Breast pathology after cryotherapy. Histological regression of breast cancer after cryotherapy

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A breast saving treatment is contemporary the preferred method of treatment with comparable results in comparing with mastectomy. In this study were evaluated the effects of cryotherapy by histological verification of changes in post treatment resection specimens. Fifty-three patients in age of 38-81 year with histologically confirmed breast cancer in needle biopsies were managed by cryotherapy between 1999 and 2007. The patients were operated between day 1 and 35 after cryotherapy.
The histologic examination of operation materials showed in all cases at least partial tumor destruction. In general in 54.7% of all handled cases (29 patient) there was no residual tumor. In 6 cases (22.2%) from group 1 and in 23 cases (88.5%) of group 2 no tumor rest was found. Cryotherapy can lead to complete destruction of tumoral tissue. In our study all 29 (54.7%) of tumor-free cases after cryotherapy were those with cT1 stage. The experience of operator and the correct selection of appropriate patients (primarily taking the tumor size into account) play the most important role for achieving the best results.

Key words: breast cancer, histology, grading, regression, cryotherapy, cryoablation.

Introduction

Breast carcinoma is the most frequent malignant tumor of women with the incidence of more than one million new cases each year. The mortality rate depends on the region and is very variable. It is about 20/100 000 in Europe and the USA, 7.5/100 000 in Korea and 5.5/100 000 in China. According to the estimation of the Robert Koch Institute, in 2012 alone the diagnosis of breast cancer was made in more than 74 500 women in Germany. About one fourth of the patients were at the time of the diagnosis younger than 55 and about one tenth younger than 45 years old. In general every one in eight women experiences the breast cancer in her life. The number of new cases in 2008 in Germany approached over 71 660, and there were 6 500 cases of *in situ* carcinoma. Nevertheless, the prognosis of breast cancer in comparison with other malignancies is relatively good. Despite the increasing disease incidence a decreasing mortality rate has been observed since the middle of 1990s. This trend is principally the results of better diagnostic techniques, adequate programs for early diagnosis and increasing awareness of the people about the breast cancer [1-3].

By increasing sensitivity of imaging procedures increasing number of small sized breast tumors are diagnosed. Contemporary the performance of percutaneous, minimally invasive, breast needle biopsy under the guidance of imaging modalities considered as a sufficient alternative method for open surgical excisional biopsy and has been integrated in the guidelines of professional German and European societies. For the monitoring of the minimal invasive procedures different imaging modalities such as stereotaxy, the ultrasonography and magnetic resonance imaging (MRI) can be used. Breast saving treatments are nowadays the preferred treatment modality with similar results in comparison with mastectomy. In recent years there is a trend to manage breast cancers with less invasive or minimal invasive treatments. The mostly used methods for local tumor ablation of breast cancer are thermal procedures such as radiofrequency ablation (RF), interstitial laser therapy (ILT) and high intensity focused ultrasound (HIFU). Cryotherapy belongs also to this group of treatments. The cryotherapy is based on local application of very low temperatures to destruct a tissue. It kills the tumor cells by rapid cooling of tissue to –180°C [4-9].

In this study we examined the histologic changes of post treatment tumor resection specimen for the possible effects of cryotherapy of breast cancers.

Material and methods

Between 1999 and 2007, cryotherapy was carried out on 53 cases with biopsy-proven breast cancer. The age spectrum was 38-81 years (mean 61). The processing of breast needle biopsies as well as tumor resection samples (breast saving or ablative) was done according to the international recommendations and S3-guideline. Each needle biopsy was embedded in a paraffin block and cut at three levels. The microscopic evaluation of HE slides was performed using an Axiophot microscope (Zeiss) at 2.5, 5, 10, 20 and 40 magnifications. The histological diagnosis in all cases was breast cancer. They included 39 invasive ductal carcinomas (IDC), 6 invasive lobular carcinomas (ILC), 3 invasive tubular carcinomas (ITC), 2 invasive ductal carcinomas with invasive lobular components, 1 case of adenoid cystic carcinoma (ACC) and 2 cases of ductal carcinoma *in situ* (DCIS). The tumor locations are depicted in Table I. For the purpose of immunohistochemical staining the slides were treated according to the standard methods. They were deparaffinized, rehydrated and stained after heat induction using microwave (boiling for 20 min in sodium citrate buffer, pH 6.0). The applied antibodies, the level of dilution and their provider are listed in Table II. The maximal dimension of tumors

