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Contrast enhanced harmonic ultrasound for differentiating breast tumors – First results

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Abstract. *Purpose*: To investigate the extent to which indeterminate lesions of the breast can be differentiated in the early and late phase after bolus injection of the ultrasound contrast medium Optison®. *Materials and methods*: Fifty female patients (mean age: 49 years) with a altogether 53 preoperatively impalpable indeterminate breast tumors, 20 fibroadenomas and 33 carcinomas, were examined by B-mode imaging and contrast medium-enhanced ultrasound with power Doppler (three patients had multifocal carcinomas). The tumors had a diameter of 5–15 mm (mean diameter: 9 mm). Histological confirmation was performed in all lesions by vacuum biopsies and/or surgical preparation. All examinations were performed with a multifrequency linear array probe (5–10 MHz, Logiq9 and Logiq 7, GE). Power Doppler (PD) and B-mode imaging as well as tissue harmonic imaging (THI) were employed. A bolus of 0.5 ml Optison® was injected intravenously and spreading of the contrast enhancement and washout in the tumors were followed for at least 20 minutes. A low mechanical index was chosen to avoid early destruction of the microbubbles. Maximum tumor size was measured and tumors vessels were evaluated in digital cine ultrasound sequences. *Results*: Without CM administration, 14 of 19 tumor lesions smaller than 10 mm could be distinguished better from the surrounding tissue with THI compared to fundamental B-mode imaging. Both benign (17/20) and malignant (30/33) tumors exhibited increased tumor marginal vessels or intratumoral vessels in the early phase after CM injection. A diffuse contrast medium accumulation was observed in the late phase (8–18 min, mean: 12 min) in 30 of 33 malignant tumors, but in none of the benign tumors. The diagnostic confirmation for this late enhancement was there with 90% for the malignant tumors. *Conclusion*: After intravenous bolus administration of Optison®, breast carcinomas appear to have a prolonged diffuse enhancement of central tumor vasularity in the late phase compared to an earlier marginal vascularity of fibroadenomas.

Keywords: Breast neoplasms, US, ultrasound, contrast media, ultrasound, power Doppler studies, ultrasound, harmonic studies

Abbreviations:
CM: contrast medium; THI: tissue harmonic imaging; DCIS: ductal carcinoma *in situ*.

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1. Introduction

Apart from morphology and the echo structure, tumor vascularity in ultrasound examination of indeterminate breast lesions may be an indication of potential malignancy [1–19]. Irregular vessels within the tumor are regarded as a sign of malignancy while marginal vessels seem to be typical for benign tumors [1,6,9,10,12,17–19]. Power Doppler best identifies vascularization after the intravenous injection of a contrast agent (CM), since this technique allows detection even of low perfusion [7–14]. As a rule, a single injection of contrast medium provides signal enhancement for a maximum of 5 minutes. However, the diagnosis of malignancy remains also uncertain if the presence of marginal, penetrating or central vessels is established [7,12]. The use of an albumin contrast agent (Optison®) and a change of the instrument settings allowed the time window for contrast medium-enhanced vascularization to be extended, enabling tumor vascularity to be detected the late phases. In a previous study, we employed this modified ultrasound technique to analyze vascularity in liver tumors. The time window for contrast medium-enhanced detection extended up to 20 minutes [20].

It was the objective of our study to investigate whether the characteristics of malignant and benign breast tumors differ in the early and late phase after intravenous bolus injection of a second-generation contrast medium.

2. Materials and methods

In a prospective study, 50 female patients (27 to 78 years; mean age: 49) with 53 impalpable indeterminate breast nodules detected either by mammography, ultrasound or by both were further investigated by ultrasound contrast medium (CM). The size of the lesions varied between five and 15 mm in diameter (mean 9 mm). Fifteen patients had previous breast-conserving surgery for carcinoma with supplementary radiotherapy and/or chemotherapy more than two years ago. In 10 of them, mammography revealed a suspect lesion, ultrasound in all 15. The tumor did not show any relation to the surgical scar. The remaining 35 patients came with a suspicious mammographic screening film. Twenty-five of them revealed one or two suspect lesions in mammography. All 35 were positive in ultrasonography. The tumor lesions were classified as BI-RADS III in 20 out of 53 lesions, as BI-RADS IV in 21 out of 53 lesions and as BI-RADS V in 12 of 53 lesions. Histological findings were obtained in all cases by ultrasound-guided biopsy. If the results in adequate percutaneous biopsy were positive, suspicious, discordant, or inconclusive with a strong suspicion for cancer in investigations, the patient was referred for surgical excision.

