

Initial experience with contrast-enhanced ultrasonography in follow-up assessment of small breast cancer treated by cryoablation

Satoki Kinoshita¹, Ryo Miyake¹, Naoko Shimada¹, Akio Hirano¹, Yoshiaki Seki², Kanichiro Shimizu², Junta Harada², Masafumi Suzuki³, Kumiko Kato⁴, Ken Uchida⁴, Hiroshi Takeyama⁴, and Toshiaki Morikawa⁴

1. Department of Surgery, Jikei University Kashiwa Hospital, Japan

2. Radiology, Jikei University Kashiwa Hospital, Japan

3. Department of Pathology, Jikei University Kashiwa Hospital, Japan

4. Department of Breast & Endocrine Surgery, Jikei University School of Medicine, Japan

RESEARCH

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Corresponding Author:

Satoki Kinoshita

Department of Surgery, Jikei University Kashiwa Hospital
163-1 Kashiwashita Kashiwa city, Chiba, 277-8567, Japan

Email: satokino0918@nifty.com

ABSTRACT

Background

Cryoablation (CA) is a nonsurgical focal therapy for small tumours. To detect residual or relapsed tumour after CA of renal cancer, contrast-enhanced imaging is generally used to identify tumour blood flow, but no definitive criteria are established for such follow-up after CA of breast cancer.

Aims

The aim of this study was to compare the usefulness of contrast-enhanced ultrasonography (CEUS) and magnetic resonance (MR) imaging for assessing residual tumours and local relapse following CA of small breast cancers.

Methods

We enrolled 4 patients treated by CA at our institution between January 2015 and December 2016 for luminal A

breast cancer with maximum tumour size of 1.5cm and neither distant metastasis nor metastatic findings in sentinel lymph node biopsy, who underwent CEUS and MR imaging before CA. In addition to our standard postoperative follow-up for breast cancer, these patients underwent CEUS every 3 months and MR imaging every 6 months after CA.

Results

Six months after CA, no patient showed enhancement at the lesion site on MR imaging, but there were two with continued enhancement on CEUS. They underwent vacuum-assisted breast biopsy under US guidance followed by histopathological examination of tissue that identified no malignancy.

Conclusion

Our findings of focal enhancement within ablated breast tissue in CEUS after CA is likely attributable to the much higher sensitivity of CEUS to that of other modalities to even slight vascularization. Further investigation in more patients is needed to clarify the utility of CEUS to detect residual or relapsed tumour after CA of small breast cancer.

Key Words

Breast cancer, cryoablation, contrast-enhanced ultrasonography

What this study adds:

1. What is known about this subject?

Cryoablation (CA), a nonsurgical therapy that involves the focal freezing of tissue, has been used successfully to treat a variety of tumours, including small breast cancer, as well as other entities. Following the CA of renal cancer, contrast-enhanced imaging is generally used to identify tumour blood flow to detect residual or relapsed tumour, but no

definitive criteria are established for such follow-up after CA of breast cancer.

2. What new information is offered in this study?

Introducing contrast-enhanced ultrasonography (CEUS) in our follow-up assessment of small breast cancers treated with CA, we observed its greater sensitivity than magnetic resonance (MR) imaging for detecting very slight vascularization within areas of ablated tissue.

3. What are the implications for research, policy, or practice?

Although further investigations are required in more patients, we believe CEUS will aid the detection of residual tumours and local relapse in the follow-up of small breast cancers after CA.

Background

Cryoablation (CA) is a nonsurgical local treatment of tumours in which biological tissues are frozen and necrosed. However, freezing at -20°C or below destroys cells and produces tissue disorder, particularly after several cycles of rapid freezing and slow thawing.¹ CA breaks down tissue by freezing intra- and extracellular fluid, and microvascular thrombi form in the frozen area. Tacke reported complete obstruction of microvasculature of 0.5mm or less.² The direct action of cell death is nucleus deviation. Intracellular freezing destroys cell membranes, and freezing of extracellular fluid increases intracellular osmotic pressure, which leads to cell death. In addition, stagnation of microvascular blood flow begins 30 minutes after thawing, and cells with low oxygen die. Macroscopically, frozen tissue changes colour from congestion and bleeding around microvessels and becomes obviously necrotic after two days.³