Table I. Tumor type and localisation

Histologic subtype	Tumor type		Localisation	
	By needle biopsy	After cryotherapy		
IDC	39	14	inferior – lateral	7
ILC	6	4	inferior – medial	4
ITC	3	0	superior – lateral	14
IDC + ILC	2	1	superior – medial	13
DCIS	2	4	superior	6
ACC	1	1	inferior	1
LN Meta.	0	3	lateral	6
All	53	27	no information	2

Table II. Applied primary antibodies

Applied primary antibodies			
Antibody	Clone	Provider	Dilution
ER	6F11	DAKO	1 : 75
PR	PgR 636	DAKO	1 : 150
HER-2	c-erbB2 oncoprotein (A0484)	DAKO	1 : 400
Ki-67	MiB-1	DAKO	1 : 100
MNF	MNF116	DAKO	1 : 200
AE1/AE3	Clone AE1/AE3	DAKO	1 : 1000

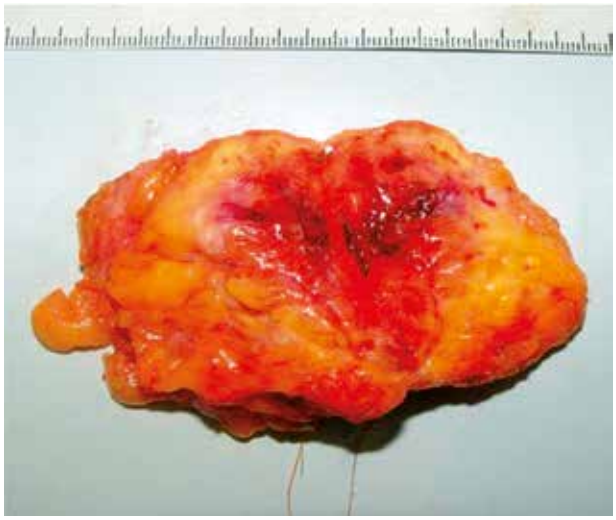

Fig. 1. Resection specimens after cryotherapy: 15 days

was determined sonographically and it ranged between 5 and 37 mm (mean 15.3 mm). Most types of carcinomas can be easily diagnosed in needle biopsy specimens. In problematic cases it is possible to use immunohistochemistry. The evaluation of hormone and Her2 status is obligatory any way.

All 53 patients underwent ultrasonographic guided cryotherapy under the application of local anesthesia. The size of the ice ball was measured sonographically with intervals of one minute (21.7-39.5 mm, mean 31 mm, in 5 cases no data).

The patients were operated on between 1 and 35 days after cryotherapy. The method of operation was in 48 cases breast saving and in 5 cases mastectomy. In 2 cases the breast saving surgery was not successful and the first operation was followed by mastectomy to remove the residual tumor tissue.

The mastectomy specimens were labeled by sutures on two facets and the segmentectomy or quadrantectomy specimens were labeled in most cases on three facets. The processing was done after fixation of specimens in 4% buffered formalin by lamellar cuts in about 5 mm thick sections along the longest axis. In mastectomy specimens the tumor was embedded totally along with an approximately 5 cm adjacent zone of macroscopic unremarkable breast tissue. The segmentectomy and quadrantectomy specimens were embedded totally in paraffin blocks, cut and examined microscopically after HE-staining. In three cases additional immunostaining was carried out including pancytokeratin (3 cases), AE1/AE3 (1 case) and Ki67 (8 cases).

In 26 cases the ipsilateral axillary lymph nodes (mean number of lymph nodes in each case 11) and in 27 cases the sentinel lymph nodes were removed and examined.