The tissue specimen either was obtained either by the surgical removal of the tumor or by means of an 8 G or 11 G vacuum assisted biopsy (Mammotome® RR, Ethicon, Norderstedt) with at least 12 specimen cylinders aspirated for each lesion. Histological examination (Table 1) revealed 20 benign tumors (all fibroadenomas) and 33 malignant tumors: 28 invasive ductal carcinomas (three of them with two lesions), two lobular carcinomas, and three ductal carcinomas *in situ* (DCIS), all non-commedocarcinomas (Table 1). No benign tumor showed signs of malignancy during follow-up examinations for more than eighteen months.

Mammography was performed with a Mammomat 300 (Siemens, Erlangen, Germany) and digital imaging (Fujii, Düsseldorf, Germany).

A 5 to 10 MHz linear transducer probe was used for ultrasound examination (Logiq700/Logig9, General Electric, Milwaukee, WI). Scan planes had to be adapted in all cases in which more than one tumor

Table 1
Histology and size of 53 impalpable breast tumors in 50 patients with 53 tumor lesions

Histology	Tumor size (mm)	Lesions
Fibroadenoma	<10	6
	10–15	14
Ductal carcinoma	<10	13
	10–15	15
Lobular carcinoma	10–15	2
DCIS	<10	3

DCIS = ductal carcinoma *in situ*.

lesion was detected by contrast-enhanced imaging and in which the comparability with reference scans were impaired. The transducer automatically shifts to 7 MHz when in power Doppler interrogation mode. The following settings were applied: low pulse repetition frequency of <1000 Hz, and low wall filter of <50 Hz. A low reduced mechanical index (MI) of less than 0.3 was chosen to avoid early gas bubble destruction. Color sensitivity was set to a level at which the background color was just suppressed, whereas small vessels could still be detected. The resultant power Doppler gain ranged from 55 to 65%. Flow parameters remained unchanged during the examination. Digital image documentation alternated between B scan and Tissue Harmonic Imaging (THI) to determine vascularity in power Doppler. The scan plane was selected for optimal detection of vascularity and had to be adapted in all cases in which more than one tumor lesion of one breast was examined. Care was taken not to apply too much pressure while examining the breast with the probe to avoid obliteration of small vessels. Image correlation, the dynamic range, the number and the localization of the foci remained unchanged during the examination.

All cine sequences of the suspicious lesions were stored in a digital image memory and were reviewed after the examination. Two independent readers assessed the different findings of vascularity findings such as the marginal, penetreting and central vessels. At this time histological findings were not known.

Ultrasound CM such as Optison® (FS069, Amersham, Little Chalfont, England) that contains perfluoropropane gas – large molecules that are insoluble in blood – have a long half-life compared to their predecessors containing carbon dioxide. CM containing gas are compressible and will consequently respond to different input sound fields in a non-linear manner. Up to now, we have used Optison® in over 500 cases for different examinations without seeing any adverse effects. The size of a single microbubble is about that of an erythrocyte. The contrast medium remains within the vascular lumen and does not penetrate to the interstitial space.

The ethic committee of our institution approved this study. An informed consent was signed by each patient for the ultrasound examination and before administering for the CM [20,21,23,24]. Optison® has been approved by the FDA for cardiac examinations although at a much higher dose (3 ml). We used only a single 0.5 ml dose of Optison® as CM; this was diluted to 20 ml by a standard saline solution and injected intravenously as a bolus into a 21 gauge peripheral indwelling cannula followed by a flushing injection of 10 ml of standard saline. Vascularity was recorded at intervals of 30 seconds controlled by a timer over a total duration of 20 minutes, if possible without changing the position of the transducer.

Diagnostic criteria were the visualization of marginal, penetrating, and central vessels without and with CM in the *early phase* and the visualization of the vascularity with CM in the *late* phase. We also considered the congruence of the findings for marginal and penetrating vessels as an additional diagnostic technique (Table 3b).

The results of our study were evaluated on basis of a statistical compilation and Fisher's two-tailed exact test for statistically assessing the discriminatory power of the different methods and the value of the CM employed [25].

