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Relationship Between Microvessel Density and Thermographic Hot Areas in Breast Cancer

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Abstract

Purpose. This study was conducted to evaluate the validity of thermography in breast examination.

Methods. We performed contact thermography and measured the direct temperature by inserting a needle-type thermometer into the tissue. The core temperature of the tumor (dTt) and the temperature of the tissue surrounding the tumor (dTs) were compared with normal tissue. The microvessel density (MVD) and the MIB-1 labeling index (MIB-1 LI) of the tumor were examined immunohistochemically. The subjects were 48 women with primary invasive ductal carcinoma. The area of the tumor was diagnosed pathologically, and the hot area was measured using thermography.

Results. The dTt was significantly higher than the dTs. Both the dTt and dTs were significantly higher when the thermographical hot area was positive, or when more than four lymph node metastases were found. The dTs was correlated with MVD. A correlation between MVD and tumor temperature measured directly was also confirmed. A higher dTs was related to the dissociated wide area of the thermogram.

Conclusion. These findings suggested a relationship between dTs and the high-risk group of breast cancer. We also found that abnormalities in temperature were reflected in thermography and that a higher dTs was related to the dissociated wide area of the thermogram.

Key words Breast cancer · Thermography · Microvessel density · MIB-1 labeling index

Introduction

Recent studies have presented evidence that the detection of breast cancer by thermography is only coincidental and that this modality is ineffective for the screening of breast cancer.[1–6] In contrast, Sterns et al. reported that thermal abnormality was associated with large tumors and lymph node involvement, but was not an independent prognostic indicator.[6] Thermal abnormalities in the tumor, detected by contact thermography, may be related to blood flow, microvessel density (MVD), inflammation, distribution of tumor cells, viability of tumor cells, and other physical factors such as distance from the skin to the tumors. However, the extent of the effects caused by each factor to thermal abnormalities is unknown.

Tumors of the breast are usually seen as "hot areas" on thermography, but the consistency of thermographic hot areas with the actual temperature of the breast cancer has not been established. To evaluate the validity of thermography for breast lesions, we performed liquid contact thermography and measured the direct temperature by inserting a needle-type thermometer into breast tissue. The MVD and the MIB-1 labeling index (MIB-1 LI) of tumors were examined by immunohistochemical methods as objective parameters to assess the malignant potential of each tumor.

Patients and Methods

The subjects were 48 women who underwent surgical resection of primary invasive ductal carcinomas of the breast at Kurume University Hospital between October 1997 and November 1999. The clinicopathological features of these patients are summarized in Table 1. Patients who underwent open biopsy or preoperative radiation were excluded from this study. We informed

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all the patients about the risks of the procedure and obtained consent in writing.

Liquid Crystal Contact Thermography (Terumo, Tokyo, Japan)

Liquid crystal contact thermography was performed in the afternoon, 3 or 4 days before the operation. According to the method described by Koga et al., after 30 s of cold stress caused by a wind fan, a contact plate was placed on both sides of the breasts, pressure was applied for 15 s, and an image of the plate was obtained using a camera. The image was evaluated by assessing four parameters, namely, the locoregional hot areas, the vascular hot areas, the hot area of nipple, and the hot areas after cooling load.[1,4,7] Fig. 1A is a positive thermograph image showing a locoregional hot area, a vascular hot area, and a hot area of nipple associated with cancer in the right breast. Fig. 1B is a positive thermograph image after cooling load.