The collection of data and statistical analysis was done using Microsoft Excel 2007 and SPSS 15.0. The groups were compared with each other using Mann-Whitney-U-Test. The $p \leq 0.05$ was considered significant.

Results

The procedure of cryotherapy was performed completely in 51 cases after placing the cryotherapy probe. In 2 patients the intervention had to be stopped prematurely because of technical problems such as leakage of gas. In all other cases there was no significant event which necessitated early cessation of the procedure. The macroscopic tumor size was between 0 and 55 mm (on average 27.4). In 1 case no tumor focus was found and in 2 cases no information about the tumor size was available. After the operation the specimens showed a typical macroscopic appearance. The treated tumoral area was variably deep red in color as the reflecting of cryotherapy-related intratumoral bleeding, necrosis and ongoing reparative processes (Figs. 1, 2). In all cases the histological examination showed variably pronounced tumor destruction (Tables III, IV).

In 24 cases a remnant of the carcinoma was identified after cryotherapy. The tumor remnants included IDC with components of ILC (1 case), DCIS (4 cases), ACC (1 case) and IDC (14 cases). In 3 cases there were lymph node metastases, although a local tumor remnant in the operational specimen was not detectable. Five cases had both a local tumor remnant and lymph node metastasis. In 29 tumors with the diameter of up to 19 mm complete destruction of tumor components was evident.

For evaluation of histological tumor regression we used a grading system that provides a possibility for classification of regressive changes in clearly separable groups. In this semiquantitative evaluation of response of breast cancers we used the parameters which can be identified in every tumor type (Table V, Figs. 3, 4).

Table VI shows the histological regression grade after cryotherapy on the basis of histological tumor type in breast needle biopsies and diagram 1 shows a regression grading system based on tumor size (cT).

Because of the small number of group of cases an accurate statistical analysis was very difficult and in some situations not possible.

The Mann-Whitney U-Test showed that the histological tumor type had a significant effect on the results of cryotherapy. All 29 tumor-free cases after cryotherapy were those in cT1 stage. The difference between cT1 and cT2 was statistically significant (Table VII). Grading had no influence on the effects of cryotherapy (Table VIII).

Discussion

Breast carcinoma is the most frequent malignant tumor in women in western countries. After the be-