3. Results

In ultrasound examinations without contrast medium, marginal vessels (Table 2a) were seen in 24/53 lesions (6/20 fibro-adenomas, 18/33 carcinomas), indicating an estimated sensitivity of 55%, a specificity of 70% for carcinoma and an overall accuracy of 60%. Tumor-penetrating and central vessels were seen in 15 cases (3 fibroadenomas, 12 carcinomas), corresponding to an estimated sensitivity of 36%, a specificity of 85% for carcinoma, and an overall accuracy of 55% (Table 2b).

Two different patterns emerged after CM injection: In the "early phase" the number of detectable maginal and penetreting vessels increased substantially within the first two minutes. The remained approximately constant in number and shape for a further 2 to 3 minutes and then disappeared slowly within 1 to 3 minutes (Figs 1, 2).

The majority of fibroadenomas and of carcinomas (Tables 3a, 3b) revealed a distinct increase marginal and penetrating blood vessel vascularization for up to 5 minutes (0.5 to 5 minutes; average: 3 minutes). Lesion-penetrating and central vessels were seen more often in malignant tumors. The vessel shape and the time of first appearance of the CM in the lesion did not differ between benign and malignant tumors (Figs 1–4). Combining the features of marginal and penetrating, the estimated sensitivity is 100%, the specificity 25% for carcinoma and an overall accuracy 72% (Table 3b).

There was a certain dependency on tumor size: All 18 malignant tumors larger than 10 mm showed increased tumor vascularization, whereas only 12 out of 15 carcinomas smaller than 10 mm featured at least one tumor penetrating vessel and a central tumor vessel only 4 out of 15. Tumor-penetrating vessels appeared within a maximum time span of 5 minutes.

In the second pattern ("late phase"), the vascular appearance did not change during the next 5 minutes after CM injection. In the following 2 to 3 minutes, the number and the shape of the vessels remained constant as the penetrating vessels are concerned. During the next 5 minutes, slight staining within the tumor was seen, that slowly increased. It could be readily differentiated from penetrating, central, or marginal vessels. It remained constant for 5 to 15 minutes (mean 7 minutes) and thereafter decreased in

Table 2a
Detection of vessels in breast lesions without the administration of contrast medium (CM)

	Fibroadenoma ($n = 20$)	Carcinoma ($n = 33$)
Marginal vessels	6/20 (30%)	18/33 (55%)
Penetrating vessels	3/20 (15%)	12/33 (36%)
Central vessels	0/20 (0%)	9/33 (27%)

Table 2b
Statistical results of examined breast lesions without the administration of Contrast Medium (CM)

	Sensitivity	Specificity	PPV	NPV	Accuracy
Marginal vessels	55%	70%	75%	48%	60%
Penetrating vessels	36%	85%	80%	45%	55%
Central vessels	27%	100%	100%	45%	55%

PPV = positive predictive value, NPV = negative predictive value.

