

## INFLUENCE OF HORMONAL STATUS ON THERMOGRAPHY FINDINGS IN BREAST CANCER

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**SUMMARY** – The aim of this study was to investigate the association of infrared imaging findings and hormone receptor (estrogen and progesterone) status in breast cancers. The study was carried out at Department of Surgical Oncology and Department of Pathology, Sestre milosrdnice University Hospital Center, in collaboration with licensed infrared thermography experts. The study involved 75 female patients with invasive breast tumors. Thermography findings were compared with different immunohistochemical findings (hormone status positive or negative). Seventy-five female patients aged 36 to 86 years, mean age  $64 \pm 11.36$  years, were examined. The tumor itself and the breast containing the tumor were statistically significantly warmer ( $p < 0.001$ ) than the healthy breast in all study patients. There was no statistically significant difference ( $p > 0.05$ ) between patients with positive and those with negative estrogen receptors. Unlike all previously published results of various thermographic studies, results obtained in this study on the hormone receptor status analyzed and its impact on thermographic findings indicated that estrogen negative tumors had a higher maximum and average temperature than estrogen positive tumors. It was also observed that estrogen negative tumors had lower impact on warming of the entire breast, and that maximum and average temperature of the affected breast was higher in estrogen positive tumors. Arithmetic means of maximum and average tumor temperatures were statistically significantly higher for progesterone negative tumors compared with progesterone positive tumors ( $p < 0.05$ ). Thermographic findings correlated with the specific hormonal status of breast invasive tumors, which reflects the biological behavior of tumors as well as their clinical variables.

*Key words: Infrared thermography; Hormone receptors; Breast cancer*

### Introduction

Infrared (IR) thermography as a diagnostic method for breast cancer is used in early detection, diagnosis and to determine prognosis. Thermal detection of tumors and other changes in the breast is based on the difference in temperature distribution between the two breasts, which reflects metabolic activity and vascular circulation<sup>1,2</sup>. Neoplasia, as observed by

thermography, is probably most important. It must be stated that thermal response is directly proportional to the biological significance of the tumor. The increase in blood flow as evidenced by hyperthermia and hypervascularity correlates with the degree of biologic activity. Either coming directly from the metabolism or indirectly from the host immune response, it correlates without respect to the size of tumor mass. Malignant tumors have shown various degrees of hyperthermia. On the other hand, inflammatory carcinoma has thermal disturbance observed by thermograms of greater than 6 °C when compared with the contralateral normal breast<sup>3,4</sup>. In precancer-

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ous tissue and surrounding of the evolving process of malignant breast, metabolism is still higher than in normal breast tissue. Due to the increasing need for nutrients, malignant change increases circulation to its cells by holding open blood vessels, opening collateral vessels and creating new blood vessels (neoangiogenesis). This process often results in temperature increase on the surface of the breast, which can be the first sign of precancerous changes or a sign of a formed malignant tumor<sup>5-8</sup>. Therefore, there seems to be close relationship between the temperature of the tumor covering skin and prognosis of patients with breast cancer. In previous studies, thermographic findings of breast cancer were compared with histopathologic and immunohistochemical (IHC) findings for evaluating thermographic usefulness. So far, previous thermography studies reported no evidence for breast infrared imaging association with hormone receptor (HR) status of breast cancers<sup>9-10</sup>. The aim of this study was to analyze the impact of HR status of invasive breast cancer on thermography findings.

## Patients and Methods

The study was carried out at Department of Surgical Oncology and Department of Pathology, Sestre milosrdnice University Hospital Center, in collaboration with licensed infrared thermography experts. The Ethics Committee of the School of Dental Medicine, University of Zagreb, approved the study.

The first survey included a total of 130 women, but only those with confirmed diagnosis of invasive breast cancer after biopsy and histopathologic diagnosis were included in the study (75 women). These patients were preoperatively examined using thermography. Thermography was performed using a ThermaCAM 2000 (FLIR Systems, Inc., North Billerica, MA, USA) thermographic system in outpatient conditions, in an air-conditioned 4x3-m room with constant humidity and temperature of 22-23 °C. The imaging was carried out with patients in sitting position, their arms on the back of the head, with maximum inspiration, from a distance of 80 cm. A front image was made of the thorax with axilla, both in the right and left oblique projections.