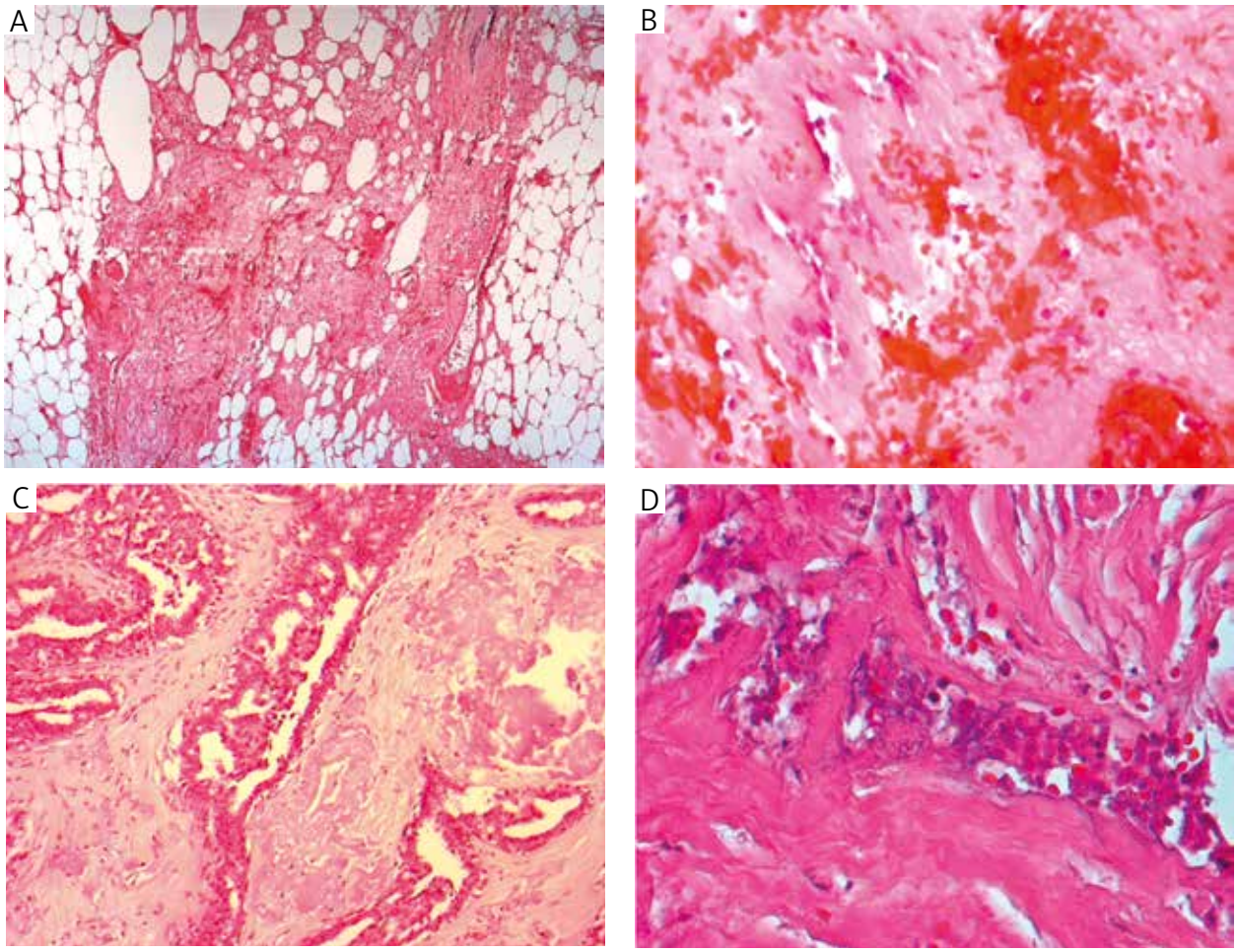

Fig. 2. Necrosis, shadow of tumor cells and avital tumor cells. **A)** HE 40×, **B)** HE 100×, **C)** HE 200×, **D)** HE 400×. Group 1

ginning of mammography screening in Germany in 2005 the number of cases were increased. Many newly detected tumors are early in their development and show evidently smaller size (many cases in T1 stage) in comparison with those tumors which are detected out of the setting of screening programs [2].

After wide application of modern imaging procedures (mammography, ultrasonography and MRI) great development in the diagnosis of benign and malignant breast lesions has been achieved. Even an increasing number of asymptomatic tumors could be detected in early stages. Despite the increasing number of patients, today a lower number of women die due to breast cancer in comparison with 20 years ago [2, 8, 9].

On the other hand, during the last 20 years the form of treatments was changed and these changes significantly enhanced the survival rate of patients. Today breast saving surgery in appropriately selected cases is the preferred method of treatment. Many randomized clinical trials from Europe and North America have shown that breast saving surgery has comparable results and a similar survival rate in comparison with mastectomy.

The neoadjuvant chemotherapy of a locally advanced breast cancer is used primarily to reduce the local tumor mass and to increase the chance of tumor operability. In recent years the target of neoadjuvant chemotherapy is expanded to cover the reduction of tumor size to make them suitable for breast saving surgeries [10]. On the other hand in the recent times the minimal invasive procedures are applied increasingly to manage breast tumors. One of these methods is cryotherapy, a method of tumor treatment which was used also successfully in the management of liver and prostate tumors [11].