CA has been applied clinically for over 20 years in Europe and the United States to treat such conditions as renal, hepatic, breast, and lung cancers, uterine fibroma, and bone tumours,⁴ and it has been applied to small breast cancer with some success.^{4,5} In Japan, the CryoHit™ cryoablation system (Galil Medical Ltd., Yokneam, Israel) was approved for percutaneous treatment of small renal cell carcinoma and laparoscopic surgery in January 2010 and national health insurance coverage on July 1, 2011. However, only two institutions in Japan, including our hospital, have introduced CA for the treatment of small breast cancers. We conducted a feasibility study of CA for small breast cancer followed by surgical resection in 2013⁶ and initiated its use in the treatment of small breast cancers without surgical

resection in 2015 with the approval of our institutional review board.

After cryoablation of renal cancer, contrast-enhanced imaging is generally used to detect blood flow that indicates residual or relapsed tumour,⁷ but no definitive criteria have been established for such follow-up assessment after the CA of breast cancers. We therefore investigated the use of contrast-enhanced ultrasonography (CEUS) with a second-generation contrast agent, Sonazoid™ (Daiichi Sankyo Co. Ltd., Tokyo, Japan), in addition to magnetic resonance (MR) imaging for this follow-up and share our initial experience with the technique here.

Method

Our institutional review board approved the protocol, and we obtained written informed consent from all patients prior to the procedure.

Among patients with primary breast cancer treated at Jikei University Kashiwa Hospital between January 1, 2015 and December 31, 2016, those who “met the criteria of clinical maximum tumour size equal to or below 1.5cm, no apparent distant metastasis, negative for metastasis in sentinel lymph node biopsy (SLNB), and luminal A type breast cancer; understood the main purpose of the clinical study; and signed a written consent” were enrolled in the study. The patients’ tumours were classified histopathologically according to the criteria of the Union for International Cancer Control and World Health Organization.

We performed immunohistochemical staining to determine the expression of steroid hormone receptors according to J-score⁸ and Hercep Test™ (DACO Corporation, Carpinteria, California, USA) to determine the expression of human epidermal growth factor receptor 2 (HER2) proteins. A score of one or two on Hercep Test™ was confirmed by dual-color fluorescence *in situ* hybridization (FISH).

All patients underwent MR imaging and CEUS before CA. At CEUS, Sonazoid™ was administered at the recommended dose of 0.015mL/kg as a bolus in the cubital vein and followed by a flush with 10mL of normal saline. Ultrasonography was performed using a LOGIQ E9 system and 9L probe (GE Healthcare, Little Chalfont, UK) in the amplitude modulation mode with the mechanical index (MI) set at 0.2 to 0.26 and the focus targeted near the deepest site of the tumour. The video image acquired was used to generate accumulation images and a time intensity curve (TIC) for assessment. In this process, an experienced

ultrasonographer visually defined a region of interest (ROI) at least 5mm in diameter within the lesion site or in and around the site of freezing. With the patient under local anesthesia, two 17-gauge cryoneedles were percutaneously inserted under US guidance, and cryoablation was performed in a double freeze-thaw procedure (10-minute freezing, 5-minute spontaneous thawing, 10-minute freezing and forced thawing) using the CryoHit™ system (Galil Medical), the only system approved by the Japanese government. During CA, ice ball formation was monitored on a 0.3-T horizontal open-configuration MR imaging system (AIRIS II, Hitachi Medical Corporation, Tokyo, Japan). Following CA, all patients underwent endocrine and radiotherapy, with acquisition of CEUS every three months and MR imaging every 6 months in addition to our standard postoperative follow-up for breast cancer.

Results

Table 1 summarizes the baseline characteristics of our 4 patients (mean age, 56 years) and their tumours (mean maximum tumour size, 11.3mm). No contrast enhancement was apparent at the former lesion site on MR imaging in any of the four patients at six months after cryoablation and on CEUS in two of the patients (Cases 1 and 2) at three and six months after CA. However, remaining enhancement on CEUS in the other two patients (Cases 3 and 4) indicated potential residual viable tumour or relapsed tumour within the site of CA, and these two underwent additional histopathological examination of tissue obtained by vacuum-assisted breast biopsy (VAB) under ultrasound guidance, which demonstrated fibrous connective tissue, fat necrosis, and granulation tissue and confirmed no malignancy in either patient.