To determine expression of estrogen (ER) and progesterone (PR) receptors in tumor cells of pri-

mary breast carcinoma, IHC staining was carried out in an automatic DakoAutostainer at room temperature. Prepared tissue slides were treated with primary mice monoclonal ER $\alpha$  antibodies (DAKO; M 7047; 1:50) and PR (DAKO; M 3569; 1:75), according to the manufacturer's protocol, by HRP/DAB method of secondary antibody conjugated with peroxidase and DAB chromogen (Dako, Denmark)<sup>11</sup>.

According to the immunohistochemical reaction of breast tumor to ER and PR receptors, the result was considered negative if reactivity was indicated for less than 10% of tumor cells, i. e. nucleus.

IR image (thermogram), i. e. measurement results were analyzed using the FlirThermaCAM-Researcher software (FLIR Systems, Inc., North Billerica, MA, USA). A 'field' analysis tool was used to measure maximum, minimum and average values and standard deviation of temperatures of tumor sites, entire tumor breasts, healthy breasts and mirror tumor sites on healthy breasts (Fig. 1).

Thermography findings were compared between different hormone receptor statuses (positive or negative) of invasive breast tumors.

Statistical analysis of data was carried out by using the SPSS for Windows 17 (SPSS Inc., Chicago, IL). The normality of distribution was tested with one-way Kolmogorov-Smirnov test. Statistical analysis included descriptive statistics and Student's t-test for dependent and independent samples. The level of significance was set at 95% probability ( $p=0.05$ ). The null hypothesis was set that there would be no significant differences between the hormone positive and hormone negative receptor tumors according to thermography measured temperature.

## Results

The study included 75 patients with invasive breast tumors, aged 36 to 86 years, mean age  $64\pm 11.36$  years. Thirty (40%) patients were aged  $\leq 60$  and 45 (60%) patients were aged  $\geq 60$ . Thirty (40%) patients had tumors in the right breast and 45 (60%) in the left breast. Histologic types and sizes of tumors in the study group of patients are shown in Figures 2 and 3. According to histologic grade, 13 patients had grade I, 38 patients had grade II, and 22 patients had grade III tumors. Fifty of 75 patients had no positive axillary metastases