This study showed that ultrasonography-guided percutaneous cryotherapy is a good minimally invasive method for treating small breast carcinomas and can cause complete destruction of the tumor. All 29 cases with tumor-free status after cryotherapy in this study (regression grade 1) were cT1 cases. That means that in 54.7% of all handled cases there was no remaining tumor after cryotherapy. The most important factors for the best result are experience of the operator and the selection of appropriate patients, in particular taking the tumor size into ac-

Table III. Histopathology

Time	Histopathology
1. Day 3 Patients	Extensive fresh bleeding Beginning of necrosis Hyperemia Shadow of tumor cells
2. Day 5 Patients	Fresh bleeding Hemorrhagic necrosis within the tumor Necrosis in the neighbouring fibrofatty tissue Beginning of inflammatory reaction Shadow of tumor cells
3. Day 4 Patients	Hemorrhagic necrosis Shadow of tumor cells Hyalinisation Inflammatory infiltrate
4. – 7. Days 16 Patients	Hemorrhagic necrosis Granulocytic infiltration and foam cells inflammation Shadow of tumor cells Well defined areas of fat necrosis Xanthogranulomatous Inflammation Plasma cell inflammatory infiltration in fatty tissue
2. Week 10 Patients	Hemorrhagic necrosis, foam cells and macrophages Shadow of tumor cells At the peripheral areas extensive necrosis with hemorrhage Sparse neutrophilic granulocytes capillary rich granulation tissue und xanthogranulomatous inflammatory reaction with giant cells
3. Week 6 Patients	No longer fresh hemorrhagic necrosis In peripheral areas bleeding, reparative changes, fibrosis, focal and small areas of granulation tissue Focal collections of foam cells und extensive fibrosis Neighbouring fatty tissue with fibrosing and xanthogranulomatous processes
4. Week 5 Patients	No longer fresh bleeding Hazy vascular structures und small foci of bleeding Large number of foam cells and capillary rich granulation tissue Beginning of scarring
5. Week 3 Patients	Fibrosis und capillary rich granulation tissue Focal giant cells and sparse inflammatory infiltrate Focal and no longer fresh necrosis with bleeding and foam cells Focal secondary vasculitis and granulocytic infiltrate Loose spindle cell proliferation, most probably myofibroblastic elements Focal scarring

Table IV. Tumor stage

Tumor Stage			
Before cryotherapy		After cryotherapy	
cT1a	3	ypT0	29
cT1b	10	ypTis	4
cT1c	28	ypT1a	3
cT2	11	ypT1c	2
no information	1	ypT2	2
≤ 15 mm	32	mypT1a	9
> 15 mm	20	mypT1b	3
		mypT2	1

count. Between 1999 and 2001 we carried out cryotherapy on 27 breast cancer patients (tumor size 8-40 mm) (group 1). Only in 6 (22.2%) of these cases did we find no tumor remnants. In comparison with this group 26 patients (tumor size 5-30 mm) were handled in a second study cryotherapy (group 2). In this group we found no tumor remnant in 23 (88.5%) cases. According to the largest study on primary neoadjuvant chemotherapy, in 20% of cases complete pathologic remission without detection of residual invasive tumor components was achieved. But the information about the rate of complete remission showed a wide spectrum of results. There is no good correlation between the clinical and pathologically defined complete remission. In only 38% of all patients with clinical complete remission were no tumor remnants detected in pathological examinations as well [12-14]. Reduction of tumor mass is considered the most important change in chemosensitive tumors in the way that the number of invasive tumor cells decreases and the amount of tumor stroma increases after treatment. A more advanced tumor regression results in loss of tumor continuity which imitates the histological appearance of a multifocal tumor growth. This pseudo-multifocality must be noticed during surgical procedures and in the assessment of resection margins, otherwise it can result in a higher rate of tumor recurrence. As accompanying pathologic changes, hyalinization, lymphocytic and plasma cell infiltration, lymphocytic ductitis, calcification and histiocytic reaction can be observed. The typical finding is the lower chemosensitivity of intraductal, intratubular and intralymphatic tumor components which can in turn result in a smaller reduction of in situ tumor elements and lesser cytopathic changes of these tumor cells. This dependence of chemosensitivity on localization in invasive tumors with intraductal components leads to that fact that after neoadjuvant chemotherapy in up to 20% of cases only intraductal tumor residues are detectable and

Table V. Classification of regression grade after cryotherapy

Classification of regression grade after cryotherapy	
Grade	**Definition**
1	No residual tumor
2	Residual in situ carcinoma without invasive component ≤ 0.5 cm
3	Residual invasive component with treatment effects in stroma or tumor cells ≤ 0.5 cm
4	Residual invasive or in situ carcinoma > 0.5 cm, also multifocal tumors

