing simultaneous measurements of MPAP and PCWP as was required in the derivation of PVR. This limitation is reflected in the high variability of the PVR values and in the fact that for eight patients at least one of the calculated PVR values was ‘clinically impossible’. However, MPAP, the alternative indicator of adequacy of pulmonary haemodynamics, was not affected by the limitations of the method used. The number of clinically significant worsenings from baseline in haemodynamic parameters was the same for the two study agents (SONOVUE: two; placebo: two). None of these changes persisted beyond the planned observation period of 10 minutes. No dose effect was observed; clinically significant worsenings occurred in two patients following administration of 2 mL and 4 mL of SONOVUE and in two patients following administration of 2 mL of placebo. With the exception of systolic systemic arterial pressure (one patient in the placebo group) only pulmonary pressure values showed clinically significant worsenings from baseline. The direction of change (increase or decrease) did not appear to be related to the dose or the study agent. The decreases in pulmonary pressure values were not accompanied by a pronounced reduction of systemic arterial pressure or compensatory increases in heart rate.

No serious adverse events were reported. Three adverse events occurred prior to injection of the study agents and six adverse events occurred in three patients after injection of the study agent, two in the SONOVUE plus placebo group and one in the placebo only group. One patient in the SONOVUE plus placebo group, who had a history of chronic renal impairment and

hypertension, experienced four adverse events (increased creatinine, urea, and uric acid, and hypotension), all of which were non-serious and of mild intensity and considered to be of unknown relationship to the study agents. Another patient in the SONOVUE plus placebo group reported one mild adverse event (right shoulder pain) which was not considered to be related to the study agent. One patient in the placebo group experienced pain at the injection site, which was considered by the investigator to have a probable relationship to the study agent, ie, placebo. No difference was seen between the tolerability of SONOVUE and placebo injections. One patient receiving 2 mL of SONOVUE and one patient receiving 2 mL of placebo had an episode of mild pain at the injection site. Episodes of local heat sensation did not occur. No changes in the physical status at the 24-hour follow-up were reported in any patients in either study group. Systolic and diastolic blood pressure changes from screening to follow-up were not clinically significant. No clinically significant changes in the ECG pattern were observed in either study group. With the exception of the one patient described above (increased creatinine, urea and uric acid) and one patient who had an improvement in potassium levels, there were no clinically significant changes in laboratory values.

Efficacy Results: No myocardial contrast activity was observed following administration of placebo in either study group. Administration of SONOVUE resulted in myocardial contrast activity during intermittent second harmonic B-mode imaging with a mean apical four-chamber view score value of 5.7 (2 mL SONOVUE) and 6.5 (4 mL SONOVUE). Due to the small number of patients, no dose response can be claimed. Only one non-responder was observed who received a dose of 4 mL of SONOVUE. Mean duration of contrast activity in the myocardium was 2.5 minutes (2 mL SONOVUE) and 2.6 minutes (4 mL SONOVUE) in patients with detectable contrast activity. The incidence as well as duration of shadowing increased with the SONOVUE dose. A better definition of coronary artery disease was seen following administration of SONOVUE, with the number of patients increasing with the dose. In both study groups all echocardiographic assessments resulted in consistently higher mean values of the left ventricular ejection fraction compared to the ones obtained with radionuclide angiography (MUGA).

Conclusion: The results of this study demonstrated that SONOVUE:

- showed no apparent risk associated with the use of single doses of 2 or 4 mL, with a cumulative dose of 6 mL, in patients with congestive heart failure of NYHA functional class II or III, with or without pulmonary hypertension as shown by:
 - a) a lack of effect on the pulmonary circulation and cardiac function;
 - b) lack of effect on the electrocardiographic pattern;
 - c) a lack of effect on the laboratory tests;
- had myocardial contrast activity at both doses of SONOVUE.

A PHASE II, SINGLE BLIND, FIXED SINGLE INTRAVENOUS DOSE STUDY TO EVALUATE THE SAFETY AND TOLERABILITY OF SONOVUE™ (BR1), A CONTRAST ENHANCING AGENT FOR ULTRASONOGRAPHY IN PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE AND <70% OF PREDICTED FORCED EXPIRATORY VOLUME IN ONE SECOND, OR FEV₁

Summary and Conclusions:

Demographics: The safety population was 100% white and predominantly male (66.7%) with a mean age of 67.8 (SD=10.65) years. After excluding respiratory abnormalities, 66.7% of patients had at least one medical history abnormality; the most commonly reported being cardiovascular (58.3%). At entry into the study, 50% had severe COPD (FEV₁ which was between 27% and 46% of predicted FEV₁) and 50% of patients had moderate COPD (FEV₁ which was between 51% and 69% of predicted FEV₁).

Safety Results: Mean FEV₁, FVC and FEF_{25-75%} levels decreased from baseline after SONOVUE and after placebo; no statistically significant differences between the study agents were observed and the results observed for the two subgroups, defined by severity of COPD at entry into the study, were similar to those observed over all patients. The percentages of patients having a substantial worsening (a decrease of at least 15% in FEV₁ or a decrease of at least 340 mL in FVC) were similar for both study agents over the post-injection time points.

The range of mean changes from baseline in oxygen saturation were -1.3% to 0.9% following SONOVUE and -1.3% to 1.0% following placebo administration; no trends in the mean changes from baseline at each post-injection time point were observed for either study agent. No substantial changes from baseline in oxygen saturation were observed for either study agent at any post injection time point. At any one post-injection time point, no more than two patients following SONOVUE and no more than one patient following placebo administration, had a marked abnormality decrease.

The mean duration of contrast effect following SONOVUE administration, was 6.5 minutes across all patients, 5.9 minutes in the patients with severe COPD and 7.1 minutes in the patients with moderate COPD.

Overall, a total of nine out of 12 patients reported 20 adverse events, when considering the adverse events reported after first injection, second injection and follow-up, ie, 24 hours after last administration of study agent. All the adverse events were of mild intensity and resolved without any sequelae. No deaths or other serious adverse events occurred and no adverse events led to discontinuation.

Three out of six patients who received SONOVUE as the first injection reported six adverse events: two of these (mild vomiting and mild lethargy) which were recorded in one patient in the severe COPD group were considered to be related to the study agent. Three out of six patients who received placebo as first injection reported seven adverse events: two of these (mild supraventricular tachycardia in one patient in the severe COPD group and mild tingling at injection site in another patient of the moderate COPD group) were considered to be related to the study agent.

Four patients in the moderate COPD group reported eight adverse events, of which three were considered possibly related and one of unknown relationship to the study agent. Five patients in the severe COPD group reported 12 adverse events, of which five were considered possibly related and one of unknown relationship to the study agent.

No difference was seen in the tolerability of SONOVUE and placebo injections. A total of two patients reported local pain at the injection site (one in each group) five minutes after injection. One patient reported severe pain after the SONOVUE injection and the other one reported mild pain after the placebo injection.

Cardiac safety parameters, ie, heart rate and systolic and diastolic blood pressure from pre-injection to 5 minutes and 1, 5 and 24 hours after the injection, 12-lead ECG and continuous ECG tracings up to 60 minutes after the injection, did not show any clinically significant changes in any patients following injection of SONOVUE or placebo at any post-injection time point. One patient had a supraventricular tachycardia after placebo.

No changes in the physical status at the 24-hour follow-up were reported in any patients.

Two patients were considered by the investigator to have clinically significant laboratory abnormalities, however, these abnormalities were not reported as adverse events. No other haematology, serum chemistry or urinalysis changes reported at the 24-hour follow-up were considered to be of clinical significance.

In conclusion, no effects were seen after SONOVUE in pulmonary function, oxygen saturation, cardiovascular function and general safety in both moderate and severe COPD patients which would raise safety concerns. The effects of 4 mL of SONOVUE were comparable to those observed following the administration of the same volumes of physiological saline. Therefore, an effect of SONOVUE on pulmonary function in this at risk population appears unlikely.

Conclusion: The results of this study have demonstrated that a single 4 mL injection of SONOVUE in patients with moderate or severe COPD:

- is safe and well tolerated as shown by:
 1. a lack of effect on the pulmonary function tests and oxygen saturation;
 2. a lack of effect on the electrocardiographic pattern;
 3. a lack of effect on the laboratory tests.
- has a mean duration of contrast activity which is in agreement with that observed in previous studies in patients with non compromised lung function.

A PHARMACOKINETIC AND SAFETY STUDY OF A BOLUS DOSE OF SONOVUE™ (sulfur hexafluoride microbubbles) IN PATIENTS WITH KNOWN DIFFUSE INTERSTITIAL PULMONARY FIBROSIS

Summary and Conclusions:

Demographics: 13 patients received SONOVUE (8 male, 5 female). Ages ranged from 36 to 80 years (mean age, 55.6 years). The majority of patients were white (85%) and had mild to moderate pulmonary impairment (85%).

Pharmacokinetic Results: Blood concentrations of SF6 declined bi-exponentially following peak concentrations. Maximum concentrations (C_{max}) ranged 10-fold from 0.35 to 3.79 µg/L and occurred between 0.97 and 4.0 minutes after administration of SONOVUE. Terminal half-life values ranged from 1.54 to 29.1 minutes, but 8 of 12 patients had terminal half-life values less than 15 minutes. Apparent blood clearance of SF6 appeared to decrease with increasing severity of pulmonary impairment; the correlation was statistically significant (p<0.05) for absolute blood clearance but not body-weight normalized clearance values. Non-parametric analysis of SF6 concentrations in expired air, ie, rate of excretion versus time, indicated elimination half-lives ranging from 17.1 to 75.6 minutes with a mean of 31.3 minutes. The actual percentage of the dose recovered as SF6 in the expired air ranged from 69.7% to 128.7% with an overall mean value of 102.2%.

Blood pharmacokinetic parameters are summarized in the following table:

Parameter	C _{max} (µg/L)	T _{max} (min)	AUC _{0-∞} (µg·min/L)	λ _z (min ⁻¹)	t _{1/2} (min)	t _{1/2,λ1} (min)	CL/F (L/min)	V/F (L)
N	12	12	12	12	12	12	12	12
Mean	1.45	2.19	5.87	0.1257	11.64	0.86	342.0	4045
SE	0.30	0.24	1.17	0.0358	2.66	0.12	68.5	943
SD	1.03	0.82	4.04	0.1241	9.23	0.40	237.2	3268
Median	1.25	2.03	4.40	0.0871	8.57	0.80	316.7	3190
Minimum	0.35	0.97	1.49	0.0238	1.54	0.37	86.8	1438
Maximum	3.79	4.00	15.08	0.4495	29.09	1.71	826.1	12761

C_{max} = maximum observed blood concentration; T_{max} = time of maximum observed blood concentration;
AUC_{0-∞} = area under the blood concentration versus time curve from time zero to infinity; λ_z = apparent blood terminal elimination rate constant; t_{1/2} = terminal half-life; t_{1/2,λ1} = distribution half-life; CL/F = apparent total body clearance; V/F = apparent volume of distribution.

Pharmacokinetic parameters from expired air data based on non-parametric analysis are summarized as follows:

Parameter	Amount (SF ₆) excreted to infinity (µg)	Percent (%) of dose recovered in expired air	λ_{z-1} (min ⁻¹)	t _{1/2,z} (min)
N	12	12	12	12
Mean	1407.9	102.2	0.0261	31.32
SE	77.0	5.32	0.0026	4.85
SD	266.8	18.4	0.0090	16.80
Median	1452.1	108.2	0.0260	26.68
Minimum	885.0	69.7	0.0092	17.06
Maximum	1827.7	128.7	0.0406	75.57

λ_z = elimination rate constant; t_{1/2,z} = elimination half-life.

Safety Results: Two patients (15.4%) experienced three adverse events: mild chest pain and pharyngitis (unknown relationship to SONOVUE) and mild headache (unrelated). There were no serious adverse events. Two patients had clinically significant changes in ECG (premature ventricular contractions). There were no clinically meaningful trends observed for physical examination, vital signs, oxygen saturation, or clinical laboratory results.

Conclusions: A dose of 0.3 mL/kg SONOVUE was safe and well-tolerated in patients with diffuse interstitial pulmonary fibrosis. The results of the pharmacokinetic analyses were consistent with those previously observed in healthy subjects (study BR1-010) and support the following conclusions:

- Blood concentrations of SF₆ and pulmonary excretion rates declined bi-exponentially following intravenous administration of SONOVUE;
- Apparent blood clearance values appeared to decrease with increasing severity of pulmonary impairment;
- The administered dose of SONOVUE was recovered in the expired air as SF₆;
- Dosage adjustment would not appear to be necessary in patients with pulmonary fibrosis.

BLINDED VALIDATION OF HOLTER TRACINGS FOR QTc MEASUREMENTS FROM PATIENTS PARTICIPATING IN SONOVUE™ STUDIES 19A/B

Summary and Conclusions:

Demographics: The majority of patients included in the 2-lead Holter ECG analysis were white men between 22 and 80 years old.

Evaluability of Patients: Holter ECG tracings were validated for 159 of the 264 patients. Complete recordings were excluded from the analyses for 105 patients (total exclusions). Reasons for total exclusions included but were not limited to technical problems/inadequate tracings, flattened T waves, IVCD >120 msec, and non-sinus rhythm. Thirtysix patients with validated Holter data had partial exclusions. Holter data for 3 of the 159 patients with validated Holter data were excluded from the analyses (for 1 patient the date of the Holter recording could not be matched to the dosing information, and 2 patients had no baseline ECG data). One additional patient who received saline had no ECG data during the first 15 minutes after the first injection; Holter data at baseline, 1 hour post dose, and during the last 5 minutes on Holter were included in the analyses.

Two-lead Holter ECG Results:

Maximum Increases in QTc Interval That Met the Criteria of Potential Clinical Importance:

The percentages of patients with maximum increases in QTc interval (Bazett's formula) of 31 - 60 msec at any 1-minute timepoint after the first injection of study agent were similar for the 3 study agent groups: 4.8% (4/84) for SonoVue, 3.0% (1/33) for saline, and 5.3% (2/38) for Albutex. The percentages of patients with maximum increases in QTc interval of >60 msec that represented either more than or less than a 2 standard deviation increase from baseline at any 1-minute timepoint after the first injection of study agent were small: 1.2% (1/84) for SonoVue, 3.0% (1/33) for saline, and 5.3% (2/38) for Albutex.

When QTc interval was calculated using Fredericia's formula, the percentages of patients with maximum increases in QTc interval of 31 - 60 msec at any 1-minute timepoint after the first injection of study agent were 1.2% (1/84) for SonoVue, 9.1% (3/33) for saline, and 5.3% (2/38) for Albutex. No patient had a QTc prolongation of >60 msec that represented more than a 2 standard deviation increase from baseline in any study agent group. Only 2 patients (1 SonoVue, 1 Albutex) had QTc prolongations of >60 msec that represented less than a 2 standard deviation increase from baseline at any 1-minute timepoint after the first injection of study agent. No patient had a prolongation in QTc interval of >60 msec after the first injection of saline.

For patients with QTc prolongations of potential clinical importance, maximum increases from baseline of >30 msec in QTc interval (Bazett's formula) were first observed during the first 3 minutes following the first injection for 4 of 5 patients receiving SonoVue, 2 of 2 patients

receiving saline, and for 2 of 4 patients receiving Albuterol. A similar pattern was generally observed when QTc interval was calculated using Fredericia's formula. Maximum increases from baseline of the same magnitude were first observed during the first 3 minutes following the first injection for 1 of 2 patients receiving SonoVue, 1 of 3 patients receiving saline, and for 2 of 3 patients receiving Albuterol.

Maximum Decreases QTc Interval That Met the Criteria of Potential Clinical Importance:

Following the first injection of SonoVue and saline, maximum decreases in QTc interval (Bazett's formula) of 31 – 60 msec occurred more frequently than maximum increases of the same magnitude. The percentages of patients with maximum increases and decreases were essentially the same following the first injection of Albuterol. When QTc interval was calculated using Fredericia's formula, similar findings were noted following the first injection of SonoVue, while maximum increases occurred more frequently than decreases following the first injection of saline and Albuterol.

No patient had maximum decreases in QTc interval (Bazett's or Fredericia's formula) >60 msec in any study agent group.

Mean Change From Baseline in QTc, Non-corrected QT, and RR Intervals by Timepoint:

Decreases from baseline in mean QTc interval calculated using either Bazett's (≤ 6.88 msec) or Fredericia's formula (≤ 3.46 msec) were observed at each 1-minute timepoint during the first 15 minutes after the first injection of SonoVue (all doses combined). In general, increases in mean QTc interval (Bazett's or Fredericia's formula) occurred more frequently and were of a greater magnitude after the first injection of Albuterol (≤ 5.86 msec) than those after the first injection of saline (≤ 0.95 msec). No time-related pattern was observed for any study agent group.