Table 1. Clinical and pathological factors in the 48 patients with invasive ductal carcinomas in the breast

Age (years)	49.9 ± 11.3 (27–76)
Tumor size	3.0 ± 1.6 cm (1.2–8.2)
LN metastasis	
positive	31 (65%)
negative	17 (35%)
UICC Stage	
I	12 (25%)
IIa	13 (27%)
IIb	13 (27%)
IIIa	9 (19%)
IIIb	1 (2%)
Menopause	
Pre-	29 (60%)
Post-	19 (40%)
Estrogen receptor	
Positive	22 (43%)
Negative	25 (49%)
Pathological type	
Papillotubular	32 (65%)
Solid-tubular	5 (12%)
Scirrhous	10 (21%)
Special type	1 (2%)
Comedo	
Positive	22 (46%)
Negative	20 (42%)

LN, lymph node

Direct Temperature Measured by a Needle-Type Thermometer (Unique Medical Tokyo, Japan)

A needle-type thermometer was inserted through the skin into the core area of the tumor (Tt), into the area surrounding the tumor (Ts), and into the normal tissue of the contralateral breast (Tc). The differences in temperature between the core area and the normal tissue ($dTt = Tt - Tc$), and between the surrounding area and the normal tissue ($dTs = Ts - Tc$), were calculated (Fig. 2).[8] To avoid inflicting emotional and physical pain on the patient, the needle was inserted under general anesthesia before breast surgery and, to prevent tumor seeding, it was inserted through the tissue that was expected to be resected surgically a few days later. The needles were not reused after being inserted into the tumor.

Microvessel Density

MVD was measured by immunostaining of paraffin-embedded tissue using the labeled streptavidin-biotin (LSAB) method. The core area and surrounding area of the tumor were selected and sliced into 4-μm thick sections on silane-coated slideglass. For antigen retrieval, the specimen was incubated in 0.1% Trypsin solution for 20 min at 37°C, before blocking endogenous peroxidase. The procedure for the LSAB method was carried out with the primary antibody, for factor VIII-related antigen (Dako, Tokyo, Japan). Counterstaining was performed by hematoxylin. Three points were exam-

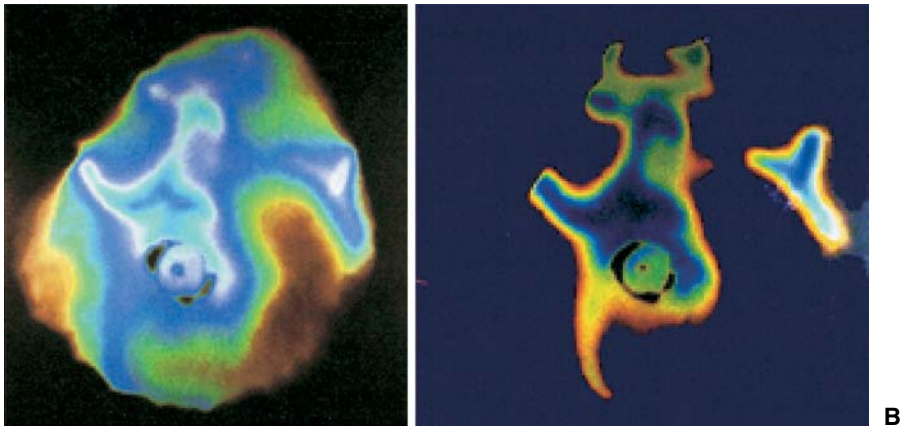

Fig. 1. Contact thermography showing **A** a positive locoregional hot area around the nipple, and **B** a positive hot area after cold load for 15 s