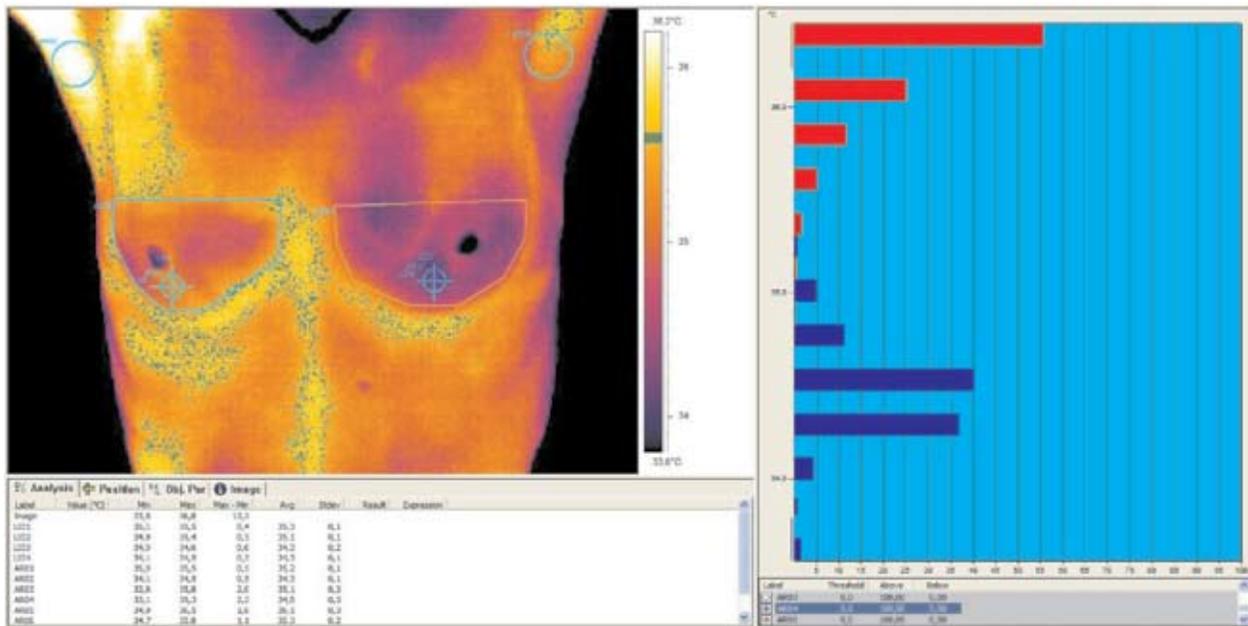


Fig. 1. Thermogram (circles indicate the areas of temperature measurement).

(68%), while 25 patients had positive axillary lymph nodes (32%). Distant metastases were found in three (4%) patients only. Fourteen (19%) patients had HER-

2 positive receptors and 59 (81%) patients had HER-2 negative receptors.

Table 1. Significance of differences between arithmetic means of temperatures measured in healthy breast and breast with tumors (Student's t-test for dependent samples)

Variable	$\bar{x}$	SD	diff	SD	t	df	p
Maximum temperature of tumor site	35.75 °C	1.05	0.83	0.79	9.17	74	<0.01
Maximum temperature in healthy breast tumor mirror site	34.92 °C	1.36					
Average temperature of tumor site	34.96 °C	1.16	0.76	0.75	8.94	74	<0.01
Average temperature in healthy breast tumor mirror site	34.2 °C	1.3					
Maximum temperature of entire breast with tumor	36.09 °C	0.94	0.24	0.56	3.7	74	<0.01
Maximum temperature of healthy breast	35.85 °C	1.08					
Average temperature of breast with tumor	34.39 °C	1.51	0.45	0.96	4.04	74	<0.01
Average temperature of healthy breast	33.94 °C	1.37					

SD = standard deviation; diff = mean of differences; df = degree of freedom

Table 2. Significance of difference between patients with tumors with positive and negative estrogen (ER) receptors (Student's *t*-test for independent samples)

	ER	N	x	SD	t	p
Maximum temperature of tumor site	-	17	35.81	0.72	0.49	0.62 NS
	+	56	35.67	1.09		
Average temperature of tumor site	-	17	35.03	0.75	0.46	0.65 NS
	+	56	34.88	1.24		
Maximum temperature of entire breast with tumor	-	17	36.02	0.67	-0.18	0.86 NS
	+	56	36.06	0.97		
Average temperature of entire breast with tumor	-	17	34.29	0.94	-0.21	0.83 NS
	+	56	34.38	1.66		
Maximum temperature of mirror tumor site in healthy breast	-	17	35.05	0.98	0.64	0.52 NS
	+	56	34.81	1.44		
Average temperature of mirror tumor site in healthy breast	-	17	34.11	1.12	-0.17	0.86 NS
	+	56	34.17	1.34		
Maximum temperature of entire healthy breast	-	17	35.69	0.69	-0.52	0.60 NS
	+	56	35.85	1.15		
Average temperature of entire healthy breast	-	17	33.78	1.13	-0.41	0.68 NS
	+	56	33.94	1.44		
Difference between average temperature of tumor site and mirror site of healthy breast	-	17	0.92	0.78	1.01	0.31 NS
	+	56	0.71	0.73		
Difference between average temperature of entire breast with tumor and entire healthy breast	-	17	0.51	0.43	0.24	0.81 NS
	+	56	0.44	1.09		

Among all patients with invasive breast tumors, 56 (77%) had estrogen positive receptors and 17 (23%) had estrogen negative receptors; 44 (60%) patients had positive progesterone receptors and 29 (40%) had negative progesterone receptors.

Table 1 shows testing for significance of differences in arithmetic means of temperatures between tumors and the contralateral side, as well as of maximum measured and average temperatures between the entire breast with tumors and healthy breast. A statistically significant difference was recorded in all cases, i.e. the tumors and the breast with tumors were statistically significantly warmer ( $p < 0.001$ ) than the contralateral side.