Increases from baseline in mean QT interval (non-corrected) were generally smaller after the first injection of SonoVue (≤ 4.68 msec) and after the first injection of saline (≤ 6.37 msec) than after the first injection of Albuterol (≤ 12.49 msec).

Increases from baseline in mean RR interval were generally smaller after the first injection of SonoVue (range: 24.22 to 42.54 msec) and after the first injection of saline (range: 24.23 to 41.42 msec) than after the first injection of Albuterol (range: 33.5 to 55.44 msec). No time-related pattern was observed for mean changes from baseline in QTc, QT, or RR intervals for any study agent group.

Mean Change From Baseline in QTc, QT, and RR Intervals by Dose:

Mean changes from baseline in QTc, QT and RR intervals by dose for each study agent group did not suggest a relationship to dose. No time-related pattern was observed for mean changes from baseline in QTc, QT, or RR intervals for any study agent group.

Conclusions: Validation to obtain a medically interpretable database of QTc prolongations from previous Holter recordings was successful. These recordings were generated from studies BR1-019A and BR1-019B. The findings from the analysis of validated Holter ECG data obtained from 156 patients in the 2 clinical trials of SonoVue have not shown that SonoVue has any effect on QTc interval. Specifically:

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- The incidence of SonoVue (1.2%) patients with maximum increases in QTc interval >60 msec that represented either more than or less than a 2 standard deviation increase from baseline at 1 or more timepoints was less than those observed for patients receiving Albutex (=5.3%) or saline (=3.0%).
 - Most of these increases occurred during the first few minutes following injection.
 - Mean values for QTc intervals decreased from baseline at most 1-minute timepoints during the first 15 minutes after the first injection of SonoVue.
 - Mean changes from baseline for QTc, QT, and RR intervals did not appear to be related to dose, and no time-related patterns were observed.

BLINDED VALIDATION OF 12-LEAD ECG TRACINGS FOR QTc MEASUREMENTS FROM PATIENTS PARTICIPATING IN SONOVUE™ STUDIES

Summary and Conclusions:

Demographics: The majority of the 477 patients included in the 12-lead ECG analysis were white males between 22 and 96 years old. The demographic and baseline characteristics profile of the 202 patients in the controlled cardiac studies was generally similar to that of the 477 patients included in the 9 cardiac studies.

Exposure to Study Agent: The majority of patients (79%; 378/477) in the 9 cardiac studies received >7.5 mL of SonoVue, 21% (99/477) received >7.5 mL.

Twelve-lead ECG Results:

Maximum Increases in QTc Interval That Met the Criteria of Potential Clinical Importance: The percentage of patients in the 9 cardiac studies with maximum increases from baseline in QTc interval (Bazett's formula) of 31 - 60 msec (11.5%) was similar to that of patients with decreases of the same magnitude (10.3%). In the controlled cardiac studies, the percentage of patients with maximum increases from baseline in QTc interval (Bazett's formula) of 31 - 60 msec was smaller for patients who received SonoVue (9.4%) than for patients who received Alburnex/saline (17%). The percentage of patients in the 9 cardiac studies with maximum increases from baseline >60 msec (5.5%) at any

postbaseline timepoint was similar to that of patients with maximum decreases >60 msec (3.6%). In the 2 controlled cardiac studies, the percentages of patients with maximum increases from baseline in QTc interval (Bazett's formula) of >60 msec were similar for the 2 study agent groups: 0.9% for patients who received SonoVue and 2.1% for patients who received Alburnex/saline. No relationship to time was observed for the postbaseline timepoints. When QTc was calculated using Fredericia's formula, the findings were generally similar to those for Bazett's formula. The percentage of patients in the 2 controlled cardiac studies with maximum increases from baseline in QTc interval of 31 - 60 msec was 5.7% for patients who received SonoVue and 8.5% for patients who received Alburnex/saline.

Maximum Increases in QT Interval That Met the Criteria of Potential Clinical Importance: The percentage of patients in the 9 cardiac studies with maximum increases from baseline in QT interval of 31 - 60 msec (22.6%) were greater than that of patients with decreases of the same magnitude (13.3%). In the controlled cardiac studies, the percentages of patients with maximum increases from baseline in QT interval of 31 - 60 msec were similar for the 2 study agent groups: 17% for patients who received SonoVue and 19.1% for patients who received Alburnex/saline. The percentage of patients in the 9 cardiac studies with maximum increases >60 msec (6.5%) at any postbaseline timepoint was similar to that of patients with maximum decreases >60 msec (5.9%). In the controlled cardiac studies, the percentages of patients with maximum increases from baseline in QT interval of >60 msec were similar for the 2 study agent groups: 3.8% for patients who received SonoVue and 1.1% for patients who received Alburnex/saline.

Mean Change From Baseline in QTc, QT, and RR Intervals:

Small changes (-0.7 to 9.3 msec) in mean QTc interval (Bazett's formula) values were observed for patients in 7 of the 9 cardiac studies at all postbaseline timepoints, except at 24 hours. At 24 hours after the last injection, mean QTc interval value increased by 16.5 msec. The magnitude of the increase at 24 hours after the last injection is largely attributed to 1 patient (Patient 107 in BR1-012) with a reported QTc interval value of 4120.0 msec. When this patient is excluded from the analysis, mean QTc interval increased by 0.4 msec. Similar findings were observed for changes in mean QT interval. Changes in mean RR interval ranged from -42.6 to 56.5 msec. No relationship to time was observed for the postbaseline timepoints.

Conclusions:

The findings from the analysis of validated 12-lead ECG data obtained from 477 patients in the 9 cardiac studies, including 106 patients who received SonoVue in the 2 controlled cardiac studies have not shown that SonoVue has any effect on QTc interval. Specifically:

- In the 9 cardiac studies, the incidence of SonoVue patients with maximum increases in QTc interval >60 msec were similar to that of patients with decreases of the same magnitude.
- In the 2 controlled cardiac studies, the incidence of SonoVue patients with maximum increases in QTc interval >60 msec was similar to that observed for patients receiving Alburnex/saline.
- In the 2 controlled cardiac studies, the incidence of patients with maximum increases in QTc interval 31 - 60 msec was lower for patients receiving SonoVue (9.4%) than for those receiving Alburnex/saline (17.0%).
- No time-related patterns were observed for maximum changes from baseline for QTc or QT intervals.
- No time-related patterns were observed for mean changes from baseline for QTc, QT, and RR intervals.

**A SINGLE-BLIND, PLACEBO-CONTROLLED, RANDOMIZED,
THREE-WAY CROSSOVER, PHASE III CLINICAL STUDY TO EVALUATE THE EFFECT
OF SINGLE INJECTIONS OF 0.1 mL/kg AND 0.5 mL/kg OF SONOVUE™ ON CARDIAC
ELECTROPHYSIOLOGY IN CORONARY ARTERY DISEASE PATIENT VOLUNTEERS**

Summary and Conclusions:

Demography: 49 subjects (25 males, 24 females) received at least one dose of study agent. The mean age of the subjects was 62.8 years, and the majority were white. All subjects had a diagnosis of CAD.

Cardiac Electrophysiology:

Primary Analysis: One subject was excluded from analysis because he was discontinued prior to receiving all three doses of study agent. There were no statistically significant differences in the maximum increase from baseline in QTcI interval between placebo and each of the SonoVue doses or between the two SonoVue doses (all 95% CI for the difference in LS mean change include zero). The results of the primary analysis are shown in the following table:

Analysis of Maximum Increase from Baseline in QTcI Interval (msec) Within 1 Hour Postdose			
Parameter	Placebo (N=48)	SonoVue 0.1 mL/kg (N=48)	SonoVue 0.5 mL/kg (N=48)
Baseline ^a			
Mean (SD)	404.6 (22.7)	403.9 (24.5)	401.8 (23.0)
Median	404.5	399.0	401.0
Min—Max	362–456	366–465	360–451
Maximum Postdose Value ^b			
Mean (SD)	422.9 (21.9)	420.7 (26.4)	419.3 (23.0)
Median	420.5	414.0	417.0
Min—Max	385–475	373–486	377–468
Maximum Increase From Baseline ^c			
LS Mean Change (SE)	18.4 (1.3)	16.8 (1.3)	17.5 (1.3)
95% CI for LS Mean Change	(15.9, 20.8)	(14.3, 19.2)	(15.0, 20.0)
SonoVue – Placebo			
Difference in LS Mean Change (SE)		-1.60 (1.60)	-0.85 (1.60)
95% CI for Difference of LS Mean Change		(-4.79, 1.58)	(-4.04, 2.33)
SonoVue 0.5 mL/kg – SonoVue 0.1 mL/kg			
Difference in LS Mean Change (SE)		0.75 (1.60)	
95% CI for Difference of LS Mean Change		(-2.43, 3.93)	
a Baseline is the mean of all technically adequate recorded values from 3 hr predose to immediately predose. b Postdose value from +1 min to +1 hr where the maximum increase from baseline occurred. c Based on an ANOVA model including dose and period as fixed effects and subject as random effect. QTc Interval Normal Range: 320 - 440 msec; QTcI = Individual subject corrected QT interval; SD = Standard deviation; SE = Standard error; LS = Least squares; CI = Confidence interval			

Additional Analyses of Continuous 12-lead ECG Data: For all three study agents, mean changes from baseline in heart rate (HR) were minimal within 1 hour postdose; however, from 1 to 12 hours postdose, mean HR values were increased relative to baseline. These changes were consistent across study agents and treatment periods. The increases in HR observed from 1 to 12 hours postdose were accompanied by decreases from baseline in PR and uncorrected QT

intervals, and small decreases in QTcI interval. There were no marked changes in QRS interval. For all parameters, the magnitude of the changes from baseline was similar for the three study agent groups. Shifts relative to the normal range for ECG parameters did not indicate a trend towards increased incidence of abnormal values with SonoVue. There was no evidence of a dose-response relationship in the incidence of changes of potential clinical importance for ECG parameters. One subject had an increase from baseline in QTcI of ≥ 60 msec (413 to 476 msec) observed 1.5 hours after administration of SonoVue 0.5 mL/kg. This was also the only subject who had QTcI interval values greater than 480 msec, which were observed both predose and postdose following administration of SonoVue 0.1 mL/kg.

Qualitative Assessments: The incidence of predose and postdose abnormalities for qualitative ECG parameters was similar for the three study agents. There was no evidence of any increases in ECG abnormalities within the first hour postdose and no apparent clinically significant differences between SonoVue and placebo.

Other Safety Results:

Seven (14.3%) subjects had adverse events (AEs) reported during study, all of which were mild to moderate in severity. There was no clear dose relationship with respect to the incidence of AEs following administration of the three study agents. One subject experienced tongue edema (verbatim: swollen tongue) 6 hours after administration of SonoVue 0.5 mL/kg that was considered probably related to study agent; the subject was subsequently discontinued from study. Another subject experienced mild, transient ventricular extrasystoles (PVCs) 1 minute after administration of SonoVue 0.5 mL/kg, for which the Investigator considered the relationship to study agent to be unknown. This subject had PVCs noted on screening ECG (ie, predose). There were no serious AEs reported.

Neurological examination did not reveal any new abnormalities following administration of study agents. There were no clinically significant changes in mini mental status examination scores at any timepoint following administration of study agent. Postdose changes from baseline in vital signs and oxygen saturation did not indicate any differences between SonoVue and placebo or any dose-related trends. Within the 24-hour postdose period, oxygen saturation values that met the criteria for potential clinical importance, ie, decrease from baseline $\geq 4\%$, were observed somewhat more frequently following administration of SonoVue 0.1 mL/kg (27.1%) and 0.5 mL/kg (32.7%) compared to placebo (20.4%). However, this overall difference was not attributable to differences at any particular timepoint within the 24 hour postdose period. The range of predose and postdose oxygen saturation values was similar for placebo and SonoVue, and was consistent with the study population, ie, subjects diagnosed with CAD. Predose to postdose changes in clinical laboratory values, including urine microscopy, did not indicate any clinically significant trends or any differences for SonoVue compared to placebo. Changes in clinical laboratory values that met the criteria for marked abnormality were infrequent following administration of study agent, and did not indicate any clinically meaningful differences between SonoVue and placebo.

Conclusions: The results of the study support the following conclusions:

- Administration of SonoVue 0.1 or 0.5 mL/kg does not appear to cause prolonged cardiac repolarization in patients with CAD undergoing B-mode echocardiography with a mechanical index of 0.7 to 0.8.

- There was no statistically significant difference between SonoVue and placebo in the maximum increase from baseline in corrected QT interval following administration of study agent and no evidence of a dose-response relationship.
- Administration of SonoVue was not associated with any increased incidence of cardiac abnormalities as evaluated by quantitative and qualitative ECG parameters.
- Consistent with the previously reported safety profile of SonoVue, there is no evidence that administration of SonoVue is associated with an increased risk of microembolism.

A SINGLE-BLIND, PLACEBO-CONTROLLED, RANDOMIZED, FOUR-WAY CROSSOVER, PHASE III CLINICAL STUDY TO EVALUATE THE EFFECT OF ULTRASOUND EXPOSURE AND SONOVUE™ ON CARDIAC ELECTROPHYSIOLOGY IN CORONARY ARTERY DISEASE PATIENT VOLUNTEERS

Summary and Conclusions:

Demography: 53 subjects (28 males, 25 females) received at least one dose of study agent. The mean age of subjects was 63.1 years, and the majority were white. All subjects had a history of CAD.

Cardiac Electrophysiology:

Primary Analysis: Three subjects were excluded from the analysis population: 2 subjects had technically inadequate ECG data due to flash card problems, and one subject had subcutaneous infiltration at the IV site on Day 1. There were no statistically significant differences in the maximum increase from baseline in QTcI interval between placebo and SonoVue at MI 0.4 or between placebo and SonoVue at MI 1.5 (95% confidence interval for the differences in least squares [LS] mean changes between placebo and SonoVue at each MI level include zero).

The results of the primary analysis are shown in the following table:

Analysis of Maximum Increase from Baseline in QTcI Interval (msec) Within 1 hour Postdose				
Parameter	Placebo MI 0.4 (N=50)	Placebo MI 1.5 (N=50)	SonoVue MI 0.4 (N=50)	SonoVue MI 1.5 (N=50)
Baseline ^a				
Mean (SD)	403.0 (24.0)	402.4 (24.1)	401.1 (24.7)	402.1 (23.8)
Median	400.0	397.0	399.5	400.0
Min—Max	358–454	360–460	349–454	363–458
Maximum Postdose Value ^b				
Mean (SD)	421.4 (26.8)	420.0 (26.5)	420.0 (26.4)	418.8 (25.7)
Median	419.0	418.0	420.5	415.0
Min—Max	364–487	378–486	365–475	375–480
Maximum Increase From Baseline ^c				
LS Mean Change (SE)	18.5 (1.3) (15.8,	17.7 (1.3)	18.9 (1.3)	16.7 (1.3)
95% CI for LS Mean Change	21.1)	(15.0, 20.3)	(16.3, 21.6)	(14.0, 19.3)
Placebo MI 0.4– SonoVue MI 0.4				
Difference in LS Mean Change (SE)			-0.49 (1.59)	
95% CI for Difference of LS Mean Change			(-3.63, 2.66)	
Placebo MI 1.5– SonoVue MI 1.5				
Difference in LS Mean Change (SE)				1.03 (1.59)
95% CI for Difference of LS Mean Change				(-2.11, 4.18)
<p>^a Baseline is the mean of all technically adequate recorded values from 3 hr predose to immediately predose. ^b Postdose value from +1 min to +1 hr where the maximum increase from baseline occurred. ^c Based on an ANOVA model including treatment and period as fixed effects and subject as random effect. QTc Interval Normal Range: 320 - 440 msec; QTcI = Individual subject corrected QT interval; SD = Standard deviation; SE = Standard error; LS = Least squares; CI = Confidence interval; Min = Minimum; Max = Maximum</p>				

Additional Analyses of Continuous ECG Data: For the secondary hypothesis tested, there was no significant difference in the maximum increase from baseline in QTcI interval between MI 0.4 and MI 1.5 for either SonoVue or placebo, as indicated by the 95% confidence intervals for the difference in LS mean changes for SonoVue at MI 0.4 versus SonoVue at MI 1.5 (95% CI: -0.85, 5.44) or for placebo at MI 0.4 versus placebo at MI 1.5 (95% CI: -2.37, 3.92).