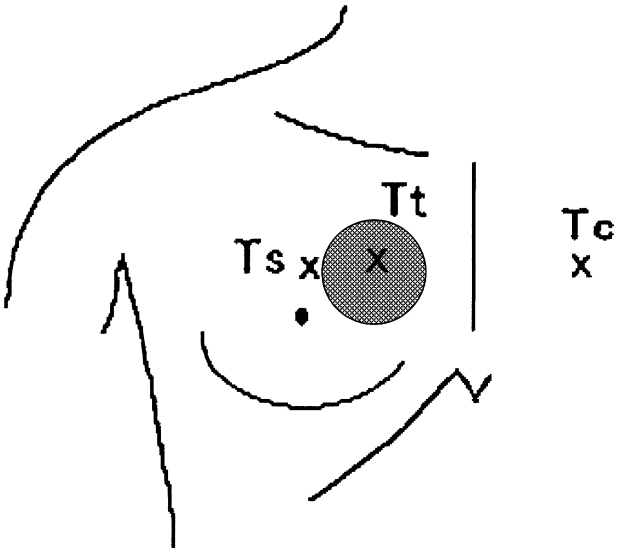

Fig. 2. Direct temperature measured by a needle. *Tt*, core of the tumor; *Ts*, 1 cm outside the surface of the tumor; *Tc*, symmetrical to Tt; *dTt*, Tt-Tc; *dTs*, Ts-Tc

ined by microscope (200×), and the number of microvessels in three viewfields (0.74 mm^2) was counted to calculate the averages for the MVDc (core area) and the MVDs (surrounding area) (Fig. 3A).[9–11]

MIB-1 Labeling Index

The MIB-1 LI was determined from immunostaining paraffin-embedded tissue using the LSAB method. The viable areas of tissue were sliced into 4-μm sections on silane-coated slideglass. For antigen retrieval, the specimen was incubated in a 0.05 M citrate buffer in a microwave oven at 100°C for 15 min before blocking the endogenous peroxidase. The procedure for the LSAB method was carried out using primary antibody: anti-MIB-1 (Dako, diluted 50×). The specimen was examined using a microscope (400×). The number of positive cells was counted from over 1000 tumor cells, and the percentage for MIB-1 LI was calculated (Fig. 3B).[12–14]

Comparison of Pathological and Thermographic Areas of the Tumor

The area of tumor diagnosed pathologically and the hot area measured by thermography were compared. The transformation of the image by formalin fixation and compression by the contact plate were taken into account. The condition in which the thermographic area was 20% wider than the pathological area was defined as a "Wide group", and the remaining condition was defined as a "Match group" (Fig. 4).

Clinicopathological Factors

The clinicopathological factors included pathological type, tumor size, lymph node (LN) metastasis, comedo, vessel invasion, and estrogen receptor status. The estrogen receptor (ER) status was measured using enzyme immunoassay.

Results

An abnormal thermogram was found in 43 (89%) of the 48 patients with invasive ductal carcinoma (Table 2). The dTt was significantly higher than the dTs, and both dTt and dTs were significantly higher when the thermographic hot area was positive (Table 3), or massive lymph node metastasis was found. No significant relationship was found between the dTt or dTs and menopausal status or ER status (Table 4). The dTt was weakly correlated with MVDc, and the dTs was weakly correlated with MVDs (Fig. 5). The dTs and MVDs of the Wide group were higher than those of the Match group (Table 5). Neither dTt nor dTs was significantly related to menopausal status, ER status, or MIB-1 LI.

Discussion

The findings of this study suggested that abnormalities in temperature were reflected by thermography. We also confirmed a correlation between MVD and tumor temperature measured directly. Thus, a relationship between tumor temperature and high-risk breast cancer defined as LN metastasis positive and comedo type is possible. Despite the distribution of microvessels, the average dTt was higher than the average dTs and higher dTs was related to the dissociated wide area of the thermogram.

Thermography was initially introduced as a potentially simple and noninvasive method of breast examination, and numerous studies have investigated whether thermographic findings are related to the prognosis or malignant potential of the tumor.[1–4,6,15] Most previous studies compared clinicopathological factors and thermographic findings with certain statistical analysis, although some reports focused on the mechanism by which the hot areas of the thermogram were produced.[1,6,16,17] Only one other report describes

Table 2. Results of contact thermography

Positive contact thermography	89% (43/48)
Locolesional hot area	81% (39/48)
Hot area related to vessel	50% (24/48)
Hot area related to nipple	10% (5/48)
Positive after cold load	83% (40/48)