In Case 3, MR imaging prior to CA showed a mass lesion 12mm in size in the right upper outer region with early intense enhancement (Figure 1a), and MR imaging six months after CA showed no intense enhancement that would indicate residual or relapsed tumour (Figure 1b). Ultrasonography in B mode prior to CA also identified an irregular low intensity echo 11mm in size, and CEUS showed an area of enhancement in the same region (Figure 1c). Analysis of the time intensity curve of the region of enhancement yielded a time to peak (TTP) of 14.5 seconds and gradient of 2.2 (Table 2); data plotting clearly differentiated the region of enhancement and its periphery (Figure 1d). Three months after CA, US in B mode indicated a tumour mass with uneven internal echo that suggested change following CA, and CEUS showed focal enhancement within the site of CA. Six months after CA, findings on US in B mode and CEUS (Figure 1e) were consistent with those at

three months. TIC analysis yielded a TTP of 13.9 seconds and gradient of 1.4 (Table 2). Data plotting resulted in a profile nearly the same as that at the periphery (Figure 1f). Because we could not rule out potential residual or relapsed tumour on CEUS, the patient underwent VAB under US guidance. Pathological examination of tissue showed panniculitis, fibrosis, and no malignancy. Histological examination showed panniculitis-associated lymphocyte infiltration, macrophage infiltration, appearance of multinucleated giant cells, and fibrosis. In addition, we observed positive cytokeratin (CK7) staining in only the breast duct without atypical cells, and positive cluster of differentiation (CD31) staining in the fibrous focus indicated capillary proliferation (Figure 2).

In Case 4, MR imaging prior to CA showed an irregular mass lesion 15mm in size in the right cranial side with early intense enhancement (Figure 3a), and absence of enhancement on MR imaging six months after CA appeared to indicate no relapse of the tumour (Figure 3b). Ultrasonography in B mode prior to CA also revealed an echo of low intensity and 13mm size in the right cranial side, and CEUS showed enhancement at the same site (Figure 3c). TIC analysis provided a TTP of 16.5 seconds and gradient of 1.4 (Table 2), and data plotting exhibited a profile that was different from that of the surrounding region (Figure 3d). Three months after CA, US in B mode indicated an echo of irregular low intensity that suggested a change after CA, and CEUS showed focal enhancement at the same site. Six months after CA, findings of US in B mode resembled those at three months with no change or blood flow signal. CEUS also showed irregular focal enhancement similar to that observed at three months (Figure 3e). TIC analysis yielded a TTP of 20.3 seconds and gradient of 0.8 (Table 2), and data plotting demonstrated a profile nearly the same as that of the periphery (Figure 3f). This patient underwent VAB under US guidance for confirmation by histopathological examination of tissue, which showed panniculitis with predominantly granulomatous changes, fibrous connective tissue, hemosiderosis that indicated old haemorrhage, and multinucleated giant cells but no malignant findings. CK7 staining showed positive signals that did not represent epidermal cells but likely indicated macrophages with keratin incorporated. In addition, positive CD31 staining in the fibrous focus indicated capillary proliferation (Figure 4).

Discussion

Contrast-enhanced imaging, commonly used to screen for tumour blood flow that might indicate residual or relapsed tumour after CA for renal cancer,⁷ will likely be useful as

well in assessing the loss of vascularity of breast cancers after CA.

Sonazoid™ consists of microbubbles of 2–3µm diameter of insoluble perflubutane coated with a yolk-derived phospholipid shell that resonate to produce contrast enhancement upon ultrasound irradiation.

A phase III clinical study demonstrated its significantly greater efficacy than B mode or MR imaging in differentiating benign from malignant mass lesions of the mammary gland.⁹ Sonazoid™ is also applied now to diagnose the spread of tumour, determine the need for needle biopsy, and assess the therapeutic effects following radiofrequency ablation (RFA) and neoadjuvant chemotherapy (NAC).¹⁰ Nevertheless, its use has not been reported in the follow-up of breast cancer after CA. Unlike gadolinium-based contrast agents, Sonazoid™ will not leak outside the vessel and thus allows pure observation of vessels and the amount of blood flow. We therefore considered that CEUS using Sonazoid™ would offer superior spatial resolution and sensitivity to those of MR imaging for evaluating residual or locally relapsed tumour after the cryoablation of breast cancer.