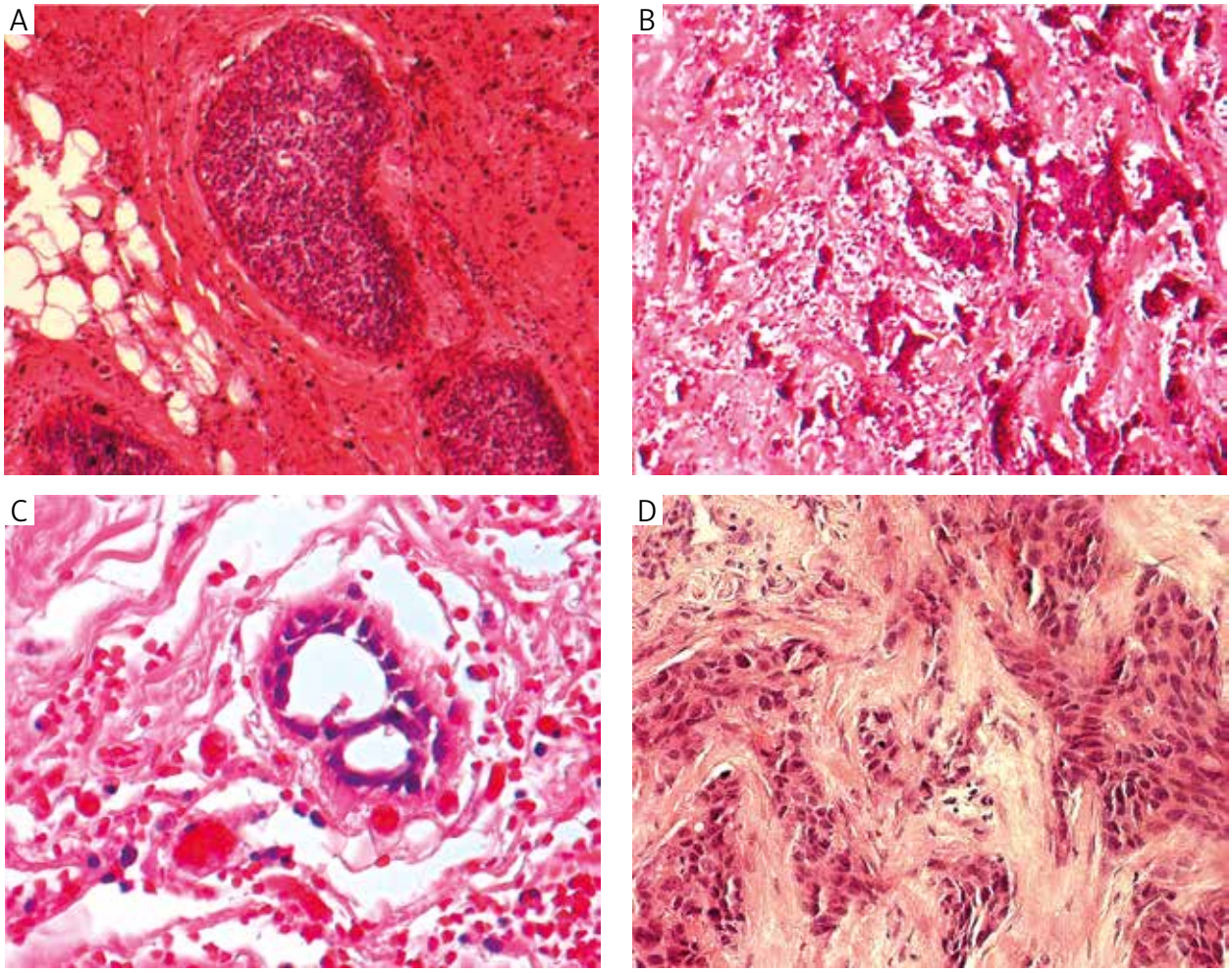

Fig. 3. A) DCIS and **B)** invasive carcinoma, HE 100×; **C** and **D)** invasive carcinoma, HE 200×. **A)** Group 2, **B** and **C)** Group 3, **D)** Group 4