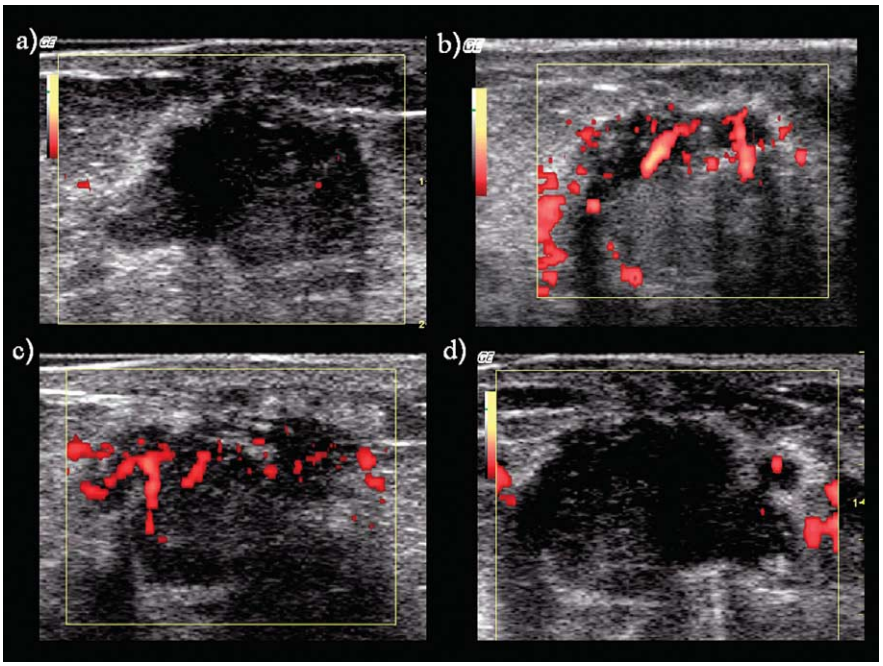

Fig. 1. A proliferating 5 × 15 mm fibroadenoma of the breast with partly irregular margins and inhomogeneous echo structures. No vascularity prior to contrast agent administration in Power Doppler with THI (a). Enhanced marginal and penetrating vessels after 30 seconds (b). No changes after 4 minutes (c). Decrease in vascularity after 8 minutes (d).

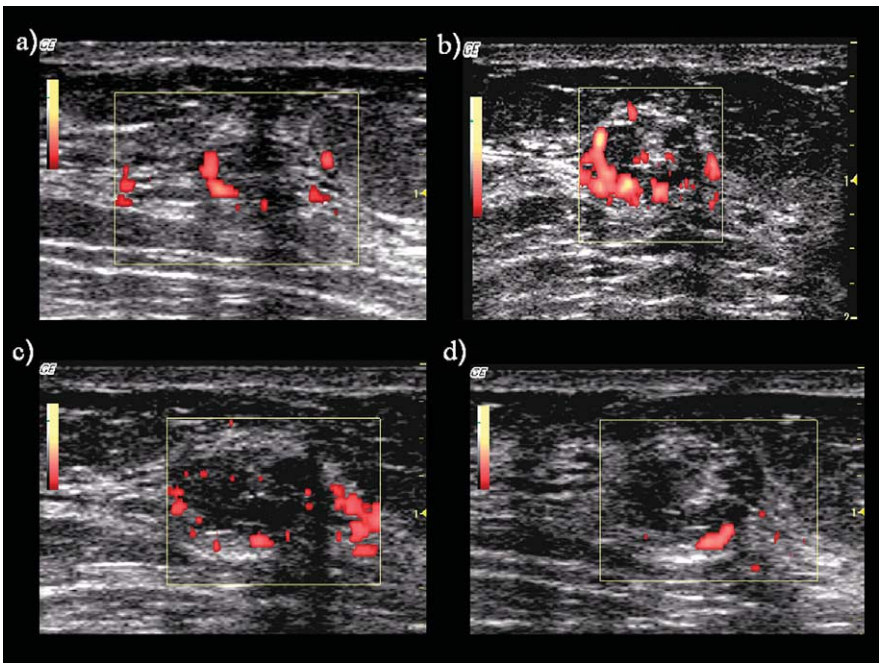

Fig. 2. A proliferating 5 × 10 mm fibroadenoma of the breast with smooth irregular edges in Power Doppler with THI. Little marginal vascularization prior to contrast agent administration (a). Increase in marginal vascularity and small intratumoral vessels (b). No significant changes after 5 minutes (c). Decrease of vascularization after 8 minutes (d).

Table 3a

"Early-phase" and "late-phase" vascularity of breast lesions after administration of contrast medium (CM)

	Fibroadenoma ($n = 20$)	Carcinoma ($n = 33$)
Early phase (1–5 min)		
Marginal vessels	17/20 (85%)	30/33 (91%)
Penetrating vessels	12/20 (60%)	30/33 (91%)
Central vessels	5/20 (25%)	22/33 (67%)
Late phase (8–20 min)		
Diffuse vascularity	0/20 (0%)	30/33 (91%)

Table 3b

Statistical results of breast lesions examined after the administration of contrast medium (CM)

	Sensitivity	Specificity	PPV	NPV	Accuracy
Early phase					
Marginal vessels	91%	15%	64%	50%	62%
Penetrating vessels	91%	40%	71%	73%	72%
Central vessels	67%	75%	81%	58%	70%
Combined mv = pv	100%	25%	69%	100%	72%
Late phase					
Diffuse vascularity	91%	100%	100%	87%	94%

– = no positive, mv = pv: equality of the findings for marginal and peripheral vessels, PPV = positive predictive value, NPV = negative predictive value.