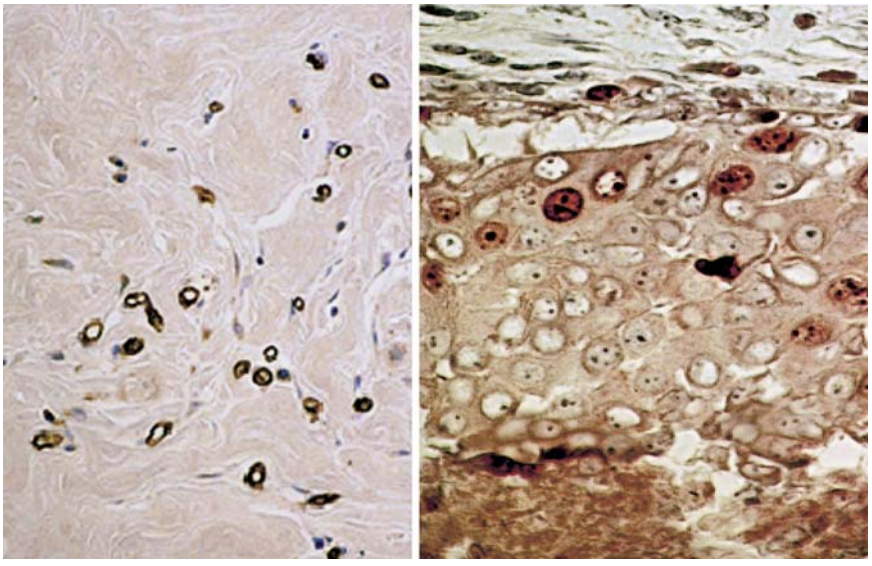

Fig. 3A,B. Microvessel density and MIB-1 using immunohistochemistry. **A** Microvessels. Microvessel density (MVD) = 144 (×200). **B** MIB-1. MIB-1 LI = 14.1 (×400)

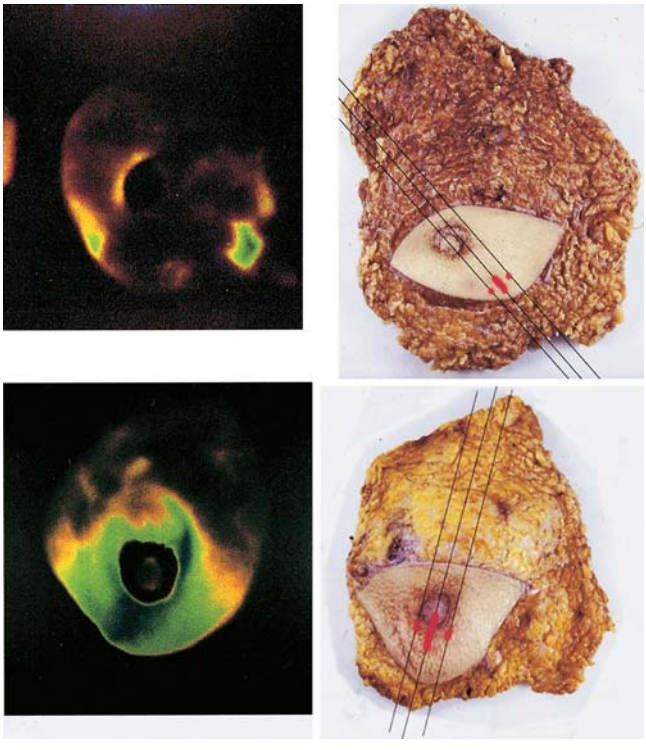

Fig. 4A,B. Comparison between the pathological area and the thermographic area of the tumor. **A** Match group ($n = 20$). Thermographic, 4.44 cm^2; pathological, 5.02 cm^2. **B** Wide group ($n = 28$). Thermographic, 10.30 cm^2; pathological, 5.44 cm^2

measuring the direct temperature of the tumor using a needle thermometer.[8] The clinical value and significance of thermography as a method of examination for breast cancer is still unclear for three reasons. First, the procedure and evaluation of thermography are cumbersome and complicated; second, the examination inflicts emotional and physical pain on the patients, especially when a needle is used; and third, there may be a risk of tumor cell seeding by needle insertion. We overcame these problems by inserting the needle under general anesthesia through the tissue that we expected to be resected by surgery a few days later. To prevent tumor seeding, the needles were not reused once they had been inserted.