Mean changes from baseline in quantitative ECG parameters were minimal within the first hour postdose; however, from 1 to 12 hours postdose, increases in heart rate were observed for all four treatment groups accompanied by decreases from baseline in PR and uncorrected QT intervals. Small decreases from baseline in QTcI interval were also observed during this time period. There were no marked changes in QRS. Shifts relative to the normal range for ECG parameters did not indicate a trend towards increased incidence of abnormal values following treatment. For all ECG interval parameters, there were no marked differences among the treatment groups with respect to the incidence of changes from baseline of potential clinical importance. No increases from baseline in QTcI interval of >60 msec were observed for any subject.

Qualitative Assessments: The incidence of predose and postdose abnormalities for qualitative ECG parameters was similar for the four treatment groups. There was no evidence of any increases in ECG abnormalities within the first hour postdose and no clinically significant differences between SonoVue and placebo at either MI level in the qualitative ECG assessments. The occurrence of postdose premature ventricular contractions (PVCs) was similar for SonoVue and placebo and no trends related to MI were observed.

Other Safety Results:

Eight (15.1%) subjects reported adverse events during study, all of which were mild in severity. One adverse event (IV site infiltration with edema) was considered probably related to study agent. There were no serious adverse events reported and no discontinuations due to adverse events. Neurological examination did not reveal any new abnormalities following administration of treatment. There were no clinically significant changes in mini mental status examination scores at any timepoint following administration of treatment. Postdose changes from baseline in vital signs and oxygen saturation did not indicate any differences between SonoVue and placebo at either MI level. Changes from baseline in blood pressure and heart rate that met the criteria for potential clinical importance were observed with similar frequency among the four treatment groups. Predose to postdose changes in clinical laboratory values, including urine microscopy, did not indicate any clinically significant trends or any differences among the four treatment groups. Changes in clinical laboratory values that met the criteria for marked abnormality were infrequent following administration of treatment, and did not indicate any clinically meaningful differences between SonoVue and placebo.

Conclusions: The results of the study support the following conclusions:

- Administration of SonoVue 0.1 mL/kg does not appear to cause prolonged cardiac repolarization in patients with CAD undergoing B-mode echocardiography using either low (0.4-0.5) or high (1.5-1.6) MI settings.

- There was no statistically significant difference between SonoVue and placebo in the maximum increase from baseline in corrected QT interval following administration of study agent during both low MI and high MI echocardiography.
- Administration of SonoVue during low MI and high MI echocardiography is not associated with any increased incidence of cardiac abnormalities including PVCs as evaluated by quantitative and qualitative ECG parameters.
- Consistent with the previously reported safety profile of SonoVue, there is no evidence that administration of SonoVue is associated with an increased risk of microembolism.

8-Risk of anaphylaxis in a hospital population in relation to the use of various drugs: an international study.

[The International Collaborative Study of Severe Anaphylaxis.](#)

PURPOSE: To evaluate the risk of anaphylaxis following exposure to medications given during hospitalization. **METHODS:** Cases with anaphylaxis and controls, matched according to length of hospital stay, were enrolled from hospitals in Hungary, Spain, India and Sweden. Exposures were recorded from the hospital record. The incidence of anaphylaxis was calculated among those exposed to various drugs, with denominators estimated from exposure rates in the controls and number of hospitalizations. **RESULTS:** Among 184 cases and 1003 controls, the incidence of anaphylaxis was in the range of 5-15 cases per 100,000 exposed patients for most analgesics and antibiotics administered orally or parenterally; for parenteral penicillin, it was 32. Incidence estimates were somewhat higher for blood, dextran, pentoxifylline and both ionic and nonionic contrast media, ranging from 35 to 95 cases. The highest estimates were for streptokinase and plasma, at 378 and 284. With no exposed controls, the incidence could not be calculated for anti-snake venom, but it was clearly higher than for other exposures.

CONCLUSIONS: Although anaphylaxis is known to occur following exposure to a large number of drugs, the incidence is not well documented. We have demonstrated a relatively low risk for dipyrone, diclofenac, paracetamol, ampicillin, cloxacillin and cephalosporins. An intermediate risk was shown for parenteral penicillin, dextran, contrast media, blood and pentoxifylline. The highest incidence was observed for plasma, streptokinase and anti-snake venom (the latter used only in India).

9-Anaphylactoid reactions to radiocontrast media.

[Cochran ST.](#)

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Adverse reactions to contrast material are a concern because iodinated contrast materials are commonly used drugs. The risk for adverse reaction is 4% to 12% with ionic contrast materials and 1% to 3% with nonionic contrast materials. The risk for severe adverse reaction is 0.16% with ionic contrast materials and 0.03% with nonionic contrast materials. The death rate, one to three per 100,000 contrast administrations, is similar for both ionic and nonionic agents. More than 90% of adverse reactions with nonionic contrast materials are anaphylactoid. The types of severe reactions seen with nonionic contrast administration were initially predominantly anaphylactoid. With the advent of helical CT angiography, the reactions are now predominantly attributable to cardiopulmonary decompensation. With the widespread use of nonionic contrast materials, adverse reactions are now seen less frequently. Skills involved in evaluating

and treating adverse reactions are not as frequently used. Periodic reviews and updates of specific treatment plans for various reactions with the physicians and staff who use contrast material are very important to ensure optimal preparedness. The key to successful treatment is preparation and early intervention.

10-Safety of gadobenate dimeglumine (MultiHance): Summary of findings from clinical studies and postmarketing surveillance.

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OBJECTIVES: Prospective studies and retrospective analyses were undertaken to evaluate the clinical safety of gadobenate dimeglumine (MultiHance) and to assess tolerability in special populations. **MATERIALS AND METHODS:** A total of 3092 subjects received MultiHance in 79 clinical trials. Data from comparisons with other contrast agents and studies in children, subjects with hepatic or renal impairment, or subjects with coronary artery disease were reviewed. Postmarketing safety surveillance data after more than 1.5 million applications were also evaluated. **RESULTS:** In total, 413 of 2982 (14%) adult subjects receiving MultiHance reported at least one adverse event (AE) definitely or potentially related to MultiHance, an incidence that was similar to that observed with placebo (21/127, 17%) or active controls (59/723, 8%). In crossover studies, 23 of 287 (8%) subjects receiving MultiHance experienced AE compared with 25 of 295 (9%) receiving gadopentetate dimeglumine (Magnevist). No increased AE rate was observed in children and no worsening of renal or liver function was observed in subjects with hepatic or renal impairment. No detrimental effect on cardiac electrophysiology could be observed from a retrospective analysis of ECG parameters in more than 1000 patients and healthy volunteers. The AE reporting rate from postmarketing safety surveillance of MultiHance was 0.05%. Serious AEs were rarely reported and included dyspnea, nausea, urticaria, hypotension, and anaphylactoid reactions. **CONCLUSIONS:** MultiHance appears to be well tolerated in adults and children and in subjects with impaired liver or kidney function or coronary artery disease. In controlled trials, MultiHance demonstrated a similar safety profile to that of Magnevist.

11-Worldwide clinical safety assessment of gadoteridol injection: an update.

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Gadoteridol injection is a low molecular weight chelate complex of gadolinium (III) which is useful as a contrast agent for magnetic resonance imaging. A total of 2481 adult and pediatric subjects were studied with gadoteridol at doses from 0.025 to 0.3 mmol/kg in phase I-IIIb clinical trials in Europe and the United States. The study population had a mean age of 49 years, and included 119 patients under 18 years of age and 747 patients over 60 years of age. After 2656 administered injections of gadoteridol a total of 233 adverse events were recorded in 176 exposures, an incidence rate of 6.6 % irrespective of relationship to drug administration. The most frequently reported adverse events were nausea (1.5 %), taste perversion (0.9 %), and headache (0.6 %). All other adverse events occurred with an incidence of 0.5 % or less. This report confirms the excellent safety profile of gadoteridol in healthy subjects and patients with a variety of known or suspected pathologies.

12-Animal models of complement-mediated hypersensitivity reactions to liposomes and other lipid-based nanoparticles.

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Intravenous injection of some liposomal drugs, diagnostic agents, micelles and other lipid-based nanoparticles can cause acute hypersensitivity reactions (HSRs) in a high percentage (up to 45%) of patients, with hemodynamic, respiratory and cutaneous manifestations. The phenomenon can be explained with activation of the complement (C) system on the surface of lipid particles, leading to anaphylatoxin (C5a and C3a) liberation and subsequent release reactions of mast cells, basophils and possibly other inflammatory cells in blood. These reactions can be reproduced and studied in pigs, dogs and rats, animal models which differ from each other in sensitivity and spectrum of symptoms. In the most sensitive pig model, a few milligrams of liposome (phospholipid) can cause anaphylactoid shock, characterized by pulmonary hypertension, systemic hypotension, decreased cardiac output and major cardiac arrhythmias. Pigs also display cutaneous symptoms, such as flushing and rash. The sensitivity of dogs to hemodynamic changes is

close to that of pigs, but unlike pigs, dogs also react to micellar lipids (such as Cremophor EL) and their response includes pronounced blood cell and vegetative neural changes (e.g., leukopenia followed by leukocytosis, thrombocytopenia, fluid excretions). Rats are relatively insensitive inasmuch as hypotension, their most prominent response to liposomes, is induced only by one or two orders of magnitude higher phospholipid doses (based on body weight) compared to the reactogenic dose in pigs and dogs. It is suggested that the porcine and dog models are applicable for measuring and predicting the (pseudo)allergic activity of particulate "nanodrugs".

14-Safety of contrast dobutamine stress echocardiography: a single center experience.

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BACKGROUND: Contrast is increasingly being used during dobutamine stress echocardiography. However, there are few data regarding the safety of this combination. **METHODS:** We retrospectively analyzed 751 consecutive stress echocardiograms, 332 without contrast and 419 with contrast (299 with Sonouve, 120 with Optison). Reported side effects and physiologic data were then compared. **RESULTS:** There were no fatalities. The incidence of side effects was similar in the 3 groups. The Optison group had a lower diastolic blood pressure compared with the noncontrast group ($P < .05$) at rest, and the Sonovue group had a higher peak heart rate compared with the noncontrast group ($P < .001$). Patients receiving Optison had more premature atrial contractions ($P < .05$) but there was no difference in the incidence of ventricular tachycardia, supraventricular tachycardia, or vagally mediated episodes. **CONCLUSION:** The use of contrast during dobutamine stress echocardiography was not associated with an increased risk of side effects.

15-Acute mortality in hospitalized patients undergoing echocardiography with and without an ultrasound contrast agent: results in 18,671 consecutive studies.

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OBJECTIVES: We sought to define acute mortality in hospitalized patients undergoing clinically indicated echocardiography with and without use of an ultrasound contrast agent. **BACKGROUND:** The U.S. Food and Drug Administration recently issued a boxed warning and new contraindications for the perflutren-containing ultrasound

contrast agents following post-marketing reports of 4 patient deaths that were temporally related to Definity (Bristol-Myers Squibb Medical Imaging, Billerica, Massachusetts) administration. To appreciate the incremental risk of any medical procedure, the ambient risk of untoward outcome in the population in question must first be defined. There are no published data on short-term major adverse cardiac events in hospitalized patients undergoing echocardiography, either with or without administration of an ultrasound contrast agent. METHODS: A retrospective analysis of hospitalized patients undergoing clinically indicated echocardiography between January 2005 and October 2007, within Saint Luke's Health System, Kansas City, Missouri, was performed. Studies were separated into 2 groups, those performed without contrast enhancement (n = 12,475) and those performed with Definity (n = 6,196). Vital status within 24 h of the echocardiographic study was available for all patients using a combination of the Social Security Death Master File and Saint Luke's Health System medical records. Incidence of death within 24 h was compared by chi-square test between Definity and unenhanced procedures. RESULTS: Of the 18,671 patient events, 72 patients died within 24 h. Of those that underwent unenhanced echocardiography, 46 died within 24 h (0.37%). Of patients receiving Definity during the echocardiogram, 26 died within 24 h (0.42%). There was no statistical difference between these 2 groups (p = 0.60). No patient died within 1 h of the echocardiographic study. In a random sampling from the unenhanced (n = 201) and Definity groups (n = 202), patients who underwent Definity-enhanced echocardiography exhibited higher clinical acuity, and more significant comorbidities. CONCLUSIONS: Approximately 0.4% of hospitalized patients die within 24 h of echocardiography. There is no increased mortality risk associated with Definity-enhanced examinations, despite evidence for higher clinical acuity and more comorbid conditions in patients undergoing contrast studies.

28-Left ventricular contrast echocardiography: role for evaluation of function and structure.

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Despite the recent introduction of tissue harmonic imaging in echocardiography, in 10%-15% of patients endocardial borders of the left ventricle are poorly defined. This may lead to erroneous assessment of regional and global left ventricular (LV) function or further diagnostic imaging with another modality leading to increased cost to the healthcare system. The recent development of second generation contrast echocardiography agents such as SonoVue has resulted in several studies showing the value of these agents to outline endocardium clearly, thereby improving assessment of LV function. The use of these contrast agents has also opened the possibility of automated and qualitative LV function assessment, resulting in more accurate and reproducible assessment. Other major clinical uses of these contrast agents are evaluation of LV masses such as thrombus and tumors, and better definition of LV structure such as

delineating LV aneurysm, pseudoaneurysm; and noncompaction of LV and apical cardiomyopathy. Thus, it is clear that contrast-enhanced LV assessment at rest will play an important role in echocardiography.

29-Contrast echocardiography: evidence for clinical use.

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The failure of echocardiography to give diagnostically useful information in a significant proportion of patients has led to the development of specific contrast agents to enhance imaging. Suitable contrast media must have the ability to modify ultrasound characteristics, be capable of crossing the pulmonary capillary bed, show stability over the duration of a procedure, offer low blood solubility with low toxicity and be rapidly eliminated. The current generation of ultrasound contrast agents comprises microbubbles of a high molecular-weight gas encapsulated in a shell of phospholipid or protein. A review of the clinical evidence shows that these agents are clinically effective in enhancing echocardiographic imaging. They enable the rescue of failed procedures, often sparing patients from invasive tests, but appear not to add to the burden of side effects. Indeed, the benefits of using contrast agents in stress echocardiography have been recommended in recently published American Society of Echocardiography guidelines. Myocardial contrast echocardiography has now developed to the stage where assessment of myocardial perfusion for the detection of coronary artery disease is possible with the same diagnostic accuracy as radionuclide imaging. However, in comparison with the latter technique, it is less expensive, is more portable, and avoids the use of ionizing radiation. It is precisely the ability of myocardial contrast echocardiography to simultaneously assess function and perfusion at the bedside that has given it a unique role in clinical practice. This review provides an overview of the clinical evidence supporting the efficacy of contrast echocardiography in the assessment of myocardial structure, function, and perfusion.

30-Clinical benefits of contrast-enhanced echocardiography during rest and stress examinations.

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Despite the recent introduction of tissue harmonic imaging in echocardiography, 10-15% of patients have poor endocardial border definition. This may lead to erroneous assessment of regional and global left ventricular (LV) function or to further diagnostic

imaging with another modality thus increasing the costs for the healthcare system. The recent development of second generation contrast echocardiography agents such as SonoVue has resulted in several studies showing the value of these agents to outline endocardium clearly, thereby improving assessment of LV function. The use of these contrast agents has also opened the possibility of automated and quantitative LV function assessment, making it more accurate and reproducible. Other major clinical uses of these contrast agents are evaluation of LV masses such as thrombus and tumors, and better definition of LV structure such as delineating LV aneurysm, pseudoaneurysm; and non-compaction of LV and apical cardiomyopathy. Furthermore, the use of these contrast agents during stress not only improved the assessment of wall motion but also made possible the evaluation of myocardial perfusion, thereby increasing diagnostic accuracy for the detection of coronary artery disease.

31-Myocardial contrast echocardiography evolving as a clinically feasible technique for accurate, rapid, and safe assessment of myocardial perfusion: the evidence so far.

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Intravenous myocardial contrast echocardiography (MCE) is a recently developed technique for assessment of myocardial perfusion. Up to now, many studies have demonstrated that the sensitivity and specificity of qualitative assessment of myocardial perfusion by MCE in patients with acute and chronic ischemic heart disease are comparable with other techniques such as cardiac scintigraphy and dobutamine stress echocardiography. Furthermore, quantitative parameters of myocardial perfusion derived from MCE correlate well with the current clinical standard for this purpose, positron emission tomography. Myocardial contrast echocardiography provides a promising and valuable tool for assessment of myocardial perfusion. Although MCE has been primarily performed for medical research, its implementation in routine clinical care is evolving. This article is intended to give an overview of the current status of MCE.

35-Multicenter evaluation of SonoVue for improved endocardial border delineation.