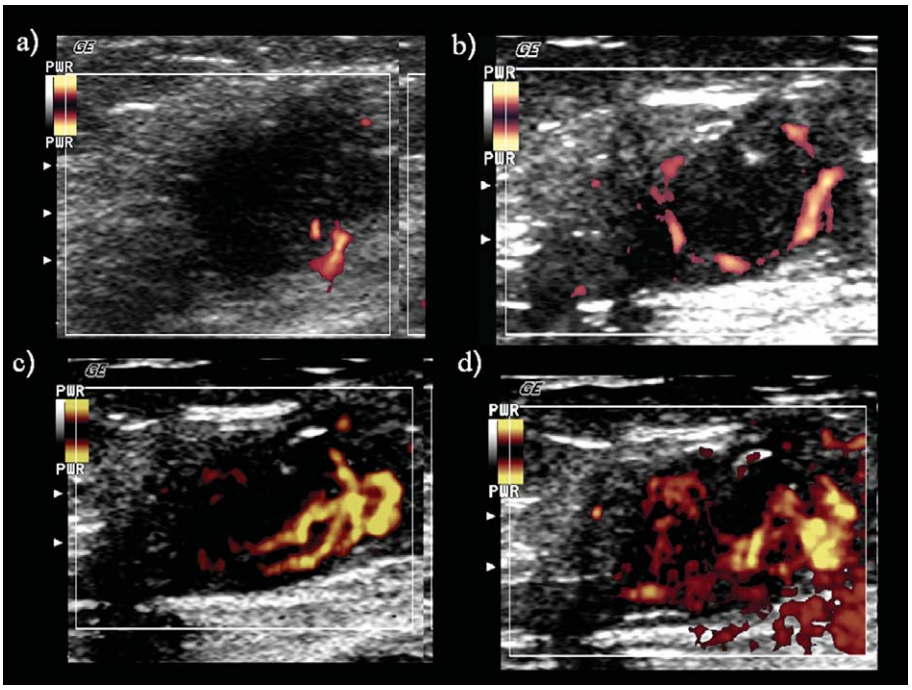

Fig. 3. Power Doppler THI image of an invasive 9 × 15 mm ductal carcinoma with undefined irregular edges and an inhomogeneous echo structure. Minimum marginal vascularity prior to contrast agent administration (a). Increased marginal vascularization after 20 seconds (b). Irregular intratumoral vessels after 5 minutes (c). Diffuse inhomogeneous intratumoral enhancement and enhanced vascularization after 8 minutes for a further 12 minutes (d).