Descriptive statistics and testing for significance of differences between measured temperatures depending on tumor positive or negative estrogen receptors is shown in Table 2. Levene test indicated equality of variances between the positive and negative group; the degree of freedom was 71. There were

no statistically significant differences ( $p > 0.05$ ) between the patients with positive ( $n = 56$ ) and negative ( $n = 17$ ) tumor estrogen receptors, with the possibility of error of 5% (i.e. 95% probability). Table 2 reveals that the ER- group had a higher maximum and average tumor temperature as compared with the ER+ group. It is also indicated that the ER+ group had a higher maximum and average temperature of the entire breast with tumor as compared with the group with ER- tumors.

Descriptive statistics and testing for significance of difference between measured temperatures depending on positive or negative progesterone receptors in tumors is shown in Table 3. Levene test indicated equality of variances between the positive and negative group; the degree of freedom was 71. Student's *t*-test for independent samples generally indicated no statistically significant differences ( $p > 0.05$ ) (with the possibility of error of 5%) between the patients with progesterone positive receptor tumors ( $n = 44$ ) and

Table 3. Significance of difference between patients with tumors with positive and negative progesterone (PR) receptors (Student's *t*-test for independent samples)

	PR	N	x	SD	t	p
Maximum temperature of tumor site	-	29	36.01	0.77	2.14	<b>0.036* S</b>
	+	44	35.51	1.11		
Average temperature of tumor site	-	29	35.31	0.84	2.5	<b>0.015* S</b>
	+	44	34.66	1.25		
Maximum temperature of entire breast with tumor	-	29	36.25	0.70	1.55	0.126 NS
	+	44	35.92	1.00		
Average temperature of entire breast with tumor	-	29	34.65	1.01	1.35	0.18 NS
	+	44	34.16	1.75		
Maximum temperature of mirror tumor site in healthy breast	-	29	35.24	1.00	1.96	0.054 NS
	+	44	34.63	1.49		
Average temperature of mirror tumor site in healthy breast	-	29	34.42	1.13	1.46	0.15 NS
	+	44	33.98	1.35		
Maximum temperature of entire healthy breast	-	29	35.98	0.72	1.07	0.29 NS
	+	44	35.71	1.23		
Average temperature of entire healthy breast	-	29	34.22	1.16	1.61	0.11 NS
	+	44	33.70	1.47		
Difference between average temperature of tumor site and mirror site of healthy breast	-	29	0.89	0.68	1.22	0.23 NS
	+	44	0.68	0.78		
Difference between average temperature of entire breast with tumor and entire healthy breast	-	29	0.43	0.42	-0.14	0.89 NS
	+	44	0.47	1.22		

those with progesterone negative receptor tumors (n=29). The only statistical significance (with the possibility of error of more than 1%) ( $p > 0.01$ ) was noted for arithmetic means of maximum temperatures and average temperatures of tumors, which were considerably higher in progesterone negative than progesterone positive tumors ( $p < 0.05$ ).

## Discussion

Clinical value of IR thermography as a prognostic factor in patients with invasive breast tumors has in all previous thermographic studies been evaluated based on individual impact of clinical, histopathologic and some IHC tumor parameters, i.e. based on the size and number of their thermobiological pathological signs. It has been noticed that by their thermobiological pathological signs, some IHC factors could determine aggressiveness of malignant breast tumors. All previous thermographic studies have indicated that larger tumors with metastases in regional lymph nodes as compared to smaller tumors, fast proliferat-

ing as compared to slow proliferating, and less differentiated as compared to well differentiated, all have evidently more pathological thermobiological indicators, which is a characteristic of more aggressive invasive tumors<sup>12-17</sup>. It has also been observed that more aggressive invasive tumors belong to the so-called group of 'warmer tumors' according to their thermographic findings, and that they directly affect shorter disease-free period and total survival of patients as compared to the so-called 'colder tumors'<sup>9,16,17</sup>. In previous studies, thermographic findings of breast cancer were compared with histopathologic and immunohistochemical findings for evaluating thermography usefulness. The present study analyzed correlation between IR thermography and predictive and prognostic parameters (ER and PR) in female patients with invasive breast tumors.

Almost all previous significant thermographic research referred to in this study found no difference in tumor hormone status (ER and PR positive or negative) on thermographic findings<sup>9-10,17,18</sup>.

