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Association of clinical, histopathological and immunohistochemical prognostic factors of invasive breast tumors and thermographic findings

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HIGHLIGHTS

- Increased temperature is more dependent on the IHC phenotypes of the tumors.
- The highest increase of temperature was found in the +HER-2,−PR and the high Ki-67.
- The tumor size and other factors had no influence on the temperature findings.

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ABSTRACT

Background: The purpose of the paper was to analyse and compare infrared thermographic findings with clinical, histopathological and immunohistochemical prognostic parameters in female patients with invasive breast tumor. *Methods:* A pre-operative thermography was made in 75 female patients with breast invasive tumors. The study analysed an individual impact of each clinical, histopathological (HP), and immunohistochemical (IHC) factors on thermographic findings, the joint impact of all factors and combined impacts according to the IHC phenotypes. *Results:* Statistically significant difference of thermographic findings between healthy and affected breast was found for positive human epidermal growth factor receptor-2 (HER-2) tumors, negative progesterone receptors (PR−) and a high proliferative activity (Ki-67 > 30%) ($p < 0.05$). Dependent on the IHC phenotype, temperatures varied from the coldest (ER+, PR+, HER-2−) tumor towards the warmest tumors (ER+, PR−, HER-2+).*Conclusions:* According to the results of this study, the increased temperature was more dependent on the IHC phenotypes of the tumor than on other clinical and histopathological prognostic factors. Moreover, tumor size had no influence on increased temperature.

1. Introduction

Infrared imaging provides information of a pathophysiological tumor induced angiogenesis and its metabolic activity. Infrared imaging has manifested itself as the earliest detection technology for breast cancer [1–5]. To reduce the mortality from breast cancer, except from early diagnostics it is also important to examine and characterize tumors of poor prognosis, to predict their biology, and ensure adequate therapy. Some previous studies involving infrared thermography of breast invasive tumors compared thermographic findings with clinical, histopathological (HP) and certain immunohistochemical (IHC) findings, but the results were not consistent and included only few IHC parameters. Thermal abnormality and its association with different clinical and IHC prognostic factors of invasive breast tumor has been unclear and controversial. Almost all previous studies reported that thermographic abnormalities (increased temperatures) were significantly associated with increased tumor size, axillary lymph nodes involvement, and high tumor grades [6–8]. So called "warmer" cancers show poorer prognosis [7]. Some studies showed better prognosis considering a survival rate of invasive breast tumor patients in less biologically active or "cooler" cancers [6–9]. Majority of thermographic studies reported no clear relationship between menopausal status, tumor location, hormonal status, and histological type of a tumor with any thermographic abnormality [6–9]. It seems that a close relationship exists between tumor temperature, tumor stage, tumor growth rate and a poor prognosis. The aim of the study was to analyse and compare infrared thermographic findings with clinical, histopathological and

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immunohistochemical prognostic parameters in female patients with invasive breast tumors.

2. Material and methods

The study was carried out at the Department of Surgical Oncology and the Department of Pathology, Clinical Hospital Centre "Sestre milosrdnice", in collaboration with licensed infrared (IR) thermography experts from the University of Zagreb, Faculty of Mechanical Engineering and Naval Architecture, Department of Thermodynamics, Thermal and Process Engineering.

2.1. Patients

The study included 75 female patients with diagnosed invasive breast cancer, who were examined throughout the year 2011. Age range of the patients spanned from 36 to 86 years. Mean age was 64 ± 11.36 years. All patients were informed about the risk of the procedures and after signed informed consent they were included in the study. In the process of clinical–pathological staging the following parameters were obtained: location of the tumor (left or right breast), histological types and tumor grade, tumor size, lymphonodal status and a presence of a distant metastatic disease. Laboratory testing included determination of the following IHC prognostic indicators: estrogen (ER) and progesterone (PR) receptors, HER-2 and Ki-67 (Table 1). According to a combination of the analysed IHC factors of invasive tumors, different groups of patients with the same IHC tumor phenotype were created according to the temperature scales starting from those with the lowest recorded average temperature of a tumor itself and of the whole breast with tumor (ER+, PR+, HER-2−), towards those with higher average temperatures (ER−, PR−, HER-2−/ER, PR−, HER-2+ ER+, PR−, HER-2−) and ultimately to the patients with the highest recorded temperatures (ER+, PR−, HER-2+).