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OBJECTIVES: Two multicenter studies were conducted to evaluate the safety and efficacy of SonoVue as a contrast agent for enhanced left ventricular endocardial border delineation (LVEBD), and to compare the efficacy of SonoVue and Albunex in adult patients with a suboptimal, nonenhanced echocardiogram. **BACKGROUND:** The use of contrast to enhance echocardiographic assessment of LVEBD is well-established. SonoVue is a new microbubble contrast agent that contains sulfur hexafluoride. **METHODS:** Patients were randomized to receive four injections of SonoVue (0.5, 1, 2, and 4 ml), or two injections of Albunex and two injections of hand-agitated saline (0.08 and 0.22 ml/kg). Echocardiographic images were evaluated at the study centers and by four blinded, offsite reviewers for degree of left ventricle opacification (LVO), duration of contrast enhancement, and LVEBD. **RESULTS:** LVO scores were significantly higher for all doses of SonoVue. Patients with complete LVO ranged from 34%-87% for SonoVue and from 0%-16% for Albunex. The mean duration of useful contrast effect ranged from 0.8-4.1 minutes for SonoVue and < 15 seconds for Albunex. Mean increases in LVEBD scores ranged from 3.8-18.2 for SonoVue and 0.1-4.3 for Albunex. SonoVue (cumulative 7.5 ml dose) was well-tolerated, with a safety profile similar to that observed in the control group. **CONCLUSIONS:** SonoVue is superior to Albunex for improving visualization of endocardial borders in patients with suboptimal noncontrast echocardiograms. Optimal increases in LVEBD, LVO, and duration of useful contrast effect were observed at the 2.0 ml dose of SonoVue.

39-Detection of left ventricular thrombus by two-dimensional echocardiography: sensitivity, specificity, and causes of uncertainty.

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To define the sensitivity, specificity and predictive accuracy of two-dimensional echocardiographic detection of left ventricular thrombus, the echocardiograms of 78 patients who had independent proof of the presence or absence of a left ventricular thrombus were interpreted without knowledge of any clinical data. The presence of thrombus was established by autopsy in four patients, by aneurysmectomy in three, and by indium-111 platelet imaging in 15; the absence of thrombus was proved by autopsy in 55 patients and by aneurysmectomy in one patient. The characteristics of true-positive

and false-positive echocardiograms, interobserver variability, and clinical features associated with proved thrombus were also defined. The echocardiogram was positive for thrombus in 22 patients, equivocal in seven and negative in 49. For detection of thrombus, a positive or equivocal echocardiogram had a sensitivity of 95% (21 of 22), a specificity of 86% (48 of 56), and a predictive value of 72% (21 of 29); the predictive value of a negative study was 98% (48 of 49). Considering positive and equivocal studies separately, the predictive value of a positive study was 86% (19 of 22), while that of an equivocal study was only 29% (two of seven). Compared with patients who had no thrombus, patients with proved thrombus had a higher prevalence of electrocardiographic transmural anterior infarction (86% vs 13%), left ventricular aneurysm (73% vs 5%), and clinical systemic emboli (36% vs 7%) (all p less than 0.05). These clinical features help to identify a subset of patients most likely to have left ventricular thrombi who may benefit from echocardiography. Two-dimensional echocardiography is highly sensitive in detecting left ventricular thrombus, but false-positive studies are relatively common. Several echocardiographic criteria derived from analysis of the true and false positives in this study may help minimize diagnostic errors.

42-The extent of microvascular damage during myocardial contrast echocardiography is superior to other known indexes of post-infarct reperfusion in predicting left ventricular remodeling: results of the multicenter AMICI study.

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OBJECTIVES: We sought to evaluate the value of the extent of microvascular damage as assessed with myocardial contrast echocardiography (MCE) in the prediction of left ventricular (LV) remodeling after ST-segment elevation myocardial infarction (STEMI) as compared with established clinical and angiographic parameters of reperfusion.

BACKGROUND: Early identification of post-percutaneous coronary intervention microvascular dysfunction may help in tailoring appropriate pharmacological interventions in high-risk patients. The ideal method to establish effective microvascular reperfusion after percutaneous coronary intervention remains to be determined.

METHODS: A total of 110 patients with first successfully reperfused STEMI were enrolled in the AMICI (Acute Myocardial Infarction Contrast Imaging) multicenter study. After reperfusion, peak creatine kinase, ST-segment reduction, and Thrombolysis In Myocardial Infarction (TIMI) and myocardial blush grade were calculated. We evaluated perfusion defects with MCE by using continuous infusion of Sonovue (Bracco, Milan, Italy) in real-time imaging. The endocardial length of contrast defect (CD) on day

1 after reperfusion was calculated. Wall motion score index, the extent of wall motion abnormalities, LV end-diastolic volume, and ejection fraction after reperfusion and at follow-up also were calculated. RESULTS: Of 110 patients, 25% evolved in LV remodeling and 75% did not. Although peak creatine kinase, ST-segment reduction >70%, and myocardial blush grade were not different between groups, in patients exhibiting LV remodeling, TIMI flow grade 3 was less frequent ($p < 0.001$), wall motion score index was greater ($p < 0.001$), and CD was greater ($p < 0.001$). At multivariate analysis, only TIMI flow grade <3 and CD with a cutoff of >25% were independently associated with LV remodeling. Among patients with TIMI flow grade 3, CD was the only independent variable associated with LV remodeling. CONCLUSIONS: Among patients with TIMI flow grade 3, the extent of microvascular damage, detected and quantitated by MCE, is the most powerful independent predictor of LV remodeling after STEMI as compared with persistent ST-segment elevation and myocardial blush grade.

43-Prognostic value of myocardial viability detected by myocardial contrast echocardiography early after acute myocardial infarction.

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OBJECTIVES: This study sought to determine whether residual myocardial viability determined by myocardial contrast echocardiography (MCE) after acute myocardial infarction (AMI) can predict hard cardiac events. BACKGROUND: Myocardial viability detected by MCE has been shown to predict recovery of left ventricular (LV) function in patients with AMI. However, to date no study has shown its value in predicting major adverse outcomes in AMI patients after thrombolysis. METHODS: Accordingly, 99 stable patients underwent low-power MCE at 7 +/- 2 days after AMI. Contrast defect index (CDI) was obtained by adding contrast scores (1 = homogenous; 2 = reduced; 3 = minimal/absent opacification) in all 16 LV segments divided by 16. At discharge, 65 (68%) patients had either undergone or were scheduled for revascularization independent of the MCE result. The patients were subsequently followed up for cardiac death and nonfatal AMI. RESULTS: Of the 99 patients, 95 were available for follow-up. Of these, 86 (87%) underwent thrombolysis. During the follow-up time of 46 +/- 16 months, there were 15 (16%) events (8 cardiac deaths and 7 nonfatal AMIs). Among the clinical, biochemical, electrocardiographic, echocardiographic, and coronary arteriographic markers of prognosis, the extent of residual myocardial viability was an independent predictor of cardiac death ($p = 0.01$) and cardiac death or AMI ($p = 0.002$). A CDI of $< \text{or} = 1.86$ and $< \text{or} = 1.67$ predicted survival and survival or absence of recurrent AMI in 99% and 95% of the patients, respectively. CONCLUSIONS: The extent of residual myocardial viability predicted by MCE is a powerful independent predictor of hard cardiac events in patients after AMI.

44-Myocardial contrast echocardiography in ST elevation myocardial infarction: ready for prime time?

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Acute myocardial infarction (AMI) continues to be a significant public health problem in industrialized countries and an increasingly significant problem in developing countries. ST elevation myocardial infarctions (STEMI) constitute approximately 40% of all AMIs with approximately 670,000 cases yearly in the United States alone. The risk of further cardiac complications such as re-infarction, sudden death, and heart failure for those who survive AMI is substantial. Thus, early assessment and risk stratification during the acute phase of STEMI is important. Furthermore, it is essential to assess the efficacy early after any initial therapeutic intervention, not only to facilitate further management, but also to enable development of new treatment algorithms/approaches to further improve the outcome. The aim of reperfusion therapy in AMI is not only to rapidly restore epicardial coronary blood flow but also to restore perfusion at the microcirculatory level. Myocardial contrast echocardiography (MCE) which utilizes microbubbles can assess myocardial perfusion in real time. Its ability to assess myocardial perfusion and function in one examination allows it to ascertain the extent of myocardial reperfusion achieved in the risk area. Furthermore, in stable patients after AMI, MCE allows assessment of LV function, residual myocardial viability, and ischaemia which are all powerful prognostic markers of outcome. Its portability, rapid acquisition and interpretation of data, and the absence of radiation exposure make it an ideal bedside technique.

45-Analysis of interinstitutional observer agreement in interpretation of dobutamine stress echocardiograms.

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OBJECTIVES. This study sought to determine the degree of interinstitutional agreement in the interpretation of dobutamine stress echocardiograms. **BACKGROUND.** Dobutamine stress echocardiography involves subjective interpretation. Consistent methods for acquisition and interpretation are of critical importance for obtaining high interobserver agreement and for facilitating communication of test results. **METHODS.** Five experienced centers were each asked to submit 30 dobutamine stress echocardiograms (dobutamine up to 40 micrograms/kg body weight per min and atropine up to 1 mg) obtained in patients undergoing coronary angiography. Thus, a total of 150 dobutamine stress echocardiograms were interpreted by each center without knowledge

of any other patient data. Left ventricular wall motion was assessed using a 16-segment model but was otherwise not standardized. No patient was excluded because of poor image quality or inadequate stress level. Echocardiographic image quality was assessed using a five-point scale. RESULTS. Angiographically significant coronary artery disease (> or = 50% diameter stenosis) was present in 95 patients (63%). By a majority decision (three or more centers), the sensitivity, specificity and accuracy of dobutamine echocardiography were 76%, 87% and 80%, respectively. Abnormal or normal results of stress echocardiography were agreed on by four or all five of the centers in 73% of patients (mean kappa value 0.37, fair agreement only). Agreement on the left anterior descending artery territory (78%) was similar to that for the combined right coronary artery/left circumflex artery territory (74%), and for specific segments the agreement ranged from 84% to 97% and was highest for the basal anterior segment and lowest for the basal inferior segment. Agreement was higher in patients with no (82%) or three-vessel coronary artery disease (100%) and lower in patients with one- or two-vessel disease (61% and 68%, respectively). Agreement on positivity or negativity of stress test results was 100% for patients with the highest image quality but only 43% for those with the lowest image quality ($p = 0.003$). CONCLUSIONS. The current heterogeneity in data acquisition and assessment criteria among different centers results in low interinstitutional agreement in interpretation of stress echocardiograms. Agreement is higher in patients with no or advanced coronary artery disease and substantially lower in those with limited echocardiographic image quality. To increase interinstitutional agreement, better standardization of image acquisition and reading criteria of stress echocardiography is recommended.

46-Noninvasive diagnosis of ischemia-induced wall motion abnormalities with the use of high-dose dobutamine stress MRI: comparison with dobutamine stress echocardiography.

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BACKGROUND--The analysis of wall motion abnormalities with dobutamine stress echocardiography (DSE) is an established method for the detection of myocardial ischemia. With ultrafast magnetic resonance tomography, identical stress protocols as used for echocardiography can be applied. METHODS AND RESULTS--In 208 consecutive patients (147 men, 61 women) with suspected coronary artery disease, DSE with harmonic imaging and dobutamine stress magnetic resonance (DSMR) (1.5 T) were performed before cardiac catheterization. DSMR images were acquired during short breath-holds in 3 short-axis views and a 4- and a 2-chamber view (gradient echo technique). Patients were examined at rest and during a standard dobutamine-atropine

scheme until submaximal heart rate was reached. Regional wall motion was assessed in a 16-segment model. Significant coronary heart disease was defined as $\geq 50\%$ diameter stenosis. Eighteen patients could not be examined by DSMR (claustrophobia 11 and adipositas 6) and 18 patients by DSE (poor image quality). Four patients did not reach target heart rate. In 107 patients, coronary artery disease was found. With DSMR, sensitivity was increased from 74.3% to 86.2% and specificity from 69.8% to 85.7% (both $P < 0.05$) compared with DSE. Analysis for women yielded similar results. CONCLUSIONS--High-dose dobutamine magnetic resonance tomography can be performed with a standard dobutamine/atropine stress protocol. Detection of wall motion abnormalities by DSMR yields a significantly higher diagnostic accuracy in comparison to DSE.

47-Safety of stress echocardiography. The results of the international stress echo complication registry

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Abstract

Background: The safety of any diagnostic test is a major issue in deciding its practicability and cost-effectiveness.

Aim: To evaluate the safety of various stress echo modalities in the "real life" an international stress echo complication registry was started.

Methods: Up to January 2001, a simple written questionnaire was distributed to 300 echo laboratories known to perform stress echo. Of these 300, 71 echo laboratories were "responders" and reported on 85,997 examinations.

Results: Exercise was used in 26,295, dobutamine in 35,103, and dipyridamole in 24,599 cases. Life-threatening events occurred in 86 patients: during exercise in 4 patients (event rate: 1/6,574), during dobutamine infusion (low dose for viability and/or high dose for ischemia) in 63 patients (event rate 1/557), and during dipyridamole stress test in 19 patients (event rate 1/1,294). Of the 86 patients with complications, 5 died during

dobutamine stress test (ventricular fibrillation n=2 and cardiac rupture n = 3), and 1 following a dipyridamole test (uncontrollable hypotension).

Conclusion: Stress echocardiography is a safe method in the "real life," as well, but dreadful complications may occur. Possibly due to preselection criteria, exercise seems safer than pharmacological stress and dipyridamole safer than dobutamine.

52-US characterization of focal hepatic lesions with intermittent high-acoustic-power mode and contrast material.

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RATIONALE AND OBJECTIVES: This study was performed to determine whether ultrasound (US) performed with SonoVue, a contrast agent that contains microbubbles filled with sulfur hexafluoride vapor, depicts differential patterns of contrast enhancement in focal hepatic lesions. **MATERIALS AND METHODS:** Forty focal hepatic lesions (15 hepatocellular carcinomas [HCCs], 10 metastases, 11 hemangiomas, and four focal nodular hyperplasias) in 39 patients were evaluated by means of US, color Doppler US, and contrast-enhanced US performed by using intermittent high-acoustic-power mode. Contrast-enhanced helical computed tomography (11 patients) and US-guided fine needle aspiration (28 patients) were used as reference procedures. Contrast enhancement patterns were defined by means of both subjective and objective analysis, and baseline and contrast-enhanced US scans were reviewed offline. **RESULTS:** Thirteen of 15 HCCs, eight of 10 metastases, and all four hemangiomas with an atypical pattern at baseline US were correctly characterized after SonoVue injection. Two of 15 HCCs and two of 10 metastases remained indeterminate, with no characteristic baseline or contrast-enhanced patterns identified. Baseline US was essential in characterizing all hemangiomas with a typical pattern (n = 7), and color Doppler US with spectral analysis of tumoral vessels was essential in characterizing focal nodular hyperplasia. The percentage of diagnostic agreement with reference procedures was significantly increased ($P < .001$) for contrast-enhanced US compared with baseline US. **CONCLUSION:** Characteristic patterns of US contrast enhancement with SonoVue help in characterizing and differentiating focal hepatic lesions.

53-European Multi-centre Study Evaluating Contrast-enhanced US in the Characterisation of Focal Liver Lesions (FLL)

Purpose: To compare the unenhanced US versus contrast-enhanced US (CEUS) in the characterization of FLL using non-linear imaging modes

Methods and Materials: From 7 centres, 127 patients with 157 FLL were studied using top-of-the-range US scanners. Non Linear Imaging (NLI) mode, fundamental B mode & power Doppler imaging of the liver were performed at baseline. NLI of the hepatic lesion was then carried out at low mechanical index (0.1-0.3) immediately after an intravenous administration of SonoVue (dose: 2.4ml) for the first 3 minutes. Biopsy or combined CT/MR, biochemical markers and other clinical information were used as the SOR. CEUS diagnosis was established on the basis of the tumour vascularity and pattern of enhancement in the arterial and portal phases and degree of contrast uptake within the lesion relative to that of normal liver in the late phase. Diagnostic accuracy and diagnostic performance of contrast enhanced US (CEUS) in identifying the lesion (a) as benign or malignant (b) as its actual tumour type, were compared with baseline US.