Indeed, focal enhancement on CEUS of half our patients in the current study suggested possible residual viable tumour or relapsed disease within the site of CA, though MR imaging confirmed the loss of significant intense enhancement in all patients, and TIC analysis of CEUS no longer indicated a marked difference between the site of the tumour and its periphery. These findings also countered those of our previous feasibility study, which demonstrated complete disappearance of tumour within the site of CA on histopathology.⁶ We do acknowledge that our results may be influenced by slight differences in the site of the tumour or range defined as the ROI and that future efforts are warranted to establish universal criteria for defining the ROI.

For the two patients who exhibited continued enhancement within the site of cryoablation, histopathological examination of tissue obtained by VAB under US guidance revealed fibrous connective tissue, fat necrosis, and granulation tissue but no findings of malignancy, and CK7 staining demonstrated no CK7-positive viable tumour cells. On the other hand, positive signals in the fibrous focus from staining of CD31, a protein in vessels and endothelial cells that serves as a marker of vascularization, indicated capillary proliferation. Findings in our feasibility study in 2013 [6] showed similar focal enhancement within the site

of CA on CEUS one month after CA in one of 5 patients. Enhancement indicating potential viable tumour within the site treated with CA is not rare.

We suspect that the superior spatial resolution and sensitivity of CEUS may pick up even slight vascularization associated with granulomatous change after CA. At present, however, this type of enhancement requires histopathological assessment of tissue obtained by VAB or other means to rule out residual viable tumour and relapse of disease.

Compared with MR imaging, ultrasonography requires no investment in extra equipment, and the equipment itself is less expensive and easy to operate. In addition, Sonazoid™ has very few contraindications compared with gadolinium-based and other MR imaging contrast agents and is therefore expected to be useful in monitoring for residual tumours and local relapse in the follow-up of breast cancer after cryoablation. At present, we consider that only the patient with enhancement on CEUS should be received MR imaging, and only the patient with enhancement on MR imaging need to perform VAB to confirm histopathological examination. However, its use for this purpose also requires further investigation.

Conclusion

In this first use of CEUS in the follow-up of small breast cancer treated with cryoablation without surgical resection, findings of enhancement in two patients that suggested residual viable tumour within the area of freezing were not confirmed as malignant at histopathological examination. Rather, the enhancement likely reflects the much higher sensitivity of CEUS to that of other modalities to even slight vascularization. However, further investigation is required in more patients to clarify the utility of CEUS in the follow-up of breast cancer after cryoablation.

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PEER REVIEW

Not commissioned. Externally peer reviewed.

CONFLICTS OF INTEREST

The authors declare that they have no competing interests.

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ETHICS COMMITTEE APPROVAL

Ethics Committee approval was obtained from the Institutional Review Board of Jikei University (26-071).

Table 1: Characteristics of patients and tumours

Patient	Case 1	Case 2	Case 3	Case 4
Age	62	42	68	52
Tumour size (mm)	8	11	11	15
Ice ball size (mm)	46x42x36	56x36x22	51x29x27	50x38x27
Histologic type	IDC	IDC	IDC	IDC
ER	3b	3b	3b	3b
PR	3b	3b	3b	3b
HER2 (IHC)	0	2+	1+	2+
HER2 FISH (SR)	-	NA (1.5)	NA (1.3)	NA (1.4)
Ki67	1%	10%	7%	5%
SLNB	0/2	0/2	0/5	0/2
MR imaging				
EE (before CA)	positive	positive	positive	positive
EE (6 months after CA)	negative	negative	negative	negative
CEUS				
EE (before CA)	positive	positive	positive	positive
EE (6 months after CA)	negative	negative	positive	positive