the amount of tumor residue is increased in direct relation to the amount of intraductal tumor elements [10, 15]. Our study showed that the cryotherapy results in complete disappearance of the tumor in cases of small breast tumors (cT1) which are handled by experienced operators. Only in group 1 did we have 4 cases with residual DCIS ≤ 0.5 cm (grade 2) and 9 cases with residual invasive or in situ carcinoma > 0.5 cm (grade 4). Residual invasive carcinoma ≤ 0.5 cm (grade 3) was detected in 12 patients (in 9 women from group 1 and in 3 cases from group 2). In our view the presence of residual tumor in these cases is related to tumor size and also false placing of the cryotherapy probe. The amount of tumor regression after neoadjuvant chemotherapy is related to the tumor type, proportion of *in situ* component, differentiation and grading as well as tumor stroma relationship [10]. In contrast with neoadjuvant chemotherapy the application of cryotherapy has advantages. In all tumor-free cases after chemotherapy the above-mentioned factors play no role. Breast carcinoma can show a wide range of regressive changes such

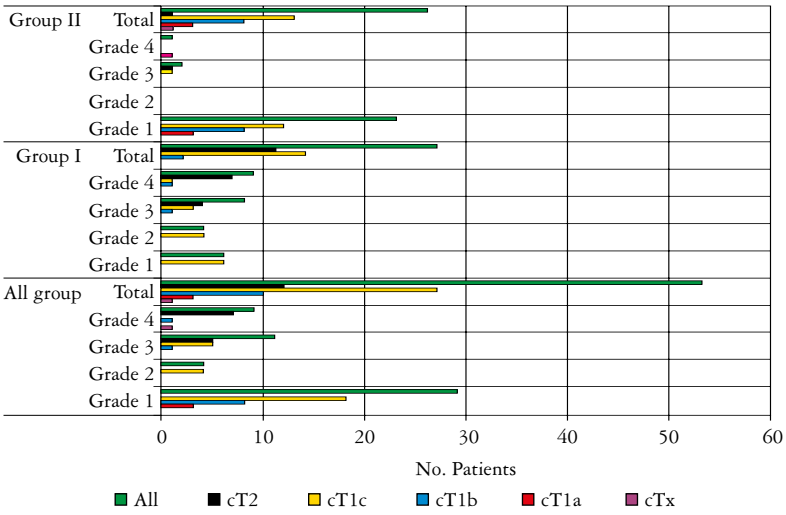

Fig. 4.

as necrosis, inflammatory infiltration and fibro-hyaline scarring. In the first week after cryotherapy there is mainly hemorrhagic necrosis, variable amounts of fibrosis, lymphocytic and plasma cell infiltration, xanthogranulomatous inflammatory reaction, giant cells and granulation tissue. The tumor remnants are diagnosed easily in histological examination. In uncertain cases an ancillary immunohistochemical examination can be performed. Our patients were operated on 1 to 35 days after cryotherapy and the tumor resection was examined histologically. It is not clear to us what happens thereafter and it has to be shown in other studies. The results of our study are extensively in concordance with other studies on the effects of cryotherapy. It is evident that this method is more successful in treatment of invasive ductal carcinomas in comparison with invasive lobular carcinomas and DCIS. In harmony with other working groups with similar tumor stages it is evident that the majority of patients could be managed successfully by cryotherapy. Sabel *et al.* reported the results of cryotherapy in 21 women with invasive tumors ≤ 2 cm. The surgical resection was performed 1 to 4 weeks later and it showed that all tumors with the

Table VI. Tumor grading

Tumor type in needle biopsy		Grade 1	Grade 2	Grade 3	Grade 4	Tumor type after cryotherapy
IDC	39	23 (43.4%)	3 (5.7%)	9 (17.0%)	4 (7.5%)	14 (58.3%)
ILC	6	3 (5.7%)	0	2 (3.8%)	1 (1.9%)	4 (16.7%)
ITC	3	3 (5.7%)	0	0	0	0
IDC + ILC	2	0	0	1 (1.9%)	1 (1.9%)	1 (4.2%)
DCIS	2	0	1 (1.9%)	0	1 (1.9%)	4 (16.7%)
ACC	1	0	0	0	1 (1.9%)	1 (4.2%)
All	53	29 (54.7%)	4 (7.5%)	12 (22.6%)	8 (15.1%)	24