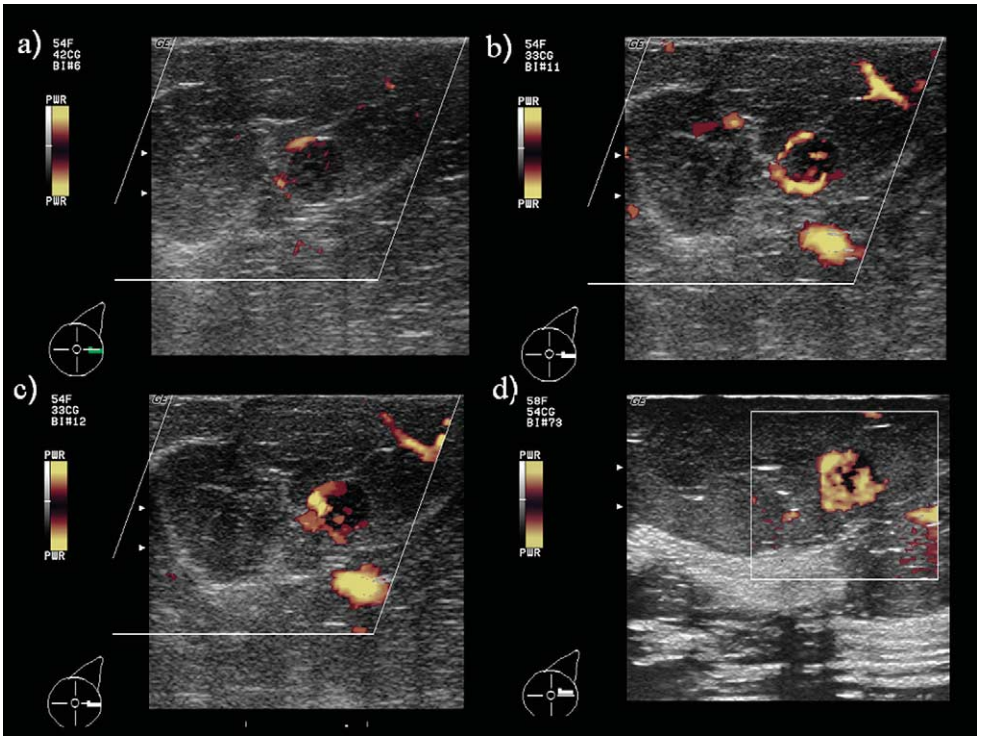

Fig. 4. Invasive metastatic 5 mm ductal carcinoma of the breast with inhomogeneous echo structure. Minimum marginal vascularity prior to contrast agent administration (a). Increase ivascularization and irregular intratumoral vessels 20 seconds (b) and 5 minutes (c) after contrast agent administration. After 8 minutes (d) enhanced vascularity for a further 12 minutes.

size and disappeared (Figs 3, 4). There was a slight, an intermediate, or an intense staining of the tumor. The entire tumor was rarely affected. There were areas of no or little enhancement. Histological findings showed tumor necrosis or fibrosis in some of the lesions.

This pattern was seen in 30 out of 33 malignant lesions (Tables 3, 4), but in not in any of fibroadenomas. In three cases of ductal carcinomas (size: 5 mm), no enhanced intratumoral vascularity was seen.

In 24 of 53 lesions enhancement of marginal and penetrating vessels were recognized better by THI than by B-scan. In the remaining lesion, there was only little difference between the two examination methods. Late CM enhancement was visualized better by THI in 22 out of 30 lesions. In the remaining cases, there was no difference between the two methods.

No adverse effects related to the CM administration have been encountered [21].

4. Discussion

Malignant tumors of the breast are known for their vascularization and their CM staining [1,7,9,12–14,22]. This fact is specially used in CM enhanced Magnetic Resonance Tomography, which is known, however, for its high sensitivity but its low specificity for carcinomas [8,28]. Not enhanced ultrasound is considered to have a high specificity and but a low sensitivity for breast cancer, as also found in our study (Tables 2a, 2b).

Intravenous injection of Levovist® (SH U 508A, Schering, Berlin, Germany), the mostly used ultrasound CM, improved the diagnostic accuracy. It was given intravenously either by bolus injection or by