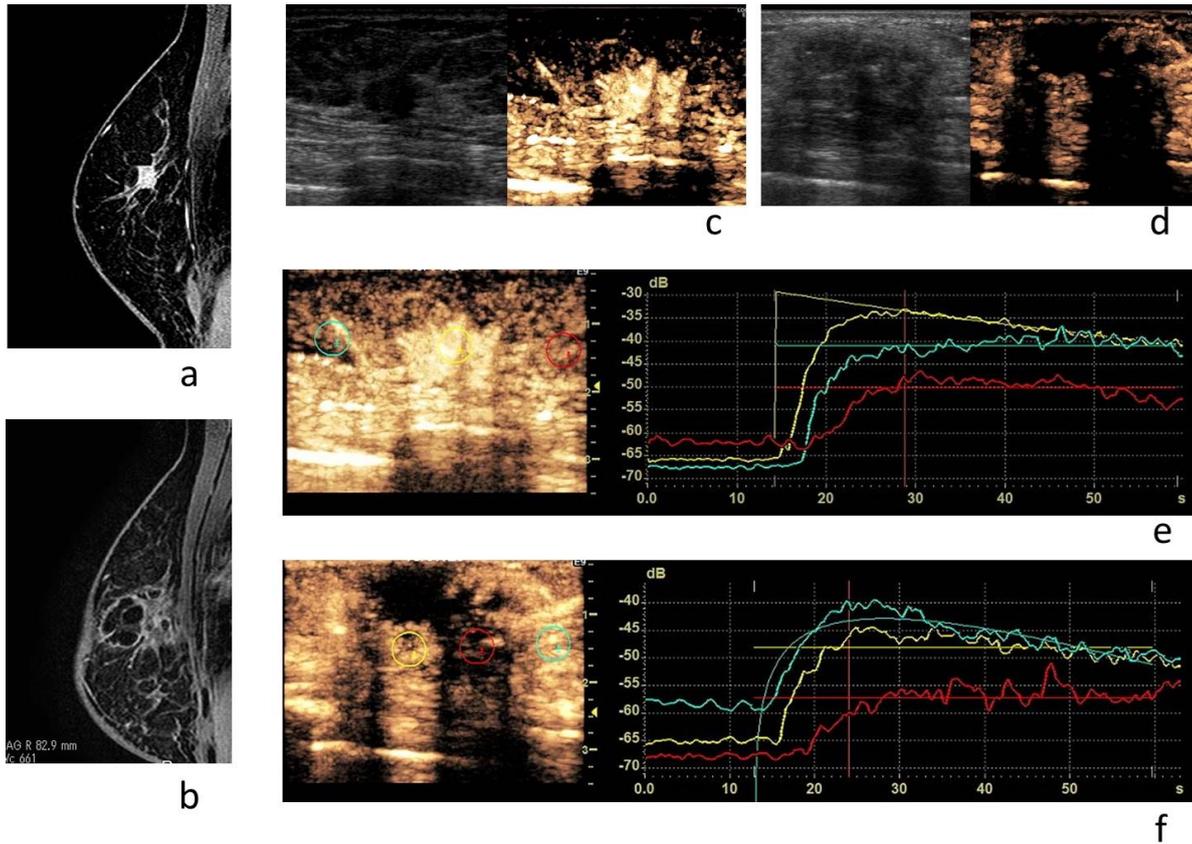
CA, cryoablation; CEUS, contrast-enhanced ultrasonography; EE, enhanced effect; ER, oestrogen receptor; FISH, fluorescence *in situ* hybridization; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; NA, not amplified; PR, progesterone receptor, SLNB, sentinel lymph node biopsy; SR, signal ratio

Table 2: Time to peak and gradient using time intensity curve analysis

Patient	Region of interest	Before cryoablation		Six months after cryoablation	
		TTP (seconds)	Gradient	TTP (seconds)	Gradient
Case 3	inside tumour	14.5	2.2	13.9	1.4
	around tumour	32.1	0.9	14.2	1.4
Case 4	inside tumour	16.5	1.4	20.3	0.8
	around tumour	23.8	0.5	20.3	0.4