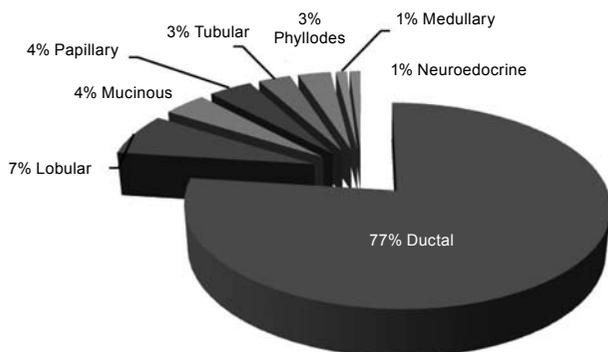


Fig. 2. Distribution of patients according to histological types of tumors.

The fact that during their aggressive growth, invasive breast tumors cause an increase in temperature, which is reflected on the body surface (skin) and which can be measured by IR thermography, was confirmed by the first thermography findings in this study. In all study patients, maximum and average temperature of both the tumor and the entire affected breast was statistically significantly higher than the maximum and average temperature of the contralateral mirror site and the entire healthy breast.

Unlike all previously published results of various thermographic studies, the results obtained in this study revealed that ER- tumors had a higher maximum and average temperature compared to ER+ tumors. It was also observed that ER- tumors had lower impact on warming of the entire breast, and that maximum and average temperature of the affected breast was higher in ER+ tumors. There are a number of possible explanations for these temperature differences, including angiogenesis, nitric oxide, inflammation, and estrogen.

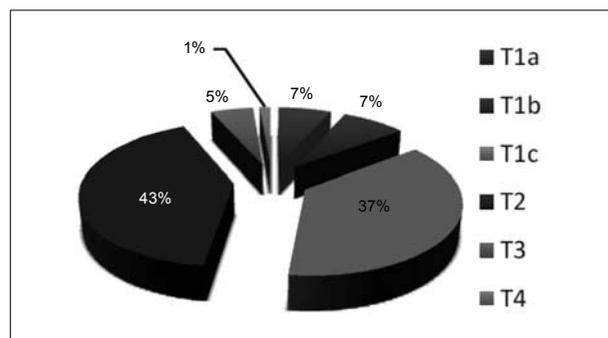


Fig. 3. Distribution of patients according to size of tumors.

Endocrine changes, inflammation, and the presence of tumors modify temperature and vascularization of the breasts. In order to grow, tumors must develop blood vessels to deliver necessary nutrients and oxygen to support their growth. These blood vessels developed in the process of pathologic angiogenesis lack smooth muscle cells, thus rendering the blood vessels incapable of normal vasoconstriction. Tumors are known for the presence of predominantly capillary network of blood vessels. During their aggressive growth, invasive breast tumors need additional blood and nutrients. Estrogen positive tumors use estrogen for increased vasodilation of the surrounding blood vessels, thus affecting temperature changes of the entire breast. Estrogen also mediates vasodilation by increasing the local production of nitric oxide, therefore estrogen imbalance could result in vasodilation of the estrogen sensitive tissues leading to localized temperature changes<sup>5-8</sup>. Probably for this reason, ER positive tumors have higher impact on warming of the entire breast. ER negative tumors probably do not have this capacity. Another question that remains to be answered is the impact of ER+ tumors on thermographic findings considering the percentage of positive tumor cells and intensity of their staining. As it is known, the ranges could be large. This could be studied in some future thermographic studies. The other part of the answer could be different biological behavior of ER+ and ER- tumors. The researches have proven that ER- tumors are more aggressive by their nature, which results in their faster growth, thus their differentiation is poorer, and they are more frequently aneuploid, with a higher mitotic activity and metastatic potential. Some studies have reported an association with HER-2 overexpression and lack of expression of PR receptors<sup>19-24</sup>.

In our study, there was no statistically significant difference in hormone receptor status between patients with ER+ and ER- tumors according to measured temperature. The results obtained in this study are similar to the results of the majority of previously published thermographic studies<sup>9,10,17</sup>. Only Sterns *et al.* found the ER- impact on thermographic findings to be greater than the ER+ impact<sup>18</sup>.

Progesterone also serves as an indicator of tumor aggression. Elevated progesterone levels indicate less aggressive tumors that are associated with a longer

time to treatment failure and longer overall survival time in metastatic disease, whereas PR- tumors are more aggressive<sup>20-23</sup>.