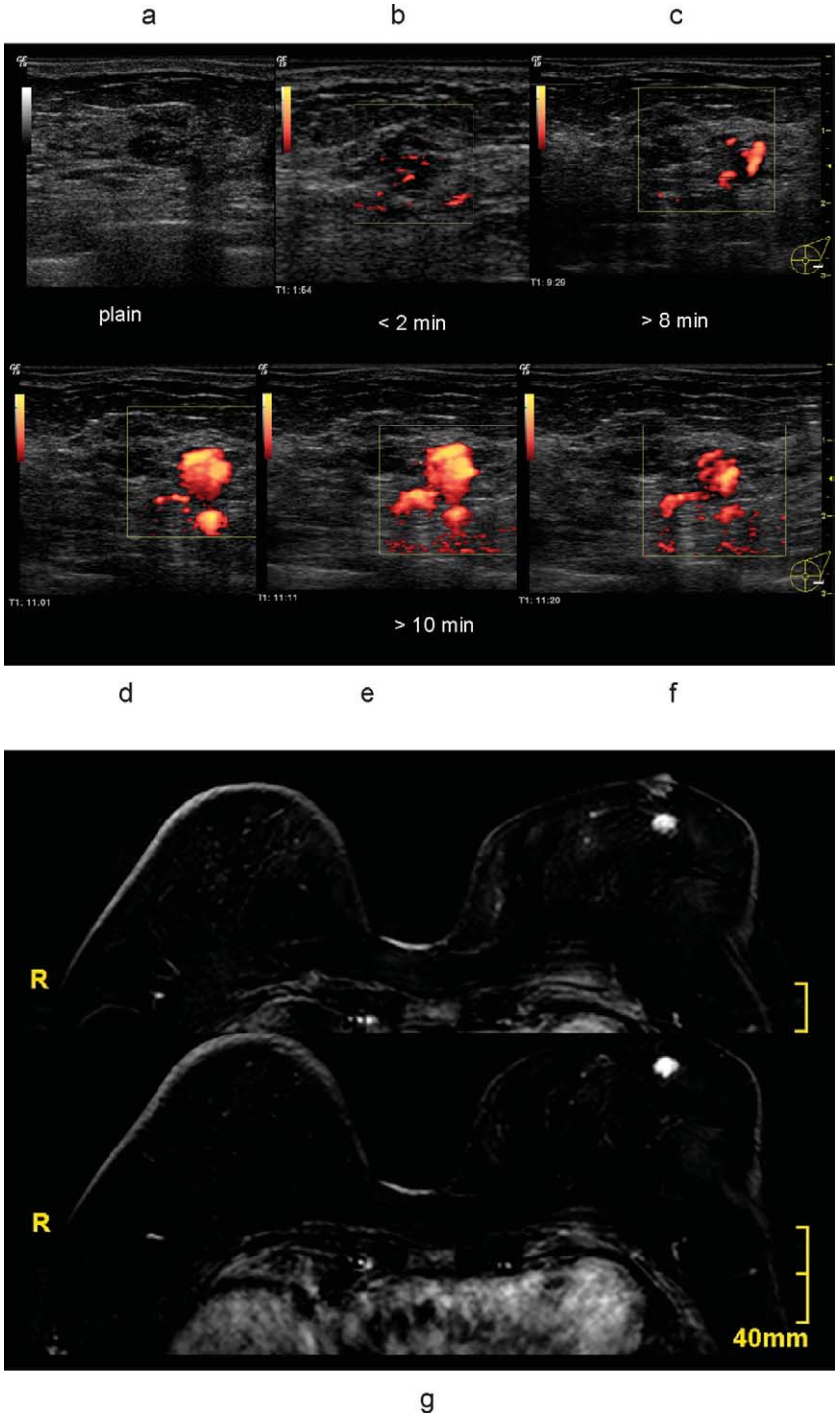

Fig. 5. Ductal carcinoma *in situ* (DCIS) of the left breast, 5 mm in diameter with irregular borders and inhomogeneous tissue (a). Only less intratumoral vascularity (b) in the "early phase" (2 minutes after CM). In the "late phase", 8 minutes after CM (c), increasing intratumoral vascularity in power Doppler with THI (d, e, f). Contrast enhanced Magnetic Resonance Tomography in subtraction technique delineates the tumor in the left breast as a bright spot (g).

Table 4
The power of the 10 diagnostic methods to discriminate between fibroadenomas and carcinomas on the basis of Fisher's two tailed exact test. (A small p-value signifies rejection of the hypothesis of non-discrimination)

Findings	Without CM		With CM	
	Reject at 95%	p-value	Reject at 95%	p-value
Early phase				
Marginal vessels	no	0.10	no	0.66
Penetrating vessels	no	0.12	yes	0.01
Central vessels	yes	0.02	yes	0.00
Vascularity	no	0.47	yes	0.01
Late phase				
Diffuse vascularity	–	–	yes	0.00

infusion [7,12,14,16,19]. Increased signals in the vessels appeared for up to 5 minutes after CM injection with a maximum at 0.5 to 2 minutes. Thereafter they declined. The number, the time of maximum enhancement, and the morphology of vessels were assessed as criteria of either the benign or the malignant nature of the tumor [7,9,10,12]. Analysis programs, which registered color pixel density, were used for the interpretation of CM dynamics [1,3,4,9,29]. A sensitivity of 84 to 95%, a specificity of 57 to 79% was attained for malignant lesions. If the morphology of the vessels (i.e. the presence of central and irregular penetrating tumor vessels) was also considered for in the analysis, diagnostic accuracy was further increased [8,11,12,14]. The degree of vascularity is also depended on tumor size: Vessels in lesions smaller than 5 mm were detected less frequently [6]. The number of tumor vessels was, however, not a reliable indicator of malignancy [9,12,13,27–29].

In our series, there was a sensitivity of 67% to 100% and a specificity of 15% to 75% for detection of cancer on the basis of tumor vascularity, a diagnostic accuracy of 62 to 72% after the intravenous application of Optison® as CM (Tables 3a, 3b) in the "early phase" and thus comparable with the results of the Levovist® studies. Differences in CM dynamics, in the localization, the number, and the shape of vessels in fibroadenomas and in carcinomas were not noted: Irregular intratumoral vessels, however, were more frequently seen in malignant tumors (Table 3a).