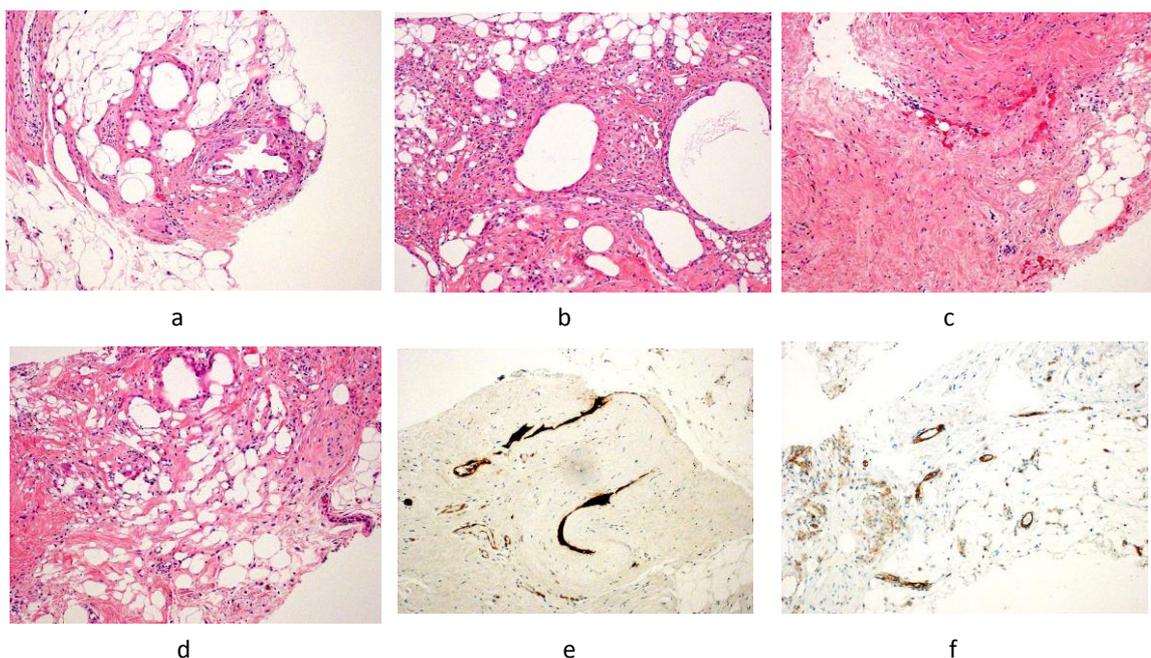
TTP, time to peak

Figure 1: Case 3, images before and after cryoablation (CA)



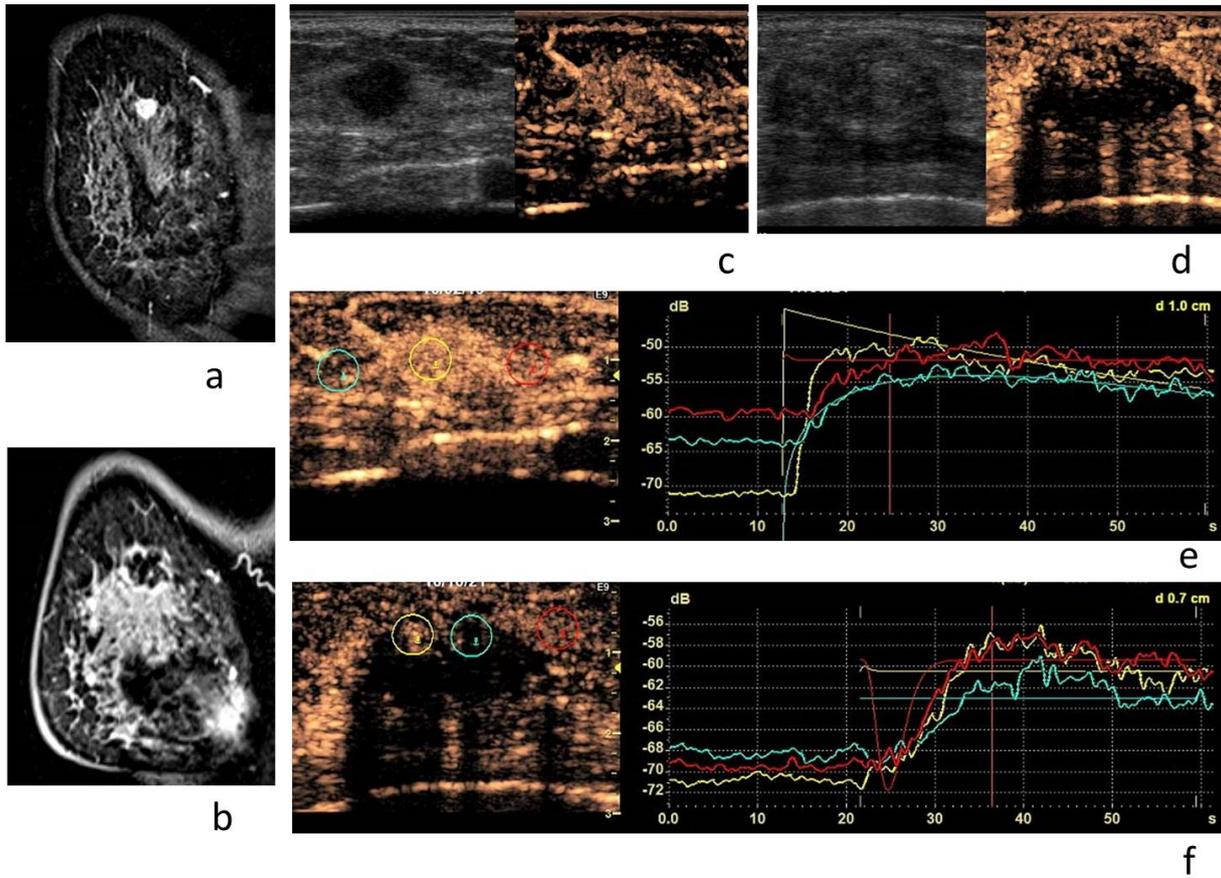
a) Magnetic resonance (MR) imaging before CA; b) MR imaging 6 months after CA; c) ultrasonography (US) and contrast-enhanced US (CEUS) before CA; d) US and CEUS 6 months after CA; e) CEUS and time intensity curve (TIC) before CA; f) CEUS and TIC 6 months after CA