Results: Of the 157 lesions, 134 had confirmed SOR diagnosis. At baseline, an US diagnosis was made in 32%, indeterminate in 67% and unknown in 2%; a CEUS diagnosis was made in 90%, indeterminate in 8% and unknown in 3%. Of the 52 benign lesions, accurate diagnosis was made in 34% of the cases on unenhanced US compared with 81% of the cases on CEUS. Of the 82 malignant lesions, accurate diagnosis was made in 28% of the cases on unenhanced US compared with 90% on CEUS. For metastases (n=31), HCCs (n=46), FNH(n=13) and haemangiomas (n=25), accurate diagnosis was made on unenhanced US in 19%, 32%, 31% and 36% respectively on unenhanced US compared with 87%, 78%, 77% and 84% respectively on CEUS. All differences were statistically significant ($p<0.001$)

Conclusion: These data suggest that CEUS improves the characterisation of focal liver lesions compared with unenhanced US and may obviate the need for further diagnostic tests

58-Diagnostic accuracy of contrast-enhanced ultrasound in focal lesions of the liver using cadence contrast pulse sequencing.

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The purpose of this study was to assess the accuracy of Cadence Contrast Pulse Sequencing (Siemens-Acuson, CA) method with injection of SonoVue (Bracco Imaging SpA, Italy) for the detection and characterisation of focal liver lesions in comparison with a reference modality during routine use. METHODS: 138 consecutive patients (62 women, 76 men; mean age: 52 years for women and 63 years for men) corresponding to 144 examinations with 381 lesions participated in this prospective study from December 2002 to November 2003. Inclusion criteria were lesions detected by conventional US and the availability of a reference imaging examination (CT or MR imaging) within three weeks. The underlying liver lesions characterised by a reference modality (including biopsy in 29 lesions) were distributed as follows: haemangioma (n = 56), focal nodular hyperplasia (n = 27), hepatocellular carcinoma (n = 44), hepatocellular adenoma (n = 5), liver metastasis (n = 174), abscess (n = 2), cysts (n = 45), other benign lesions (n = 24) and 3 peritoneal metastases. RESULTS: A significant improvement was reported in the number of detected lesions between contrast-enhanced ultrasound and baseline ultrasonography (351 lesions versus 280 lesions, respectively, $p < 0.01$), whereas no significant difference was noted between contrast-enhanced ultrasound and reference imaging (351 versus 377 lesions, respectively). On the whole, contrast-enhanced ultrasound allowed a complete diagnosis in 96 % of the detected nodules with a significant improvement compared to conventional sonography in which the diagnosis was suspected in only 52 % out of these cases ($p < 0.001$). No significant difference was noted between contrast-enhanced ultrasound and the reference modality concerning characterisation of nodules. CONCLUSION: The present study clearly indicates that contrast-enhanced sonography using Sonovue and Cadence Contrast Pulse Sequencing allows real-time imaging with high accuracy and thus will be a competitive alternative to other modalities such as CT and MR imaging for liver imaging

59-Characterization of small focal liver lesions using real-time contrast-enhanced sonography: diagnostic performance analysis in 200 patients.

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OBJECTIVE: The purpose of this study was to assess the diagnostic performance of real-time contrast-enhanced sonography in characterization of small focal liver lesions (FLLs; $< \text{or} = 3.0$ cm in diameter). **METHODS:** Two hundred small FLLs in 200 patients were examined by contrast-enhanced sonography using a contrast-specific mode of contrast pulse sequencing and a sulfur hexafluoride-filled microbubble contrast agent. The sonographic images were reviewed by 2 independent readers. A 5-point confidence level was used to discriminate malignant from benign FLLs, and specific diagnoses were recorded. The diagnostic performances were evaluated by receiver operating characteristic (ROC) analysis, and the interobserver agreement was analyzed by weighted kappa statistics. **RESULTS:** After review of contrast-enhanced sonography, ROC analysis revealed significant improvement in differentiating between malignant and benign small FLLs that the areas under the ROC curve were 0.856 at baseline sonography versus 0.954 at contrast-enhanced sonography for reader 1 ($P < .001$) and 0.857 versus 0.954 for reader 2 ($P = .003$). The sensitivity, negative predictive value, and accuracy for both readers also improved significantly after contrast agent administration (all $P < .001$). A better result of specific diagnosis was obtained (38.5% [77/200] at baseline sonography versus 80.5% [161/200] at contrast-enhanced sonography for reader 1 and 34.5% [69/200] versus 80.5% [161/200] for reader 2; both $P < .001$) after contrast agent administration, and a better interobserver agreement was achieved ($\text{kappa} = 0.425$ at baseline sonography versus 0.716 at contrast-enhanced sonography). **CONCLUSIONS:** Real-time contrast-enhanced sonography improves the diagnostic performance in small FLLs compared with baseline sonography

60-Characterization of hepatic tumors: value of contrast-enhanced coded phase-inversion harmonic angio.

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OBJECTIVE: Our purpose was to evaluate the value of contrast-enhanced coded phase-inversion harmonic imaging in showing the characteristic intranodular hemodynamics of hepatic tumors. **SUBJECTS AND METHODS.** Using a microbubble contrast agent we performed coded harmonic angio in 163 patients with 192 hepatic tumor nodules: 153 hepatocellular carcinomas, 13 metastases, 14 hemangiomas, eight dysplastic nodules, and four focal nodular hyperplasias. After injecting Levovist, we performed real-time scanning, interval-delay fast low-angle shot imaging, and sweep scanning in the early arterial phase, late vascular phase, and postvascular phase, respectively. **RESULTS:** On contrast-enhanced coded harmonic angio, the typical hemodynamic pattern of hepatocellular carcinomas was shown as abundant tumor vessels supplied from the periphery to the center of the tumor and dense parenchymal tumor staining with fast

washout (sensitivity, 92.8%; specificity, 92.3%). The characteristic hemodynamic pattern of metastases was peripheral tumor vessels with a rim parenchymal stain in the vascular phase followed by a perfusion defect in the postvascular phase (sensitivity, 69.2%; specificity, 100%). Hemangiomas were hypovascular in the early arterial phase with gradual spotty or cotton-wool pooling continuing to the late vascular phase (sensitivity, 92.9%; specificity, 100%). Dysplastic nodules were shown as having no early arterial supply with isovascularity in the late vascular phase (sensitivity, 75%; specificity, 100%). Focal nodular hyperplasias were shown to have a spoked wheel pattern of blood vessels accompanied by dense staining in interval-delay scanning (sensitivity, 100%; specificity, 100%). CONCLUSION: Contrast-enhanced coded harmonic angio is a promising method to provide useful information for the differential diagnosis of hepatic tumors.

61-Contrast-enhanced ultrasound of histologically proven liver hemangiomas.

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Differentiation of small and atypical hemangiomas from other hepatic masses using imaging methods can be difficult, especially in patients with underlying malignant disease. Therefore, contrast-enhanced ultrasound was assessed in patients with histologically confirmed hemangiomas with respect to contrast-enhancing kinetics and tumor characteristics. In 58 patients with indeterminate hepatic lesions demonstrated with at least 2 imaging methods (ultrasound/computed tomography/magnetic resonance imaging), ultrasound-guided liver biopsy revealed hemangioma. In all patients a hepatic neoplasm had been suspected because of underlying malignant disease (n=41), liver cirrhosis (n=15), or growth of the lesion (n=2). All patients underwent nonlinear, low mechanical index real-time contrast-enhanced ultrasound scanning with bolus injections of SonoVue. Peripheral nodular arterial enhancement was detected in 43 patients (74%), whereas the typical metastatic peripheral rim-like enhancement was not observed at all. Strong homogenous arterial enhancement was found in 9 of 58 (16%) patients. In 6 patients (10%), the arterial contrast enhancement pattern could not be determined because of the very small size of the lesions or fibrotic nodules. Forty-five (78%) of the hemangiomas showed homogenous centripetal filling within 180 seconds. CONCLUSION: Contrast-enhanced ultrasound demonstrates typical hemangioma imaging characteristics, that is, peripheral nodular contrast enhancement and iris-diaphragm sign in a high percentage of patients with undetermined lesions. This technique may therefore improve noninvasive functional characterization and differentiation of hemangiomas.

62-Differentiation of focal nodular hyperplasia and hepatocellular adenoma by contrast-enhanced ultrasound.

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Non-invasive differentiation of focal nodular hyperplasia (FNH) and hepatocellular adenoma (HCA) is difficult. The aim of this study was to assess the accuracy of contrast-enhanced phase inversion ultrasound to differentiate between histologically proven FNH and HCA, analysing the arterial and (early) portal venous phase. 32 patients with histological proven FNH (n=24) or HCA (n=8) have been included in this prospective study. Examination technique: Siemens Elegra, phase inversion harmonic imaging (PIHI) with low mechanical index (MI)<0.2-0.3 using SonoVue (BR 1). The contrast enhancing tumour characteristics were evaluated during the hepatic arterial (starting 8-22 s) and early portal venous phase (starting 12-30 s). The image analysis was performed by three examiners. In 23 of 24 patients with FNH the contrast pattern revealed pronounced arterial and (early) portal venous enhancement. Homogeneous enhancement was detected during the hepatic arterial phase in all eight patients with HCA. In contrast to patients with FNH, no enhancement was seen during the portal venous phase. In conclusion, contrast-enhanced phase inversion ultrasound demonstrated pronounced arterial and portal venous enhancement in patients with focal nodular hyperplasia. In contrast, after homogeneous enhancement during hepatic arterial phase, no enhancement during hepatic portal venous phase was detected in patients with hepatocellular adenoma. Therefore, this technique might improve the functional characterization of benign hypervascular focal liver lesions.

63-Contrast-enhanced sonography for the characterisation of hepatocellular carcinomas-- correlation with histological differentiation.

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AIM: We aimed to characterise the vascularisation patterns of hepatocellular carcinomas in contrast-enhanced sonography in correlation to the histological differentiation of hepatocellular carcinomas (HCC), and we also compared the diagnostic value of contrast-enhanced sonography in addition to B-scan sonography and power Doppler sonography. METHODS: 51 hepatocellular carcinomas (21 well differentiated, 27 moderately differentiated, 3 poorly differentiated) were examined: by B-scan sonography, power Doppler sonography and contrast-enhanced sonography using phase or pulse inversion

harmonic imaging with a low mechanical index (< 0.3) and second generation microbubble contrast medium (Optison, SonoVue) as echo-enhancing agent. Lesion vascularity and the kinetics of contrast enhancement within the lesions in the early arterial phase, arterial phase, portal phase and late phase were analysed. The final diagnosis of a HCC was assessed after B-scan sonography, power Doppler sonography and contrast-enhanced sonography. **RESULTS:** Hypervascularisation and/or irregular tumour vascularisation could be detected in 18/48 HCCs (37.5%) by power Doppler sonography. After contrast application, 46/51 HCCs (90.2%) were identified as hyperechoic lesions during the early arterial or arterial phase with no correlation to histological differentiation. In the portal phase and late phase, the echogenicity of HCCs after contrast application was variable. A hypoechoic appearance was noted in 17/51 HCCs (33.3%) in the portal phase and in 21/51 HCCs (41.2%) in the late phase. Moderately differentiated HCCs were more often hypoechoic than well differentiated HCCs ($p = 0.04$). **CONCLUSION:** Contrast-enhanced sonography is highly efficient for the detection of tumour vascularity in HCCs. The majority of HCCs--regardless of histological differentiation--can be characterised as hypervascular lesions in the early arterial and arterial phase with irregular tumour vessels using contrast-enhanced sonography. In addition to B-scan sonomorphology, contrast-enhanced sonography may offer helpful information in patients with liver cirrhosis and focal liver lesions.

64-Analysis of neuroendocrine tumour metastases in the liver using contrast enhanced ultrasonography.

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BACKGROUND: Imaging of liver tumours might be improved by contrast-enhanced ultrasonography, which allows much better demonstration of the microvascular system. The aim of this study was to assess the sonographic morphology and vascularity of neuroendocrine liver metastases. **METHODS:** Forty-eight patients with histologically proven neuroendocrine tumours (NET) and suspected liver metastases--as well as 50 consecutive patients with liver metastases of other origins--were included in a prospective study to evaluate tumour characteristics using B-mode, colour Doppler (CDI) and contrast-enhanced ultrasound (CEUS). **RESULTS:** In 4/48 patients with NET, liver biopsy revealed hemangiomas. The typical B-mode appearance was that of both echo-rich and echo-poor combined, also inhomogeneous depending on the size, and often centrally cystic. With CDI, neuroendocrine metastases appeared hypervascular (66%) or isovascular (34%). Metastases of another origin were hypovascular in 82%. With CEUS, neuroendocrine metastases showed increased arterial enhancement in 38 patients and hypoechoic appearance in the portalvenous phase in 39 patients. In liver metastases of another origin, the sensitivity for malignancy due to a hypoechoic appearance during the portalvenous phase was 100%. In liver metastases of NET origin the sensitivity for malignancy was 39/48 (82%). **CONCLUSIONS:** Neuroendocrine tumour metastases might show characteristics which are similar to hemangiomas. In patients with liver

cirrhosis and severe fatty liver disease the identification of NET with CEUS as a malignant lesion is more difficult. The sensitivity of CEUS in identifying malignancy based on the lack of portalvenous enhancement is higher for metastases of other origin.

65-Characterization of focal liver lesions using contrast-enhanced sonography with a low mechanical index mode and a sulfur hexafluoride-filled microbubble contrast agent.

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PURPOSE: To characterize focal liver lesions (FLLs) using real-time contrast-enhanced sonography (CEUS) with a low mechanical index mode and a sulfur hexafluoride-filled microbubble contrast agent. **METHODS:** CEUS was performed in 190 patients with FLLs, including hepatocellular carcinoma (HCC) (n = 107), liver metastasis (n = 21), intrahepatic cholangiocarcinoma (ICC) (n = 7), liver hemangioma (n = 37), focal nodular hyperplasia (FNH) (n = 11), regenerative nodule (n = 6) and liver lipoma (n = 1). The cadence contrast pulse sequencing technique and the contrast agent SonoVue(R) were used for CEUS examination. The enhancement patterns during the arterial, portal, and late phases were evaluated. **RESULTS:** HCC showed hyperenhancement in 100 (93.5%) of 107 nodules during the arterial phase and hypoenhancement in 102 (95.3%) during the late phase. Liver metastases showed homogeneous enhancement in 8 of 21 (38.1%) nodules and a peripheral regular rim-like enhancement in 11 of 21 (52.4%) nodules during the arterial phase and marked hypoenhancement in 16 of 21 (76.2%) nodules during the late phase. ICC exhibited irregular rim-like enhancement in 4 of 7 (57.1%) nodules during the arterial phase and hypo-enhancement in 7 of 7 (100%) nodules during the late phase. Hemangioma showed peripheral nodular hyperenhancement, and progressive centripetal enhancement was seen in 35 of 37 (94.6%) lesions during the arterial phase. All 11 cases of FNH exhibited homogeneous hyperenhancement during the arterial phase and hyperenhancement (n = 1) or isoenhancement (n = 9) during the late phase. The sensitivity, specificity, and positive predictive value, respectively, were 88.8%, 89.2%, and 91.3% for HCC; 81%, 100%, and 100% for liver metastasis; 57.1%, 100%, and 100% for ICC; 94.6%, 100%, and 100% for liver hemangioma; and 90.9%, 97.8%, and 71.4% for FNH. **CONCLUSIONS:** Low-mechanical index CEUS permits real-time, complete assessment of vascularity in FLLs, which in turn facilitates their characterization. Copyright 2006 Wiley Periodicals, Inc.

66-Prospective multicenter trial evaluating a novel method of characterizing focal liver lesions using contrast-enhanced sonography.

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OBJECTIVE: The purpose of this study was to assess the clinical value and potential impact of SonoVue-enhanced sonography in the characterization of focal liver lesions. **SUBJECTS AND METHODS:** This study included 127 patients with 82 malignant and 52 benign lesions in the liver. Contrast-enhanced sonography was performed using nonlinear imaging modes at low mechanical index (0.1-0.3) to enable real-time visualization of arterial, portal, and late-phase enhancement. Digital recordings of unenhanced sonography and contrast-enhanced sonography were reviewed by on-site investigators and two off-site blinded interpreters. The final diagnosis was based on consensus interpreting of all examinations by another two expert observers with access to CT, MRI, and histologic data; the diagnostic accuracy of contrast-enhanced sonography in identifying the lesion as benign, malignant, or indeterminate and as actual tumor type was compared with baseline sonography. **RESULTS:** For on-site investigators, contrast-enhanced sonography reduced the number of indeterminate diagnoses by 67% and improved the sensitivity and specificity to 90.2% and 80.8%, respectively ($p < 0.001$). For off-site interpreters, contrast-enhanced sonography reduced the number of indeterminate diagnoses by 51-56% ($p < 0.001$); significantly improved sensitivity and specificity to 90.8-95.4% and 83.7-89.8%, respectively ($p < 0.001$); eliminated observers' variability (kappa coefficient: 0.66-0.77); and showed no significant difference in all comparisons in the analysis of lesions measuring less than 1.5 cm, 1.5-2.5 cm, and all sizes combined. Contrast-enhanced sonography did not rely on availability of clinical history to enable the diagnoses, and it reduced the need for further imaging investigations 23.7% to 90.4%. **CONCLUSION:** Contrast-enhanced sonography improves the characterization of focal liver lesions and may limit the need for further investigations.