Table VII. Tumor size

Tumor size (cT)	p – Value
T1a vs. T1b	p = 0.4185 (ns)
T1a vs. T1c	p = 0.2400 (ns)
T1a vs. T2	p = 0.0002
T1b vs. T1c	p = 0.4370 (ns)
T1b vs. T2	p = 0.0001
T1c vs. T2	p = 0.0001

Table VIII. Grading and histologic subtype

Grading			Histologic subtype (needle biopsy)		
			IDC	ILC	ITC
Gx	1	3.4%	0	0	0
G1	6	20.7%	3	0	3
G2	12	41.4%	9	3	0
G3	10	34.5%	10	0	0
All	29		22	3	3

maximal size of less than 1 cm could be eradicated completely. That means there was histologically no tumor remnant. In tumors with diameter of > 1.5 cm residual DCIS and invasive carcinoma have been found [16]. As confirmed by surgery one month after the cryotherapy histologically verified invasive breast carcinomas (≤ 2 cm) could be successfully managed. Roubidoux *et al.* reported their experience with cryotherapy of small (< 2 cm) breast tumors. By ultrasonographic examination 7 of 9 patients had no residual tumor [17]. In the study of Pusztaszeri *et al*. only 2 patients had a complete remission without residual vital tumor cells. Seven patients had partial remission with fractions of remaining invasive carcinoma. Two patients had intraductal carcinoma. The authors believe that the tumor destruction has no relation to tumor size (clinically < 2 cm) or the histological parameters. Their cases included only 1 invasive lobular carcinoma and 10 invasive ductal carcinomas [18]. Promising results have also been obtained in a pilot study by Vlastos *et al.* [6].

Many authors have reported that cryotherapy can be applied successfully in treatment of benign tumors such as fibroadenoma. In comparison with open surgery percutaneous cryotherapy provides better patient comfort, shorter hospitalization and lower costs. In addition, as the results of a 2-3 year follow-up showed, the tendency for scar formation can be reduced by cryotherapy of fibroadenomas without a detectable volume deficit by visual examination or on palpation [19]. In the case of malignant histological findings evaluation of HER-2 and hormone receptor status on a needle biopsy sample as well as a minimally invasive sentinel lymph node resection which are usual adjuvant processes in breast saving surgery have to be carried out. It can be followed by radiation therapy, or if indicated (on the basis of hormone receptor and HER-2 as well as lymph node status) by adjuvant antihormone or chemotherapy [20]. The histopathologic evaluation of a needle biopsy specimen is very important, because after successful cryotherapy no residual tumoral tissue may be present for the evaluations.

Cryotherapy is a safe, effective, practical and painless treatment option with good cosmetic results primarily in the management of fibroadenomas. For benign breast tumors cryotherapy is a preferred method, particularly in women who want to have management without an open operation [19]. Similar results are also expected with management of malignant breast tumors. Before widespread application of this it is necessary to validate through other larger studies that the local tumor control of invasive carcinoma by this minimally invasive method is reproducibly possible. Accordingly, larger studies with more cases and long-term follow-up are necessary.

The present study showed that percutaneous, ultrasonography-guided cryotherapy is a simple and safe method for ambulatory management of small breast carcinomas (cT1). The contraindications are multicentricity, less than 10 mm distance of tumor from skin and localization in the area of the nipple. Before the cryotherapy we had 6 patients with ILC, after cryotherapy in 4 cases a residual tumor in the form of ILC and all of them were more than 2.3 cm in size. It has to be pointed out that ILC are often accompanied by numerous foci of tumor and in our view are not good candidates for successful cryotherapy. In addition the informed consent of the patient is necessary.

Authors declare no conflict of interests.

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