Figure 2: Case 3, histopathological examination by vacuum-assisted breast biopsy



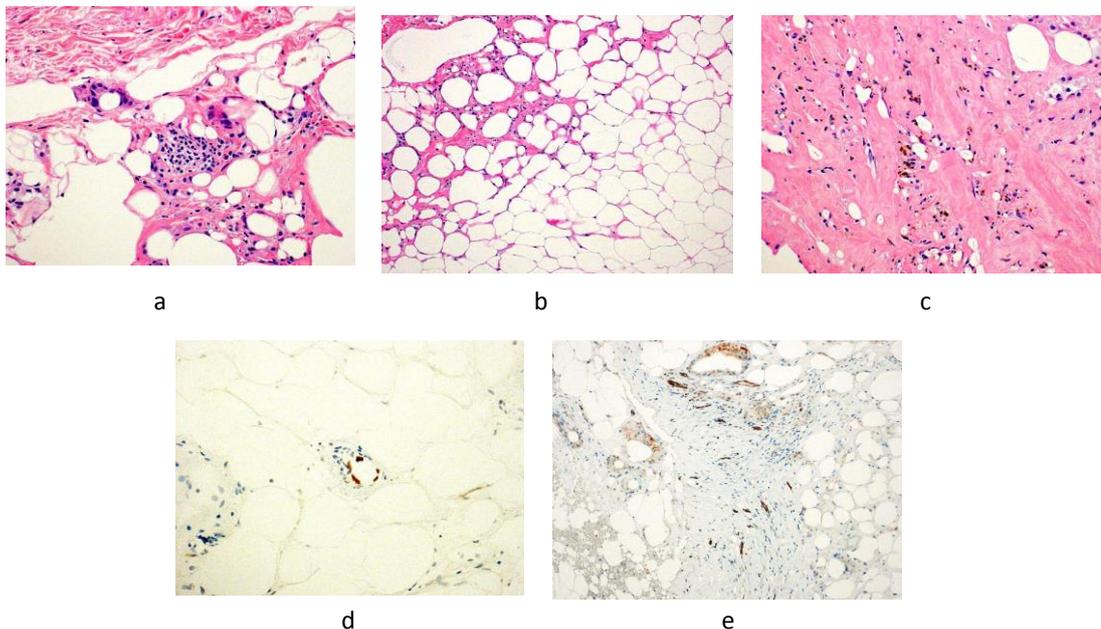
a) Multinucleated giant cell; b) xanthogranuloma; c) fibrosis; d) fat necrosis; e) cytokeratin (CK7); f) cluster of differentiation (CD31)

Figure 3: Case 4, images before and after cryoablation (CA)



a) Magnetic resonance (MR) imaging before CA; b) MR imaging 6 months after CA; c) ultrasonography (US) and contrast-enhanced US (CEUS) before CA; d) US and CEUS 6 months after CA; e) CEUS and time intensity curve (TIC) before CA; f) CEUS and TIC 6 months after CA

Figure 4: Case 4, histopathological examination by vacuum-assisted breast biopsy



a) Multinucleated giant cell; b) fat necrosis; c) fibrosis and hemosiderin deposition; d) cytokeratin (CK7); e) cluster of differentiation (CD31)