Our findings demonstrated that the hot area was related to the dTs rather than to the dTt. Moreover, high dTs values were related to high MVDs, which in turn suggested the malignant potential of the tumor. MVD was more a significant factor in increasing the temperature of the breast tumor than proliferation factors such as MIB-1 LI.

We suspect that a tumor and its surrounding tissue produce signals, which are caused by the high density of vessels and blood flow. On the other hand, several factors may result in noises, including the distance from the skin to the tumor, age and menopausal status, menstrual cycle, and abnormal peripheral vascular hemodynamic behavior due to diabetes mellitus or liver cirrhosis.[18] Because the intensity of these noises may be strong, and the procedure for reading images of a thermogram is complicated and subjective, it is still difficult to apply thermography as a diagnostic procedure clinically. In 1979, Beahrs et al. of the National Canser Institute recommended excluding thermography from the routine examinations for diagnosing breast cancer.[19] However, in more than 20 years since their statement, many advances have been made in the devices for thermographic imaging to detect the signal and evaluate the image.[16,17] Improvement in the sensitivity and specificity of thermography in diagnosing breast cancers has been achieved, and therefore, re-evaluation of thermography is justified.

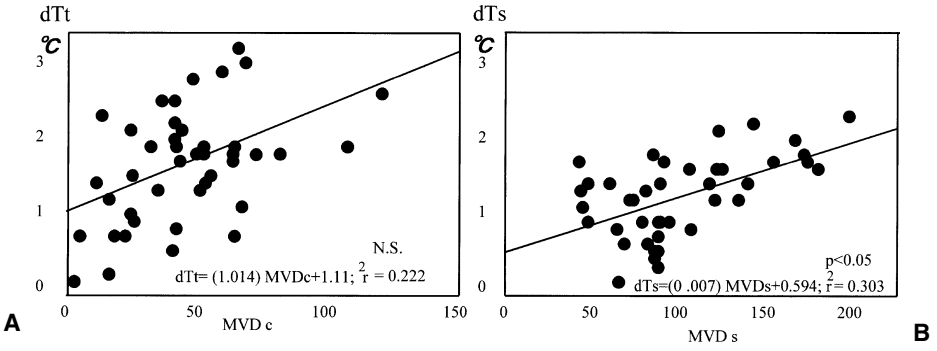

Fig. 5A,B. Correlation between MVD and dTt, dTs. **A** MVD-dTt; **B** MVD-dTs

Table 3. Hot areas and clinicopathological factors, MVD, MIB-1 LI, and dTt, dTs

Hot area	Positive	Negative	*P* value
No. of patients	39	9	
Age (years) (mean ± SD)	49.4 ± 11.5	53.0 ± 11.0	NS[a]
Tumor size (cm)	3.0 ± 1.7	2.7 ± 1.2	NS[a]
Pre/postmenopause	24/15	5/4	NS[b]
Node positive/negative	25/14	6/3	NS[b]
ER positive/negative	17/21	5/4	NS[b]
Comedo positive/negative	18/16	4/4	NS[b]
MVDc	78.26 ± 38.52	71.88 ± 31.64	NS[a]
MVDs	93.62 ± 35.54	72.55 ± 27.20	0.093
MIB-1 LI	18.6 ± 8.9	15.4 ± 7.7	NS[a]
dTs (°C)	1.79 ± 0.88	1.11 ± 0.73	0.045
dTs (°C)	1.30 ± 0.61	0.82 ± 0.51	0.042