2.2. Thermography

The thermography was carried out using the Therma CAM 2000″ (FLIR Systems, Inc. North Billerica, MA, USA) under ambulatory conditions, in an air-conditioned room with constant humidity and temperature between 22 and 23 °C. The imaging was carried out with patients in a sitting position from a distance of 0.8 m. A frontal image was made accompanied with additional 2 images, both in the right and the left oblique projections. The infrared (IR) image (thermogram), namely the measurement results were analysed using a computer software: "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 deviations of a temperature of tumors sites, entire tumor breasts, entire healthy breasts and the mirror tumors sites in healthy breasts. The following differences were obtained: the difference between the average temperature of a tumor site (affected breast) and the average temperature of a mirror site (healthy breast), as well as the difference between the entire affected breast and the entire healthy breast.

2.3. Immunohistochemistry (IHC)

The IHC staining was carried out in an automatic Dako Autostainer at room temperature to determine the expression of ER and PR in tumor cells of the primary breast cancer. Prepared tissue slides were treated with primary mice monoclonal ERα 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 chromogene (Dako Danmark). According to the immunohystochemical reaction of breast tumor to ER and PR, the result was considered negative if reactivity was indicated for less than 10% tumor cells.

The HER-2 expression determination (Kit HER-2, DAKO, Danmark, K 5207; ready to use) was done routinely using Hercep-Test® according to the manufacturers' protocol. Grading was done using the 0 to 3+ scale (United States Food and Drug Administration (FDA)-approved grading system). Positive findings for HER-2 receptors were those with 3+ or 2+, confirmed by a chromogenic in situ hybridization (CISH) or, when the CISH findings were not clear, by additional fluorescence in situ hybridization (FISH). The tumor proliferation rate was measured immunohistochemically using the Ki-67 monoclonal antibody. We divided proliferative activity of tumors into low (<15%), intermediate (15–30%) and high (>30%).

2.4. Statistical analysis

Statistical analysis was carried out by using the SPSS 17 for Windows (SPSS Inc., Chicago, Il). The statistical analysis included descriptive statistics, Pearson's coefficients of correlation, the Student *t*-test for independent samples, the one-way ANOVA (post hoc Sheffe), and multifactorial analysis. The level of significance was set at 95% probability ($p = 0.05$).

3. Results

There was no significant correlation between the tumor size and any of the analyzed variables (Table 2). The ER+ tumors were positively correlated with the PR+ tumors, negatively with the high Ki-67 and the high tumor grade. The PR+ tumors were positively correlated with the ER+ tumors, negatively with the high Ki-67, the high grade and the HER-2+. The HER-2+ tumors were positively correlated with the high grade, and negatively with the PR+. Tumors with the high Ki-67 were positively correlated with the high grade, negatively with the ER+ and the PR+. Tumors with high grades were positively correlated with the high Ki-67 and the HER-2+, and negatively with the ER+ and the PR+ (Table 2).

The statistically significant impact on the increase of a temperature was recorded for the following variables: HER-2+ [average temperature of a tumor ($p = 0.035$), maximum temperature of entire breast with tumor ($p = 0.012$), maximum temperature of

Table 1
Clinicopathological and immunohistochemical characteristics of the tumors in the examined patients.

AGE		SIDE		TYPE		T		N		M		GRADE		ER		PR		HER-2		Ki-67	
<60	40%	R	40%	Ductal	77%	T1a	7%	+	32%	+	4%	I	18%	+	77%	+	60%	+	19%	<15%	25%
>60	60%	L	60%	Lobular	7%	T1b	7%	–	68%	–	96%	II	52%	–	23%	–	40%	–	81%	15–30	29%
				Other	16%	T1c	37%					III	30%							>30%	46%
						T2	43%														
						T3	5%														
						T4	1%														

T = tumor size; N = axillary lymph nodes; M = distant metastasis; ER = estrogen receptors; PR = progesterone receptors.