67-Characterization of focal liver lesions with contrast-specific US modes and a sulfur hexafluoride-filled microbubble contrast agent: diagnostic performance and confidence.

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PURPOSE: To assess whether characterization of solid focal liver lesions could be improved by using ultrasonographic (US) contrast-specific modes after sulfur hexafluoride-filled microbubble contrast agent injection, as compared with lesion characterization achieved with preliminary baseline US. **MATERIALS AND METHODS:** Four hundred fifty-two solid focal hepatic lesions that were considered indeterminate at baseline gray-scale and color Doppler US were examined after microbubble contrast agent injection performed by using low-acoustic-power contrast-specific modes during the arterial (10-40 seconds after injection), portal venous (50-90 seconds after injection), and late (100-300 seconds after injection) phases. Two readers independently and retrospectively reviewed baseline and contrast material-enhanced US scans and classified each depicted lesion as malignant or benign according to standard diagnostic criteria. Sensitivity, specificity, accuracy, and positive and negative predictive values and areas under the receiver operating characteristic curve (Az) were calculated by considering histologic analysis (317 patients) or contrast-enhanced helical computed tomography followed by serial US 3-6 months apart (135 patients) as the reference standards. **RESULTS:** Different contrast enhancement patterns were observed according to lesion characteristics. During the late phase, benign lesions were predominantly hyper- or isoechoic relative to the adjacent liver parenchyma, whereas malignant lesions were predominantly hypoechoic. Review of the contrast-enhanced US scans after baseline image review yielded significantly improved diagnostic performance ($P < .05$). Overall diagnostic accuracy was 49% before versus 85% after review of the contrast-enhanced scan for reader 1 and 51% before versus 88% after review of the contrast-enhanced scan for reader 2. Diagnostic confidence—that is, the Az—was 0.820 before versus 0.968 after review of the contrast-enhanced scan for reader 1 and 0.831 before versus 0.978 after review of the contrast-enhanced scan for reader 2. **CONCLUSION:** The use of contrast-specific modes with a sulfur hexafluoride contrast agent led to improved characterization of solid focal liver lesions. Copyright RSNA, 2004

69-Contrast ultrasound imaging in focal liver lesions: diagnostic value and guidelines

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The recent introduction of high quality scanners and contrast agents for ultrasound deeply modifies diagnosis strategy in focal liver lesions by using validated criteria. Non-linear imaging methods using low mechanical index ($MI < 0.2$) and second generation contrast agents allow real-time continuous imaging with concomitant limitation in background tissue signal and also in agent collapse for a high quality contrast imaging giving dramatic improvement in detection and characterization of lesions. Interpretation is based on the presence of contrast agent within the lesion or not (hyper-, hypo- or isosignal) and the delay after injection (arterial, portal or parenchymal or late phase) as previously used by non-ultrasound methods. This allows an easy differentiation of benign from malignant lesions. Moreover, this allows complete characterization in 85 to 95% of all focal liver lesions and 75% in hepatocellular carcinomas. Those results markedly improve ultrasound accuracy compared to conventional sonography and so put contrast-enhanced sonography among recommended non-invasive imaging methods for focal liver lesions with changes in diagnostic strategy according to the lesion type and actual place of US methods. It is recommended to use contrast ultrasound methods in cancer staging for an optimal detection of liver metastases as well as in characterization of lesions detected during conventional sonography with a consecutive decrease of cost-diagnosis ratio.

71-The use of contrast-enhanced ultrasound in the management of the cirrhotic patient and for detection of HCC.

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Hepatocellular carcinoma (HCC) is the leading cause of death in liver cirrhosis. Ultrasound (US) is widely accepted as the screening imaging modality of choice for HCC in patients with a history of chronic liver disease. However, the US characteristics of HCCs are non-specific and thus, other imaging techniques or biopsy are usually necessary to characterize focal liver lesions (FLL) and confirm malignancy. Blood flow to HCC is mainly arterial, making dynamic CT and MRI the most commonly used techniques to detect the characteristic arterial hypervascularization. Recently, the development of second-generation US contrast agents and microbubble-specific software has changed the role of US in real-time evaluation of the macro and microvascularization

of FLLs. With this technology, the accuracy of US in the diagnosis of HCC and its differentiation from other FLLs such as regenerating nodules has improved dramatically. In addition, contrast-enhanced ultrasound may also be a useful tool in the staging of HCC and in the evaluation of percutaneous treatment.

72-Characterization of small nodules in cirrhosis by assessment of vascularity: the problem of hypovascular hepatocellular carcinoma.

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In a prospective study, we examined the impact of arterial hypervascularity, as established by the European Association for the Study of the Liver (EASL) recommendations, as a criterion for characterizing small (1-3 cm) nodules in cirrhosis. A total of 72 nodules (1-2 cm, n = 41; 2.1-3 cm, n = 31) detected by ultrasonography in 59 patients with cirrhosis were included in the study. When coincidental arterial hypervascularity was detected at contrast perfusional ultrasonography and helical computed tomography, the lesion was considered to be hepatocellular carcinoma (HCC) according to EASL criteria. When one or both techniques showed negative results, ultrasound-guided biopsy was performed. In cases with negative results for malignancy or high-grade dysplasia, biopsy was repeated when an increase in size was detected at the 3-month follow-up examination. Coincidental hypervascularity was found in 44 of 72 nodules (61%; 44% of 1-2-cm nodules and 84% of 2-3-cm nodules). Fourteen nodules (19.4%) had negative results with both techniques (hypovascular nodules). Biopsy showed HCC in 5 hypovascular nodules and in 11 of 14 nodules with hypervascularity using only one technique. All nodules larger than 2 cm finally resulted to be HCC. Not satisfying the EASL imaging criteria for diagnosis were 38% of HCCs 1 to 2 cm (17% hypovascular) and 16% of those 2 to 3 cm (none hypovascular). In conclusion, the noninvasive EASL criteria for diagnosis of HCC are satisfied in only 61% of small nodules in cirrhosis; thus, biopsy frequently is required in this setting. Relying on imaging techniques in nodules of 1 to 2 cm would miss the diagnosis of HCC in up to 38% of cases. Any nodule larger than 2 cm should be regarded as highly suspicious for HCC.

73-Evaluation of hepatocellular carcinoma using SonoVue, a second generation ultrasound contrast agent: correlation with cellular differentiation.

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The appearance of hepatocellular carcinoma (HCC) with contrast-enhanced ultrasound (CEUS) in the vascular phase is described and evaluated as to whether the enhancement pattern correlates with the degree of cellular differentiation. One hundred four HCCs were prospectively evaluated with CEUS using coherent-contrast imaging (CCI) and SonoVue with a low mechanical index (<0.2). The enhancement of HCCs in the vascular phase was analyzed according to the degree of pathological differentiation obtained by fine-needle biopsy. In the arterial phase, all HCCs except for four well differentiated ones (96.2%) showed enhancement ($P<0.05$). Histological differentiation of hypoechoic lesions in the early portal phase (7 HCCs; 16%) significantly differed from hyperechoic (1 HCC; 1%) or isoechoic lesions (87 HCCs; 83.6%) ($P<0.05$), with a significant probability of a worse differentiation in hypoechoic lesions. Histological differentiation of isoechoic lesions in the late phase (30 HCCs; 28.8%) significantly differed from hypoechoic lesions (74 HCCs; 71.2%) ($P<0.05$), with a significant probability of a better differentiation in isoechoic lesions. CEUS using CCI and SonoVue revealed enhancement in the arterial phase in >95% of HCCs, with a few well-differentiated cases not being diagnosed due to the absence of enhancement. Echogenicity in the portal and late phases correlated with cellular differentiation.

74-Liver metastases from rectal carcinoma: disease progression during chemotherapy despite loss of arterial-phase hypervascularity on real-time contrast-enhanced harmonic sonography at low acoustic energy.

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We used a new sonographic technique, real-time contrast-enhanced harmonic sonography at low acoustic energy, to evaluate liver perfusion and liver metastases from colorectal cancer in a 73-year-old woman after chemotherapy. After 6 weeks of chemotherapy, liver metastases that had been clearly visible on conventional sonography before chemotherapy were no longer detectable on conventional sonography but were still

evident on contrast-enhanced sonography. At about 6 months after initiation of chemotherapy, the lesions were all visible again on conventional sonography and had become significantly larger, although some no longer showed contrast enhancement during the arterial phase. In this case, changes in arterial perfusion over time did not parallel the response of liver metastases to chemotherapy. Copyright 2003 Wiley Periodicals, Inc.

75-Comparison of contrast-enhanced ultrasonography versus baseline ultrasound and contrast-enhanced computed tomography in metastatic disease of the liver: diagnostic performance and confidence.

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AIM: The aim of this study was to compare contrast-enhanced ultrasonography (CEUS) to baseline US and contrast-enhanced computed tomography (CT) in metastatic disease of the liver diagnosed or suspected by US during presurgical staging or postsurgical follow-up for primary malignancies. **MATERIALS AND METHODS:** Two hundred-fifty-three patients considered suitable for US due to the complete explorability of the liver and with one to five proven or suspected liver metastases at baseline US were included. All patients underwent US before and after microbubble injection, and multiphase contrast-enhanced CT. Independent panels of readers reviewed US and CT scans and recorded liver metastases according to a 5-grade scale of diagnostic confidence. Sensitivity, specificity (diagnostic performance) and area under the receiver operating characteristics (ROC) curve (diagnostic confidence) were calculated.

RESULTS: Reference standards revealed no metastases in 57/253, more than five in 59/253, and one to five in 137/253 patients. In patients with one to five metastases, CEUS versus baseline US revealed more metastases in 64/137 and the same number in 73/137 patients while CEUS versus CT revealed more metastases in 10/137, the same number in 99/137, and lower number in 28/137. Sensitivity, specificity, and area under ROC curve of CEUS (83%, 84%, 0.929, respectively) differed from baseline US (40%, 63%, 0.579, respectively; $P < 0.01$) while did not differ from CT (89%, 89%, 0.945, respectively; $P > 0.05$). **CONCLUSION:** CEUS improved liver metastases diagnosis in comparison with baseline US while it revealed similar diagnostic performance and confidence to contrast-enhanced CT in patients considered suitable for US and with proven or suspected liver metastases at baseline US.

77-Differential patterns of contrast enhancement in different focal liver lesions after injection of the microbubble US contrast agent SonoVue

[Article in English, Italian]

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PURPOSE: To identify differential contrast enhancement patterns in different focal hepatic lesions after injection of the microbubble contrast agent SonoVue using high or low acoustic power imaging. **MATERIAL AND METHODS:** Forty-seven focal hepatic lesions (1-8 cm) were detected in 45 patients at unenhanced gray-scale ultrasound (US) and evaluated by color Doppler (CD) US with spectral analysis of tumoral vessels. Lesions were subsequently evaluated by US contrast specific modes after IV bolus administration of 2,4-4,8 ml of SonoVue, by intermittent high acoustic power (18 patients) or by continuous low acoustic power imaging (27 patients), during arterial, portal and late phase. Subjective evaluation of lesions appearance before and after SonoVue injection was performed. For final diagnosis multiphase helical CT (21 patients) and/or fine needle US guided biopsy (24 patients) were considered as the reference procedures. **RESULTS:** Final diagnoses comprised 22 hepatocellular carcinomas (HCCs; 1,5-6 cm), 2 macroregenerative nodules (RNNs; 1-2 cm), 10 metastasis (2-3,5 cm), 10 hemangiomas (2-6 cm) and 3 focal nodular hyperplasias (FNHs; 1-3 cm). On CD evaluation HCCs revealed peripheral basket shaped (12/22) or intranodular (10/22) arterial pattern while, after SonoVue injection HCCs revealed diffuse contrast enhancement during arterial phase with contrast washout during portal and late phase. Metastases did not reveal flow signals on CD or contrast enhancement after SonoVue injection, except for 2 metastases which revealed peripheral and central vessels on CD and a diffuse contrast enhancement during arterial phase, appearing hypoechoic to the adjacent liver during portal and late phase. RNNs revealed dotted contrast-enhancement during portal and late phase with isoechoic appearance to the adjacent liver. Hemangiomas revealed some peripheral venous flows on CD and a peripheral nodular contrast enhancement during arterial phase with a centripetal fill-in during portal and late phase. FNHs revealed low resistance peripheral or central arterial vessels and a diffuse contrast enhancement during arterial phase, preceded or not by central spoke wheel shaped contrast enhancement, and a persistent iso-hyperechogenicity during portal and late phase. **CONCLUSIONS:** SonoVue injection has showed to identify differential contrast enhancement patterns in different focal hepatic lesions.

78-Focal liver lesions: can SonoVue-enhanced ultrasound be used to differentiate malignant from benign lesions?

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OBJECTIVE: To evaluate whether contrast-enhanced ultrasound (CEUS) with SonoVue could differentiate malignant focal liver lesions (FLLs) from benign lesions and provide lesion type diagnoses. **MATERIALS AND METHODS:** Four hundred fifty-six patients with 554 FLLs were examined by CEUS with SonoVue using low mechanical index, nonlinear imaging techniques. Each lesion was characterized by 2 independent off-site readers as malignant or benign and given specific lesion type diagnosis, if possible, both at baseline ultrasound (US) and after SonoVue administration (CEUS). The final diagnosis was achieved by histopathology obtained from biopsy or surgical specimens, or by typical manifestation on contrast-enhanced CT or MRI. **RESULTS:** The diagnostic accuracies of the 2 readers were 41.9% and 35.2% for baseline US, which improved significantly to 87.2% and 87.9% for CEUS ($P < 0.05$). Interreader agreement also increased with CEUS compared with baseline US (κ value changed from 0.49 to 0.77). The accuracy for lesion type diagnosis was 38.4% and 32.5% for baseline US, which increased to 77.6% and 78.0% for CEUS ($P < 0.05$). **CONCLUSIONS:** CEUS with SonoVue improves differentiation between malignant and benign FLLs, and also provides improved lesion type (differential) diagnosis.

79-Characterization of focal liver lesions: comparative study of contrast-enhanced ultrasound versus spiral computed tomography.

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The purpose of this study was to compare the diagnostic accuracy of contrast-enhanced ultrasound (CEUS) with spiral computed tomography (SCT) for the characterization of focal liver lesions (FLL) and to determine the degree of correlation between the two techniques. Seventy-seven FLL (45 hepatocellular carcinomas; 12 metastases; ten hemangiomas; two regenerating/dysplastic nodules; eight focal nodular hyperplasias) detected with ultrasound (US) were prospectively evaluated by CEUS using a second-generation contrast agent and SCT (with an interval of no more than one month between the two techniques). Independent observers made the most probable diagnosis and the results were compared with the final diagnoses (histology $n = 59$; MRI $n = 18$). Statistical analysis was performed by the Chi-square and Kappa tests. CEUS provided a correct,

specific diagnosis in 69/77 (90%) of the FLL, while SCT did so in 67/77 (87%). The sensitivity, specificity, and diagnostic accuracy for malignancy were 91%, 90%, and 91%, respectively, for CEUS and 88%, 89%, and 88%, respectively, for SCT. No statistically significant difference was found between CEUS and SCT in the characterization of FLL ($p > 0.05$). In addition, agreement between the two imaging techniques was good ($k = 0.75$). We conclude that CEUS and SCT provide a similar diagnostic accuracy in the characterization of FLL, with a good degree of correlation between the two techniques.

80-Characterization of focal liver lesions with a new ultrasound contrast agent using continuous low acoustic power imaging: comparison with contrast enhanced spiral CT.