MVDc, contralateral microvessel density; MVDs, surrounding microvessel density; dTt, core area temperature difference; dTs, surrounding area temperature difference; MIB-1 LI, MIB-1 labeling index; NS, not significant
[a] Student *t*-text
[b] χ^2-test

Table 4. dTt, dTs, and clinicopatholgical factors

	dTt (°C)		dTs (°C)	
Premenopausal ($n = 29$)	1.72 ± 0.41		1.14 ± 0.28	
Postmenopausal ($n = 18$)	1.56 ± 0.44	NS	1.06 ± 0.36	NS
>3 positive LN ($n = 24$)	1.45 ± 0.63		0.82 ± 0.36	
>4 positive LN ($n = 14$)	1.82 ± 0.48	NS	1.39 ± 0.49	$P < 0.05$
ER positive ($n = 22$)	1.52 ± 0.31		1.22 ± 0.48	
ER negative ($n = 25$)	1.68 ± 0.38	NS	1.09 ± 0.29	NS
Comedo positive ($n = 22$)	1.77 ± 0.62		1.40 ± 0.50	
Comedo negative ($n = 25$)	1.40 ± 0.54	NS	1.05 ± 0.26	$P < 0.1$

LN, lymph node; ER, estrogen receptor; NS, not significant (Student *t*-test)

It was suggested that the proliferative activity of the tumor such as MIB-1 LI, proliferating cell nuclear antigen LI, and mitotic index could exhibit a positive correlation with the temperature of tumor; however, in this study, there was no such correlation between MIB-1LI and dTt or dTs. The main factor that potentially raises the temperature of the tumor is the increase in blood flow from the core area of the body. Other possible factors include the resolution of necrotic tissue by phagocytosis and the activity of muscle fibers. In contrast, the main factor that reduces the temperature of the tumor is a decrease in blood flow from the core area of the body.[18,20,21]

We noted a certain diversion in the distribution of MIB-1 positive cells and microvessels. In some cases, microvessels were distributed significantly more in the surrounding area of the tumor, whereas in others, microvessels were distributed almost uniformly within the tumor. In each focus of the tumor, MIB-1-positive cells were either distributed significantly more in the peripheral area close to stromal tissue, or they were distributed almost uniformly. The focus in which MIB-1-positive cells were distributed significantly more in the peripheral area tended to have necrosis in the center of the tumor nest. This necrosis or decrease in proliferating activity (or possible tumor "dormancy") of tumor

Table 5. Dissociation between the pathological area and the thermographic hot area

		P value[a]
dTt (°C)		
Match group	1.35 ± 0.68	
Wide group	1.80 ± 0.96	0.123
dTs (°C)		
Match group	0.96 ± 0.47	
Wide group	1.39 ± 0.68	0.004
MVDc		
Match group	66.48 ± 33.47	
Wide group	80.50 ± 26.14	0.149
MVDs		
Match group	72.47 ± 25.76	
Wide group	105.25 ± 34.91	0.001

[a] Student *t*-test

cells may be due to hypoxia. In this study, we confirmed that the change in microvessel density by tumors was correlated to the temperature of the tumor.

In the clinical management of breast cancer, thermography may play two potential roles. First, it may be utilized as a method of screening for breast lesions, either malignant or benign; and second, it may be able to differentiate malignant from benign lesions that have been detected by other methods.[1,2,7] The advantage of thermography lies in the fact that it is noninvasive and does not require irradiation. As a measure for detecting tumors of the breast, it has a false-negative rate of nearly 10%, which is similar to that of mammography or ultrasound. The main disadvantages of thermography include the complexity of procedure and the subjectivity in reading the image. Advances in image analysis such as analyzing the moving image of a cold-loaded thermography using computer technology may help to improve thermography, by making it more objective and simple with higher sensitivity and specificity. The mechanism by which dTt was higher than dTs, even though MVDc was lower than MVDs, needs to be reinvestigated.

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