Considering the impact of the progesterone receptor status on thermographic findings, it is obvious that PR- tumors, compared to PR+ tumors, had a statistically significant impact on two measured temperatures, i.e. maximum and average tumor temperature. Other findings also suggested a tendency of PR- tumors to exert greater impact on the measured temperatures than PR+ tumors.

The correlations of negative receptor status with poor differentiation, high proliferation rate and other unfavorable characteristics provided the rationale for studies of HR expression as a possible prognostic factor for patients with invasive breast cancer. On the other hand, tumors that are initially HR negative are accompanied by a more aggressive tumor behavior, loss of endocrine control, and poor survival<sup>18-21</sup>. As mentioned above, it has been noticed that by thermobiological pathological signs some IHC factors could determine aggressiveness of malignant breast tumors, i.e. that less aggressive tumors are colder and more aggressive tumors are warmer in thermographic findings. The present study showed the biological nature of the tumor considering different hormone receptor status of the tumors to exert different impact on thermographic findings. Based on the present study results, the patients with hormone negative tumors belonged to the group of warmer tumors, whereas the patients with hormone positive tumors belonged to the group of colder tumors, which has not been indicated by observations from other thermographic studies<sup>9-10,16-18</sup>.

## Conclusion

Based on the introduction section, the results of this study, and previous discussion, it is concluded that the tumors with positive hormone receptors (ER+, PR+) by thermographic findings are colder, which implies biologically less aggressive tumors compared to tumors with negative hormone receptors (ER-, PR-), which are by thermographic findings warmer and could be classified into biologically more aggressive tumors. It can be said that thermographic findings correlated with the specific hormonal status

of invasive tumors, as reflected in their different biological behavior.

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### Sažetak

#### UTJECAJ HORMONSKOG STATUSA NA TERMOGRAFSKI NALAZ KOD RAKA DOJKE

Z. Zore, I. Boras, M. Stanec, T. Orešić i I. Filipović Zore

U ovom istraživanju analizirao se utjecaj hormonskog receptorskog statusa, tj. estrogenskih (ER) i progesteronskih (PR) receptora na termografski nalaz kod bolesnica s karcinomom dojke. Rad je napravljen u KBC "Sestre milosrdnice" na Zavodu za onkološku kirurgiju i Zavodu za patologiju u suradnji s licenciranim stručnjacima za termografiju sa Zavoda za termodinamiku, toplinsku i procesnu tehniku Fakulteta strojarstva i brodogradnje u Zagrebu. Istraživanje je obuhvatilo 75 prijeoperacijski termografski snimljenih bolesnica s invazivnim tumorom dojke, starosti 36-86 godina, prosječne dobi 64±11,36 godina. Rezultati istraživanja su pokazali da je dojka s tumorom statistički značajno toplija ( $p < 0,001$ ) u odnosu zdravu dojku kod svih bolesnica. Nije bilo statistički značajne razlike ( $p > 0,05$ ) između bolesnica s pozitivnim u odnosu na one s negativnim estrogenskim receptorima. Za razliku od svih prethodno objavljenih rezultata različitih termografskih istraživanja invazivnih tumora dojke i utjecaja hormonskog receptorskog statusa na termografske nalaze, iz rezultata dobivenih u ovom istraživanju može se primijetiti da su ER- tumori imali višu maksimalnu i prosječnu temperaturu u odnosu na ER+. Primjetno je da su ER- tumori imali manji utjecaj na zagrijavanje cijele dojke, kao i to da je maksimalna i prosječna temperatura cijele dojke bila viša u ER+ tumorima. Aritmetičke sredine za maksimalne i prosječne temperature tumora statistički su značajno više kod progesteronski negativnih tumora u usporedbi s progesteronski pozitivnim tumorima ( $p < 0,05$ ). U zaključku, za razliku od ranijih termografskih istraživanja, rezultati termografske analize invazivnih tumora dojke u ovom istraživanju pokazali su da postoji razlika po utjecaju na termografske nalaze s obzirom na status hormonskih receptora. Ove rezlike ukazuju na danas dokazane različite imunohistokemijske, patohistološke i biološke osobine tumora dojki s obzirom na status hormonskih receptora.

Ključne riječi: *Infracrvena termografija; Status hormonskih receptora; Invazivni tumori dojke*