[Article in English, Italian]

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PURPOSE: To evaluate the concordance of the enhancement patterns of a new ultrasound contrast agent (SonoVue) with those obtained with dual-phase contrast-enhanced spiral CT (CE-CT) in the characterization of focal liver lesions (FLLs).
MATERIALS AND METHODS: Sixty-two patients with focal liver lesions discovered at ultrasound and also studied with CECT underwent contrast-enhanced ultrasound using continuous low acoustic power imaging after receiving a 2.4 ml bolus of the new US contrast agent SonoVue, consisting of a dispersion of sulphur hexafluoride microbubbles. The examinations were made using ATL HDI-5000, Acuson SEQUOIA and Aloka 5500 Prosound ultrasound systems with 5.2 MHz curved-array probes. The concordance between US and CE-CT images was evaluated on site by two radiologists blinded to CT
RESULTS: The FLLs were assessed in the arterial (20 s after CM injection), portal (after 45-60 s) and late (after 120 s) phases for: 1) presence/absence of enhancement 2) distribution of enhancement (homogenous or target distribution, centripetal or centrifugal flow, and other), 3) qualitative enhancement pattern (hyperechoic, hypoechoic, or isoechoic) versus normal liver parenchyma. **RESULTS:** The concordance between SonoVue-enhanced US and CE-CT was 85%. Moreover during portal venous phase with CEUS it was possible to differentiate between malignancy or benignity of 91% of lesions.
CONCLUSIONS: The preliminary data obtained in this study suggest that continuous low acoustic power imaging and contrast-enhanced US show similar results to CT in contrast distribution and contrast enhancement patterns.

81-Usefulness of contrast-enhanced perfusional sonography in the assessment of hepatocellular carcinoma hypervascular at spiral computed tomography.

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BACKGROUND/AIMS: Diagnosis of hepatocellular carcinoma (HCC) relies strongly on the detection of hypervascularity in the arterial phase and, in this setting, spiral computed tomography (CT) is the most widely used method. This prospective study aimed to investigate the usefulness of low mechanical index harmonic ultrasound (US), using a second generation contrast-enhanced technique, in the assessment of vascular pattern of HCC shown to be hypervascular at spiral CT. **METHODS:** A total of 79 cirrhotic patients with 103 nodules (mean \pm -SD 28 \pm -13 mm) with arterial hypervascularity at spiral CT were studied. US examination was performed by perfusional sonography, using a new dedicated technology (CnTI-Esaote trade mark), operating at low mechanical index, after injection of a second generation contrast agent (SonoVue trade mark -Bracco), allowing detection of tumoral flow during arterial phase. **RESULTS:** Selective arterial enhancement on perfusional sonography was observed in 94 /103 nodules (91.3%), with a sensitivity of 66.6, 87.5, 91.7, and 97.3% in nodules \leq 1 cm, $>1\leq$ 2 cm, $>2\leq$ 3 cm, and >3 cm respectively. **CONCLUSIONS:** Perfusional sonography shows good diagnostic agreement with spiral CT in hypervascular HCC and may be proposed for the immediate vascular characterization of nodules detected at US and used as second imaging technique to confirm hypervascularity in cirrhotic nodules.

82-Contrast-enhanced sonographic appearance of hepatocellular carcinoma in patients with cirrhosis: comparison with contrast-enhanced helical CT appearance.

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OBJECTIVE: We sought to investigate the efficacy of contrast-enhanced sonography using a second-generation contrast agent for the evaluation of hepatocellular carcinoma in patients with cirrhosis by comparing the results to those obtained with contrast-enhanced helical CT. **SUBJECTS AND METHODS:** Between October 2002 and March 2003, 74 patients with cirrhosis (60 men and 14 women; age range, 47-80 years; mean age, 67 years) who had a single nodule of hepatocellular carcinoma were selected to be studied from a cohort of 437 patients with cirrhosis. The size range of the 74 nodules was 9-65 mm (mean, 28.2 mm). Twenty-eight (38%) were 20 mm smaller (range, 9-20 mm; mean, 16.6 mm), and 46 (62%) were larger than 20 mm (range, 21-65 mm; mean, 35.2 mm). Contrast-enhanced sonography was performed at a low mechanical index after IV administration of the contrast agent SonoVue. CT scans were obtained in all patients. The enhancement pattern related to tumor hypervascularity was analyzed. The chi-square test was used for statistical analysis. **RESULTS:** For the 28 hepatocellular carcinomas 20 mm or smaller, contrast-enhanced sonography showed 15 (53.6%) as hypervascular and 10 (35.7%) as avascular; three (10.7%) carcinomas were missed. On CT, 12 (42.9%) of the 28 hepatocellular carcinomas appeared hypervascular, 13 (46.4%) appeared hypovascular, and three (10.7%) were missed. For the 46 hepatocellular carcinomas larger than 20 mm, contrast-enhanced sonography showed 42 (91.3%) as hypervascular and four (8.7%) as avascular. On CT, 35 (76.1%) hepatocellular carcinomas appeared hypervascular, eight (17.4%) appeared hypovascular, and three (6.5%) were missed. Differences between CT appearance of hepatocellular carcinomas and contrast-enhanced sonographic appearance of the carcinomas were not statistically significant. Concordance between contrast-enhanced sonographic and CT appearances was observed in 61 (82.4%) of 74 cases. **CONCLUSION:** Contrast-enhanced sonography is similar to CT for detecting hepatocellular carcinoma hypervascularity. It could be complementary to conventional unenhanced sonography for evaluation of liver nodules.

83-Characterization of hepatic tumors with contrast-enhanced ultrasound and digital grey-scale analysis]

[Article in German]

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PURPOSE: The characterization of different liver tumors is of therapeutic and prognostic relevance and has been the purpose of several studies. Although ultrasound offers the opportunity to detect hepatic tumors without ionizing radiation, its previous techniques did not lead toward a definitive differentiation of different tumor entities. The purpose of this study was the clinical evaluation of contrast enhanced ultrasound followed by quantitative digital analysis in patients with focal hepatic tumors. **MATERIALS AND METHODS:** In a prospective study, 50 patients (18 females, 32 males, age 28 to 83 years, mean age 59.4 years) with liver tumors previously detected by CT (n = 47) or MRI

(n = 3) were examined by ultrasound of the upper abdomen using conventional technique and phase inversion technique after intravenous application of sulfur-based contrast enhancer SonoVue. At scheduled intervals after application of the contrast enhancer, a digital image was stored and the characteristic signal course of each lesion determined semiquantitatively. The gold standard was either resection (n = 17), percutaneous needle biopsy (n = 19) or the clinical course (n = 14). RESULTS: While the percentage of tumors correctly characterized by CT/MRI amounted to 78 %, the percentage increased from 60 % using conventional ultrasound to 86 % using contrast enhanced ultrasound including grey-scale analysis. Typical graphs were achieved for different tumor entities on digital grey-scale analysis. The optimal intervals for the differentiation of particular entities were 20 and 100 seconds after injection. CONCLUSION: Quantification of contrast enhanced ultrasound is an addition to the previous diagnostic procedure in hepatic tumors. It offers the possibility of an investigator-independent characterization of lesions and should be evaluated in further studies.

84-Characterization of focal liver lesions: comparison of pulse-inversion harmonic contrast-enhanced sonography with contrast-enhanced CT.

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PURPOSE: To compare the efficacy of contrast-enhanced pulse-inversion harmonic sonography for the characterization of focal liver lesions with that of contrast-enhanced helical CT. METHODS: Real-time contrast-enhanced sonography (CEUS) using Sonovue and contrast-enhanced CT (CECT) were performed on 109 patients with focal liver lesions, including 61 hepatocellular carcinomas, 15 liver metastases, 5 cholangiocellular carcinomas, 12 hemangiomas, 5 regenerative nodules, 3 adenomas, 3 focal nodular hyperplasias, 4 focal necroses, and 1 angiomyolipoma prior to surgery or percutaneous needle biopsy. The diagnostic performance was assessed by using histopathological results as reference standards. RESULTS: Three cases were missed on CEUS, and 7 cases were missed on CECT. These 10 missed cases were excluded from paired statistical comparison. Ten cases were misdiagnosed on CEUS and 17 cases were misdiagnosed on CECT. The overall accuracy was 89.9% (89/99) for CEUS and 82.8% (82/99) for CECT. The difference between CEUS and CECT was not statistically significant. Concordance between CEUS and CECT was observed in 90.9% (90/99) cases. CONCLUSION: Real-time pulse-inversion harmonic CEUS with Sonovue is comparable with CECT in the characterization of focal liver lesions. Copyright 2007 Wiley Periodicals, Inc.

87-The safety of Sonovue® in abdominal applications: Retrospective analysis of 23188 investigations

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Abstract

The aim of the present retrospective study was to assess the incidence of adverse events (AE) of a second-generation ultrasound contrast agent in real clinical practice. A total of 28 Italian Centres provided data on the postmarketing use of SonoVue® (Bracco Spa, Milan, Italy) in abdominal examination performed between December 2001 and December 2004. A total of 23 188 investigations were reported. No fatal event occurred. AEs were reported in 29 cases, of which only two were graded as serious; the rest, 27, were nonserious (23 mild, three moderate and one severe). The overall reporting rate of serious AE was 0.0086%. Overall, only four AEs required treatment (two serious, two nonserious including one moderate and one severe AEs). In conclusion, the present large-scale retrospective analysis showed that SonoVue has a good safety profile in abdominal applications, with an AE reporting rate lower than or similar to that reported for radiologic and magnetic resonance contrast agents. (E-mail: Piscagl@med.unibo.it)

88-Transcranial color-coded duplex sonography.

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Transcranial color-coded duplex sonography (TCCS) enables the reliable assessment of intracranial stenoses, occlusions, and cross-flow through the circle of Willis without using potentially hazardous compression tests. Transpulmonary ultrasound contrast agents (UCAs) increase the number of conclusive TCCS investigations, which suggests that UCAs may provide the conclusive evaluation of intracranial arteries in most patients with ischemic cerebrovascular disease. Further, contrast-enhanced TCCS may become an important tool both for the management of acute ischemic stroke by assessing intracranial hemodynamics and the displacement and diameter changes in supratentorial ventricles. TCCS is useful for the detection and monitoring of intracranial vasospasm, may visualize larger supratentorial hematomas with subcortical location and hemorrhagic transformation of ischemic infarcts, and provides the incidental detection of cerebral aneurysms and arteriovenous malformations. Second-generation UCAs and new ultrasound machines are very likely to further increase the frequency of conclusive TCCS

studies. Power-based three-dimensional, contrast-enhanced TCCS is an important further development, which would make the method much less operator dependent. Site-targeted UCAs appear to provide a new and exciting method for ultrasonic diagnosis and management of patients with ischemic cerebrovascular disease.

89-Diagnosis of arterial disease of the lower extremities with duplex ultrasonography.

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The development of duplex scanning carries the prospect of an entire non-invasive work-up of patients with peripheral arterial occlusive disease. To obtain the best available estimates of its diagnostic accuracy, a meta-analysis of 71 studies evaluating duplex scanning was performed. Independent methodological judgement left 16 studies for data extraction. Pooled estimates (95 per cent confidence interval of sensitivity and specificity for detection of a stenosis greater than or equal to 50 per cent or occlusion in the aortoiliac tract were 86 (80-91) per cent and 97 (95-99) per cent respectively. The results for the femoropopliteal tract compared well with this, with a sensitivity of 80 (74-85) per cent and a specificity of 96 (94-98) per cent. The accuracy of detection of a stenosis greater than or equal to 50 per cent or an occlusion in the infragenicular arteries was lower with a sensitivity and specificity of 83 (59-96) per cent and 84 (69-93) per cent respectively. Duplex scanning is an accurate tool for assessment of atherosclerotic lesions in the aortoiliac and femoropopliteal tract and can replace routine preinterventional angiography in a substantial number of patients.

90-Vascular surgery without arteriography: use of Duplex ultrasound.

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Although contrast arteriography has served as the historical 'gold standard' for diagnosis of arterial disease, recent improvements in noninvasive diagnostic methods have made it possible to plan surgical treatment without subjecting patients to this invasive procedure. This approach avoids both the risks and costs associated with arteriography. Duplex scanning has become the standard noninvasive test for extracranial carotid artery disease, and it can also be used to directly evaluate the lower extremity arteries. In addition to the standard duplex criteria for classification of carotid stenosis, new criteria are available that reflect the stenosis thresholds identified in randomized clinical trials. Clinical experience has clearly shown that carotid endarterectomy can be performed safely based

on the duplex scan alone in the majority of patients: however, arteriography is still indicated in selected cases. The evaluation of lower extremity arterial disease requires examination of multiple arterial segments, and most vascular surgeons still rely on the anatomic detail provided by arteriography for preoperative planning. Still, it may be possible to avoid formal preoperative arteriography in selected patients by using a combination of lower extremity duplex scanning and intraoperative arteriography. Further developments in noninvasive testing will continue to reduce the need for diagnostic arteriography prior to direct arterial surgery.

91-Interobserver variability in aortoiliac and femoropopliteal duplex scanning.

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PURPOSE: The interobserver variability of aortoiliac and femoropopliteal duplex scanning in peripheral arterial occlusive disease was assessed. **METHODS:** Two experienced, independent vascular technologists investigated in random order 61 consecutive patients sent to the vascular laboratory for investigation of the aortoiliac or femoropopliteal arteries. In each aortoiliac vessel segment, the proximal, mid, and distal peak systolic velocities (PSVs) were measured, and corresponding PSV ratios were calculated. The superficial femoral artery was divided in 10-cm segments with a roll-centimeter taped from the upper patella margin. Interclass correlation coefficients (ICCs) were calculated as a means of appreciating the measurement variability of the PSV ratio values as a continuous variable and the Kappa value for the PSV ratio categories of less than 2.0, 2.0 through 3.0, more than 3.0, and occlusions. **RESULTS:** The overall ICC and Kappa values were 0.72 (95% CI, 0.63-0.79) and 0.53 for the aortoiliac tract and 0.85 (95% CI, 0.79-0.89) and 0.73 for the femoropopliteal tract. Agreement in the PSV ratio categories was 85% in the aortoiliac and 87% in the femoropopliteal tract. Interobserver variation increased markedly with increasing PSV ratio. In the PSV ratio category between 2.0 to 3.0, indicating a borderline stenosis, a substantial disagreement was found (aortoiliac, 1 of 8 agreement; femoropopliteal, 2 of 8 agreement). **CONCLUSION:** A moderate interobserver agreement was found in the duplex investigation of the aortoiliac and femoropopliteal arteries. One should be aware of this in clinical decision making, especially in cases of borderline stenoses. In these cases, repetition of the measurement or additional diagnostics is advocated.

92-Transcranial color-coded duplex sonography, magnetic resonance angiography, and computed tomography angiography: methods, applications, advantages, and limitations.

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Transcranial color-coded duplex sonography (TCCD), magnetic resonance angiography (MRA), and computed tomography angiography (CTA) are novel noninvasive or minimally invasive techniques for the study of the intracranial circulation. TCCD is relatively inexpensive and permits bedside examination. It improves the accuracy and reliability of conventional transcranial Doppler studies. The main limitation of TCCD are the ultrasonic windows. They restrict the area of insonation to the major cerebral arteries and the proximal part of its branches, lower the spatial resolution, and may prevent transtemporal insonation. Using MRA, both large and small intracranial arteries and veins can be imaged by selecting the appropriate imaging parameters. MRA provides morphologic information about the cerebral vessels, relying on blood flow as the physical basis for generating contrast between stationary tissues and moving spins. MRA is highly sensitive for the detection of occlusive disease in large intracranial arteries. However, with bright blood techniques the degree of stenosis tends to be exaggerated. Flow direction, eg, in collaterals, can be determined by selective or phase-contrast MRA. Perfusion imaging techniques provide information about blood flow at the capillary level. Diffusion imaging depicts molecular motion. TCCD and MRA used in combination or alone may eliminate the need for intra-arterial digital subtraction angiography (DSA) in most patients studied for occlusive cerebrovascular disease. DSA may be reserved for those patients where there is disagreement among the noninvasive techniques, and for the diagnosis of cerebral aneurysms and arteriovenous malformations. CTA relies on spiral CT technology and intravenous contrast injection. To date, intracranial use has been predominantly for the diagnosis of aneurysms. The role of CTA for the detection of nonaneurysmal intracranial vascular disease has yet to be established.

93-Transcranial color-coded duplex sonography in the evaluation of collateral flow through the circle of Willis.

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PURPOSE: To determine the sensitivity, specificity, and positive and negative predictive values of transcranial color-coded duplex sonographic (TCCD) evaluation of cross flow through the anterior (ACoA) and posterior (PCoA) communicating arteries in patients with occlusive cerebrovascular disease. **METHODS:** We studied prospectively 132 patients (37 women, 95 men; mean age, 60 years) with stenoses of more than 69% reduction in vessel diameter (n = 93) and occlusions (n = 52) of the internal carotid artery, and three occlusions of the basilar artery. The sonographer was aware of extracranial sonographic findings but was blinded to the results of cerebral angiography. **RESULTS:** Nine patients (7%) with thick bones preventing transtemporal insonation and three patients (3%) with occlusions of the middle (n = 3) and anterior (n = 1) cerebral

arteries were excluded. Sensitivity of TCCD for detection of collateral flow through the ACoA in patients with occlusive carotid artery disease was 98%, specificity was 100%, positive predictive value was 100%, and negative predictive value was 98%. The corresponding values for the PCoA were 84%, 94%, 94%, and 84%, respectively. All three functional PCoAs were identified in patients with occluded basilar arteries. CONCLUSION: TCCD is a valuable method for noninvasive evaluation of cross flow through the ACoA in patients with adequate sonographic windows. However, TCCD evaluation of cross flow through the PCoA is less reliable, because hemodynamic criteria may cause falsely positive and falsely negative results.