In the "late phase" of 8 to 20 minutes after the injection of CM, a CM enhancement was seen in more than 90% of carcinomas; this was somewhat diffuse and in most instances coverd only part of the lesion. However, in some cases it was also observed in the entire lesion. No fibroadenoma shows this diffuse enhanced vascularity.

Optison®, the CM we used, is *in vitro* more stable, associated with less disruption and greater induction of apoptosis in leukocytes after relatively high-pressure insonation in the range for diagnostic use than phospholipid-stabilized microbubbles [30]. In ultrasound cardiac imaging, also a late contrast enhancement of the myocardium for up to 15 minutes was achieved by this CM [23]. Various stimuli can initiate a vascular-capillary matrix–tissue match [32]. Earlier experience in the examination of liver tumors suggested that differences of CM accumulation might improve the detection of liver tumors [20]. An explanation of this "late phase" CM enhancement may be that intratumoral vascularity, such as arterio-veneous shunts of benign and malignant tumors are different. Perhaps the improvement of blood fluidity and thus oxygen transfer in the tumor-vascular-microcirculation after stimulation with contrast enhanced Power Doppler and harmonic imaging caused an prolong enhancement [32].

US contrast media of the second generation contain perfluorocarbon gases; these are large molecules that are highly insoluble in blood, making them long lived compared to their predecessors. Gas-containing contrast media are compressible and will therefore respond to different input sound fields in a nonlinear manner. US can now cause microbubbles to oscillate differently on the basis of the characteristics of the US field. *In vivo* studies showed that Optison® microbubbles respond differently to externally applied US fields. This response can be modulated by the strength of the external field [33].

With reduced MI harmonic imaging we found a better visualization of the marginal, penetrating and central tumour vessels after enhancement with CM. When central necrosis was present, contrast enhancement was limited to the non-necrotic parts of the tumor lesion. In the late phase, THI with power Doppler facilitated lesion delineation and increased contrast medium accumulation when compared the power B scan mode. *In vivo* studies the harmonic mode with lower imaging frequencies caused Optison® to produce more intensive myocardial enhancement [33]. Further studies in normal subjects showed that intermittent harmonic imaging was superior to either conventional fundamental or continuous harmonic imaging in determining perfusion [34].

Our initial results suggest that further studies of the kinetics of Optison® will be worthwhile. It was remarkable that a dose of only the sixth of the dose (0.5 ml) provided for cardiac workup (3.0 ml) was sufficient to obtain a satisfactory detection. Patients must still be informed in detail about potential adverse effects as well as contrast medium application, if the contrast medium is used for non-cardiac studies. Advantages of a second-generation albumin based CM seems its high potential for signal enhancement at a low dose and possibly tumor tissue specificity.

Only the CM-enhanced vascularity in the late phase shows statistically differences between the two lesion types. All fibroadenomas are correctly classified. Three carcinomas less than 10 mm in size with regular margins and only marginal vessels are considered as probably benign. The combination of two methods (Table 3b) improves the diagnosis based on marginal vessels with CM in every respect and provides results equivalent to those obtained for penetrating and central vessels with contrast medium to enhance accuracy.

We finally assess the discriminating power of different diagnostic methods between benign and malignant lesions by applying the two-sided version of Fisher's exact test. Penetrating vessels, central vessels and the combined method, all with CM show a significant difference for benign and malignant tumors after CM in power Doppler (Table 4).

An increased vascularity of a putative tumor lesion for up to 20 minutes could be additional criterion for the histological confirmation of impalpable tumors. The results are limited by the fact that only fibroadenomas of benign breast tumors were examined. The characteristics of other tumors are under evaluation.

Conclusion

Improved ultrasound techniques, changes in basic equipment settings and advanced ultrasound contrast media allow for the differentiation between friboadenomas and malignant tumors particularly on the basis of tumor vascularity in the early phase. They can be discriminated even better on evidence from contrast medium accumulation in the late phase. Our ultrasound studies on breast lesions showed that in the first 5 minutes after injection of a contrast medium, vascularization of fibroadenomas and carcinomas are difficult to differentiate within the first 5 minutes after injection of a contrast medium, whereas contrast medium accumulation with diffuse enhanced intratumoral vascularization after more than 5 minutes seems to be characteristic of carcinomas.

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