98-SonoVue (BR1), a new long-acting echocontrast agent, improves transcranial colour-coded duplex ultrasonic imaging.

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BACKGROUND AND PURPOSE: Visualization of normal or pathological flow in the intracranial arteries using transcranial colour-coded duplex sonography (TCCS) is occasionally obstructed by insufficient temporal bone windows, unfavourable insonation angles, or low flow velocities or volumes. SonoVue (BR1) is a new, long-acting echocontrast agent, which could be helpful in these instances. **METHODS:** SonoVue was studied in two separate multicentre investigations of patients with insufficient bone windows (BR1-014 with 73 patients and BR1-017 with 40 patients) using an identical protocol. The agent was given intravenously as a bolus in four different doses (0.3, 0.6, 1.2 and 2.4 ml). Evaluation was performed on-site and off-site with video clips. **RESULTS:** The contrast agent was well tolerated. As compared to the precontrast scans, SonoVue facilitated the visualization of vessel patency, stenosis, occlusion, and collateral flow, decreased the need for additional tests, and had an impact on the patient's treatment. In 66-74%, a non-diagnostic investigation was converted into a diagnostic investigation. The highest dose (2.4 ml) allowed for a clinically useful signal enhancement of a median 1.9-6.3 min. **CONCLUSIONS:** SonoVue at a single dose of 1.2 or 2.4 ml is effective in increasing the detection of normal or pathological flow in the intracerebral arteries in patients who do not have a fully diagnostic unenhanced TCCS examination. Copyright 2002 S. Karger AG, Basel

99-Prognostic value of transcranial sonography in acute stroke patients.

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Accurate assessment of stroke is critical for patient prognosis and selection of appropriate treatment regimens in order to optimize patient outcomes. Advanced neurosonologic techniques are straightforward, portable, and cost-effective, representing significant advantages over other noninvasive imaging modalities for monitoring of the hemodynamic status of acute ischemic stroke. Ultrasound findings acquired both early (<3 h from onset of stroke) and later (6-24 h after stroke) have demonstrated feasibility and validity for the detection of stenosis/occlusion of key intracranial structures, such as the middle cerebral artery, and for immediate and unambiguous indication of flow velocities, particularly when contrast enhancement is used. In addition, the target of thrombolysis can be identified and localized, and the success of therapy monitored, by transcranial ultrasound. Finally, transcranial ultrasound can be used to gauge the appropriateness of more complex and costly imaging studies, thereby optimizing utilization of health care resources. Copyright 2008 S. Karger AG, Basel.

100-Prognostic relevance of ultra-early doppler sonography in acute ischaemic stroke: a prospective multicentre study.

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BACKGROUND: Ischaemic stroke can result from a temporary or permanent occlusion of intracranial arteries. In the hyperacute stage of the disease cerebrovascular ultrasound can be used to determine the vascular pathology, but the significance of very early findings on ultrasound is unclear. The present study aimed to assess the prognostic value of doppler ultrasonography within the first hours after stroke for functional outcome. **METHODS:** In a prospective multicentre design, patients with clinical signs of ischaemic anterior-circulation stroke were examined by doppler ultrasonography of the intracranial and extracranial arteries. Patients were separated into three groups according to the findings: normal middle-cerebral artery (MCA); branch occlusions; or a main-stem occlusion. The primary endpoint was functional outcome at 3 months. Logistic regression was used to test the association between the ultrasound diagnosis and functional outcome. **RESULTS:** 361 patients were identified with moderate to severe clinical deficits (National Institutes of Health Stroke Scale score 5-25). Of these, 121 (34%) had a normal

MCA, 176 (48%) had branch occlusions, 7 (2%) had severe MCA stenosis, and 57 (16%) had a main-stem occlusion. 50 of the 57 (88%) patients with main-stem occlusion were dead or dependent 3 months after stroke. An occlusion of the main stem of the MCA within 6 h after stroke was an independent predictor for poor outcome ($p=0.0006$). 50% of patients with ultrasonographic diagnosis of branch occlusions and 63% with normal MCA had a good outcome. Combination of CT scan without early signs of infarction and a normal MCA resulted in a predictive value of 71% for a good functional outcome. INTERPRETATION: Cerebrovascular ultrasonography provides additional functional prognostic information in the hyperacute stage of ischaemic stroke. The technique is practical in a well-resourced unit, can be used to identify patients with high risk for poor functional outcome, and thus would be an appropriate investigation for future trials.

101-The Eligible study: ultrasound assessment in acute ischemic stroke within 3 hours.

[Malferrari G, Bertolino C, Casoni F, Zini A, Sarra VM, Sanguigni S, Pratesi M, Lochner P, Coppo L, Brusa G, Guidetti D, Cavuto S, Marcello N; Eligible Group; SINV Group.](#)

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BACKGROUND: Aims of the study: to identify with echo color Doppler ultrasound of the supra-aortic vessels and transcranial color-coded duplex sonography (TCCD) various patterns of vessel occlusion within 3 h from stroke onset, to compare each group defined at the admission with clinical findings and outcome, and to study the recanalization process, independent of therapy. METHODS: We enrolled 89 consecutive patients (mean age 68.9 years). Ultrasound evaluation was done within 3 h from stroke onset, and was repeated at 3-6 and 24-36 h, at day 5, and at 3 months. At admission, patients were divided into the following groups: internal carotid artery occlusions and stenoses (<50%, 50-69%, > or =70%, near occlusion), middle cerebral artery stenoses and occlusions, tandem occlusions and T occlusions. Vascular recanalization in each group was evaluated. Subgroups were compared for NIH Stroke Scale (NIHSS) and the outcome measures mortality, Barthel index (BI) and modified Rankin scale (mRS). Favorable outcome was defined as mRS < or =2 and BI > or =90. RESULTS: Each subgroup differed significantly for baseline NIHSS ($p < 0.0001$), 3-month mortality ($p = 0.0235$), BI at day 5 ($p = 0.0458$) and mRS at 3 months ($p = 0.0028$), even after adjustment for treatment. T and tandem occlusions were the subgroups with the highest NIHSS scores and the poorest outcomes, and the same subgroups had the worst recanalization rates. CONCLUSIONS: TCCD in the acute setting of stroke patients allows identification of the presence and site of clots, prediction of outcome and study of the dynamic process of vessel recanalization, in both the acute phase and follow-up. (c) 2007 S. Karger AG, Basel

102-Diagnostic transcranial ultrasound perfusion-imaging at 2.5 MHz does not affect the blood-brain barrier.

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The purpose was to assess whether standard ultrasound (US) perfusion-imaging by means of contrast-enhanced transcranial color-coded sonography (TCCS) affects the blood-brain barrier (BBB) in patients with small-vessel disease (SVD). One week after a screening MRI to exclude a preexisting BBB disruption, unilateral TCCS phase inversion harmonic imaging (PIHI) was performed in an axial diencephalic plane after intravenous bolus application of 2.5 mL SonoVue (IGEA, Bracco, Italy). Magnetic resonance imaging (MRI) was performed immediately after US. In five patients, PIHI was performed applying a mean mechanical index (MI) of 0.7 +/- 0.1 for a time period of 2.5 min. MRI was started 12 +/- 2 min after US contrast injection. Comparisons of initial and post-US MRI by four blinded readers did not show any signs of BBB disruption. It is concluded that standard contrast-enhanced US perfusion imaging in patients with SVD did not lead to MRI-detectable BBB changes. This gives further evidence for safety of diagnostic US. Future investigations with larger sample sizes and higher-field MRI might give further insights into potential bioeffects of diagnostic, as well as therapeutic, contrast-enhanced transcranial US.

103-Sustained complete response and complications rates after radiofrequency ablation of very early hepatocellular carcinoma in cirrhosis: Is resection still the treatment of choice?

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If liver transplantation is not feasible, partial resection is considered the treatment of choice for hepatocellular carcinoma (HCC) in patients with cirrhosis. However, in some centers the first-line treatment for small, single, operable HCC is now radiofrequency ablation (RFA). In the current study, 218 patients with single HCC ≤ 2.0 cm (very early or T1 stage) underwent RFA. We assessed 2 primary end points that could be easily compared with those reported for resective surgery: (1) the rate of sustained, local,

complete response and (2) the rate of treatment-related complications. The secondary end point was 5-year survival in the 100 patients whose tumors had been considered potentially operable. After a median follow-up of 31 months, sustained complete response was observed in 216 patients (97.2%). In the remaining 6, percutaneous ethanol injection, selective intraarterial chemoembolization, or resection were used as salvage therapy. Perioperative mortality, major complication, and 5-year survival rates were 0%, 1.8%, and 68.5%, respectively. Conclusion: Compared with resection, RFA is less invasive and associated with lower complication rate and lower costs. RFA is also just as effective for ensuring local control of stage T1 HCC, and it is associated with similar survival rates (as recently demonstrated by 2 randomized trials). These data indicate that RFA can be considered the treatment of choice for patients with single HCC \leq 2.0 cm, even when surgical resection is possible. Other approaches can be used as salvage therapy for the few cases in which RFA is unsuccessful or unfeasible.

108-Guidance and monitoring of radiofrequency liver tumor ablation with contrast-enhanced ultrasound.

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Radiofrequency (RF) treatments of non-resectable hepatic tumors are generally guided with real-time sonography, which, however, cannot differentiate necrotic changes from viable tumor. To achieve complete treatment of hepatic tumors, accurate imaging techniques are needed for close treatment follow-up. Usually contrast-enhanced computed tomography (CT) and magnetic resonance imaging (MRI) are used; however, they can be performed only at the end of treatment sessions. In this field, contrast-enhanced ultrasound (CEUS) has shown to improve the sensitivity of plain ultrasonography. Recently, further developments of contrast-enhanced US technique have significantly increased its clinical utility. Continuous mode, low MI scans performed with harmonic imaging and contrast specific software appears as a very useful technique for the visualization of both macro- and microcirculation with depiction of tumor vascularisation. In our hospital, we have been employing contrast-enhanced sonography with sulphur hexafluoride microbubbles (SonoVue, Bracco, Italy) before, during and immediately at the end of RF ablation procedures to monitor and assess the therapeutic result prior to closing the treatment session. The results obtained in a group of 109 patients with hepatocellular carcinoma (HCC) in liver cirrhosis (192 lesions) and in 53 patients with liver metastases (97 lesions) undergoing a single session of percutaneous RF tumor ablation, showed that the sensitivity of CEUS for the detection of residual tumor was almost equivalent to that of contrast-enhanced helical CT. More importantly, since the introduction of intraoperative CEUS the rate of partially unablated tumors has dropped from 16.1 to 5.9%. Cost-effectiveness and reduction of patients' discomfort related to the need of re-treatment are the two most outstanding advantages of CEUS in this field.

109-Monitoring of liver metastases after stereotactic radiotherapy using low-MI contrast-enhanced ultrasound--initial results.

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The purpose of this study was to monitor liver metastases after radiotherapy using contrast-enhanced ultrasound (CEUS). In 15 patients, follow-up examinations after stereotactic, single-dose radiotherapy were performed using CEUS (low mechanical index (MI), 2.4-ml SonoVue) and computed tomography (CT). Besides tumor size, the enhancement of the liver and the metastases was assessed at the arterial, portal venous, and delayed phases. The sizes of the tumor and of a perifocal liver reaction after radiotherapy measured with CEUS significantly correlated with those measured at CT ($r=0.93$, $p<0.001$). CEUS found a significant reduction of the arterial vascularization in treated tumors ($p<0.05$). In the arterial phase, the perifocal liver tissue was hypervascularized compared to the treated tumor ($p<0.001$); in the late phase, it was less enhanced than the liver ($p<0.001$) and more than the tumor ($p<0.01$). The perifocal liver reaction was also seen in CT, but with a variable enhancement at the arterial (50% hyperdense compared to normal liver tissue), venous, or delayed phase (each with 70% hyperdense reactions). CEUS allows for the assessment of tumor and liver perfusion, in addition to morphological tumor examination, which was comparable with CT. Thus, changes of tumor perfusion, which may indicate tumor response, as well as the perifocal liver reaction after radiotherapy, which must be differentiated from perifocal tumor growth, can be sensitively visualized using CEUS.

111-Comparing the outcomes of radiofrequency ablation and surgery in patients with a single small hepatocellular carcinoma and well-preserved hepatic function.

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GOALS: To compare the efficacy of radiofrequency ablation (RFA) and surgical resection in a group of patients with a Child-Pugh score of 5 and a single HCC less than 4 cm in diameter. **BACKGROUND:** Radiofrequency ablation (RFA) has become a popular

method for treatment of hepatocellular carcinoma (HCC) and has been applied as an alternative primary therapy to surgical resection. **STUDY:** We compared outcomes for 148 patients treated with RFA (n = 55) and those treated surgically (n = 93). **RESULTS:** The rate of local recurrence among patients in the RFA group was significantly higher than in the surgery group (P = 0.005), while the incidence of remote recurrence was similar between the two groups (P = 0.30). The cumulative 1- and 3-year overall survival rates (P = 0.24) and the cumulative 1- and 3-year recurrence-free survival rates (P = 0.54) were not significantly different between the two groups. **CONCLUSIONS:** Despite a higher rate of local recurrence, RFA was found to be as effective as surgical resection for the treatment of single small HCC in patients with well-preserved liver function, in terms of the incidence of remote recurrence and the patients' likelihood of achieving overall and/or recurrence-free survival.

114-Doppler US with perfusion software and contrast medium injection in the early evaluation of radiofrequency in breast cancer recurrences: a prospective phase II study.

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INTRODUCTION: The aim of this study was to determine the efficacy of Doppler ultrasonography (US) with perfusion software and contrast agent injection (DUPC) during radiofrequency (RF) treatment of local recurrent breast cancer. **MATERIALS AND METHODS:** Ten patients were included in this monocentric prospective phase II study. DUPC was performed for each patient the day before treatment and immediately after RF in the operating suite. RF ablation was followed by a total mastectomy. The results of DUPC were compared to the histologic analysis of the operative specimen. **RESULTS:** Before RF, contrast uptake exceeded 70% in 5 lesions and was less than 50% in 5 lesions. Immediately after RF, no vascularization was detected with DUPC in 9 of the 10 lesions. Contrast uptake attaining 30% was depicted in the remaining lesion. At histologic analysis, complete tumour destruction was confirmed in 7 of the 10 operative specimens. **CONCLUSION:** In this study, DUPC is highly efficient and better adapted for the confirmation of tumour destruction in tumours that are hypervascularised before RF compared to hypovascularised lesions.

115-Doppler US with perfusion software and contrast medium injection in the early evaluation of isolated limb perfusion of limb sarcomas: prospective study of 49 cases.

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BACKGROUND: The aim of this study was to prospectively evaluate the use of Doppler ultrasonography with perfusion software and contrast agent injection (DUPC) during isolated limb perfusion (ILP) with high-dose chemotherapy and TNF- α (biochemotherapy) in patients with locally advanced extremity soft tissue sarcoma (STS). **PATIENTS AND METHODS:** Fifty-two patients were prospectively included in this monocentric imaging trial. Three were excluded because the study was incomplete in two patients and one tumour did not exhibit any contrast uptake. DUPC was performed before ILP and on days 1, 7, 15, 30 and 60 after ILP. A total of 292 DUPC were performed on 55 target lesions in 49 evaluable patients. The percentage of contrast uptake was evaluated at each tumour site by two radiologists. The criterion tested was a decrease of more than 50% in intra-tumour contrast uptake compared to the pre-ILP examination. Results were compared with both MRI and histological analysis after resection of residual disease. **RESULTS:** According to MRI and the histological analysis, 25 (51%) patients were good responders (no difference between the four treatment arms) with tumour necrosis exceeding 90% and 24 (49%) were poor responders. As of day +1, the accuracy of DUPC in predicting tumour response was 82% (18/25 good responders and 22/24 poor responders) increasing to 91% at day +7, 95% at day +15 and 96% at day +30. At day +15, DUPC was predictive of a good response in 100% of the cases. **CONCLUSION:** DUPC is a simple technique, allowing early prediction of tumour response after ILP. A new treatment planning scheme can be proposed based on the results of this study.