Infrared Physics & Technology 68 (2015) 201–205

Contents lists available at ScienceDirect

Infrared Physics & Technology

journal homepage: www.elsevier.com/locate/infrared

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.

ARTICLE INFO

Article history:
Received 3 September 2014
Available online 16 December 2014

Keywords:
Infrared thermography
IHC
Prognostic factors
Breast cancer

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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http://dx.doi.org/10.1016/j.infrared.2014.12.009
1350-4495/

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.

Table 2
Correlations between tumor size, ER and PR receptors, HER-2, Ki-67 and tumor grade.

Variable		T (size)	ER	PR	HER-2	Ki-67	Grade
Tumor size	Pearson correlation		0.03	−0.16	−0.12	0.11	0.17
	Sig. (2-tailed)		0.79 NS	0.18 NS	0.32 NS	0.35 NS	0.14 NS
	N		73	73	73	73	73
ER	Pearson correlation	0.03		**0.679****	−0.23	**−0.336****	**−0.424****
	Sig. (2-tailed)	0.79 NS		**<0.0001**S**	0.06 NS	**<0.0001**S**	**<0.0001**S**
	N	73		73	73	73	73
PR	Pearson correlation	−0.16	**0.679****		**−0.387****	**−0.353****	**−0.469****
	Sig. (2-tailed)	0.18 NS	**<0.0001**S**		**<0.0001**S**	**<0.0001**S**	**<0.0001**S**
	N	73	73		73	73	73
HER-2	Pearson correlation	−0.12	−0.23	**−0.387****		0.23	**0.269***
	Sig. (2-tailed)	0.32 NS	0.06 NS	**<0.0001**S**		0.06 NS	**0.02*S**
	N	73	73	73		73	73
Ki-67	Pearson correlation	0.11	**−0.336****	**−0.353****	0.23		**0.647****
	Sig. (2-tailed)	0.35 NS	**<0.0001**S**	**<0.0001**S**	0.06 NS		**<0.0001**S**
	N	73	73	73	73		73
Tumor grade	Pearson correlation	0.17	**−0.424****	**−0.469****	**0.269***	**0.647****	
	Sig. (2-tailed)	0.14 NS	**<0.0001**S**	**<0.0001**S**	**0.02*S**	**<0.0001**S**	
	N	73	73	73	73	73	

S = significant; NS = not significant.
* Significant $p < 0.05$.
** Significant $p < 0.01$.

Table 3
Significance of the differences between different age of patients (<60 years, >60 years), different tumor types, positive or negative lymphonodal status, positive or negative distant metastasis, different tumor grades, ER positive and negative tumors, PR positive and negative tumors, HER-2 positive and negative tumors, and Ki-67 positive and negative tumors for maximum and average temperature of the tumor, maximum and average mirror site temperature, maximum and average temperature of affected breast and maximum and average temperature of healthy breast, as well as for the temperature difference between the tumor site and the mirror site, and the affected breast and healthy breast (independent Student's *t* test or one way ANOVA, post hoc Sheffe).

	AGE *p*	T -type *p*	N *p*	M *p*	Grade *p*	ER *p*	PR *p*	HER-2 *p*	Ki-67 *p*
Maximum tumor temperature	0.50NS	0.55NS	0.99NS	0.98NS	0.15NS	0.62NS	**0.036*S**	0.055NS	0.16NS
Average tumor temperature	0.98NS	0.91NS	0.81NS	0.87NS	0.09NS	0.65NS	**0.015*S**	**0.035*S**	0.17NS
Maximum temperature of entire affected breast	0.87NS	0.91NS	0.89NS	0.74NS	0.39NS	0.86NS	0.126NS	**0.012*S**	0.47NS
Average temperature of affected breast	0.97NS	0.94NS	0.30NS	0.79NS	0.11NS	0.83NS	0.18NS	0.11NS	0.29NS
Maximum temperature of tumor's mirror site in healthy breast	0.97NS	0.41NS	0.73NS	0.95NS	0.67NS	0.52NS	0.054NS	0.365NS	0.93NS
Average temperature of tumor's mirror site in healthy breast	0.31NS	0.75NS	0.90NS	0.49NS	0.52NS	0.86NS	0.15NS	0.284NS	0.94NS
Maximum temperature of entire healthy breast	0.71NS	0.94NS	0.81NS	0.90NS	0.74NS	0.60NS	0.29NS	**0.029*S**	0.95NS
Average temperature of entire healthy breast	0.38NS	0.2NS	0.67NS	0.85NS	0.72NS	0.68NS	0.11NS	0.06NS	0.94NS
Difference between the average temperature of tumor site and mirror site	0.07NS	0.36NS	0.88NS	0.15NS	0.12NS	0.31NS	0.23NS	**0.048*S**	**0.011*S**
Difference between average temperature of entire affected breast and healthy breast	0.19NS	1.00NS	0.10NS	0.89NS	0.10NS	0.81NS	0.89NS	0.86NS	0.15NS

T-TYPE – tumor histological type, N – axillar nodes, M – distant metastasis, GRADE – tumor grade, ER – estrogen receptors, PR – progesterone receptors, HER-2 protein, Ki-67 – proliferative activity; S* = significant; NS = not significant.

entire healthy breast ($p = 0.029$) and the difference between the average temperature of the tumor site and the mirror site of the healthy breast ($p = 0.048$)]; PR− [maximum temperature of a tumor ($p = 0.036$) and the average temperature of a tumor ($p = 0.015$)]; Ki-67 [the difference between the average temperature of the tumor site and the mirror site ($p = 0.011$)] (Table 3).

Multifactorial analysis of all analysed clinical, HP and IHC parameters revealed statistically significant model ($p = 0.047$) with statistically significant impact of positive HER-2 on the increased tumor temperature ($p = 0.013$), as well as a significant impact of the combination of positive HER-2 tumors and the high Ki-67 (>30%) ($p = 0.046$).

According to the ICH findings and the registered tumor temperature and the tumor size, they are arranged from the coldest to the warmest tumors (Table 4).

4. Discussion

Infrared imaging has been found to provide reflection of a biological activity in the breast. Several studies have shown that infrared imaging is a good method for the risk assessment of a breast cancer [1–5]. Another application of infrared imaging of a breast cancer may be for predicting a prognosis. To date, the thermal mechanisms associated with different clinicopathological and some IHC predictive and prognostic factors of a breast cancer have not been fully understood and explained. The data obtained from earlier thermographic studies is inconsistent. Almost all previous studies reported that thermographic abnormalities were significantly associated with a large tumor size, axillary lymph nodes involvement and a high tumor grade [6,7]. However, no clear relationship was found between thermographic abnormality and menopausal status, hormonal status, tumor location and tumor types [6,7]. Some studies exploring cancer biology showed that amount of thermovascular activity in the breast was directly proportional to the aggressiveness of a tumor. A clear association between thermographic findings and clinical, HP and IHC prognostic parameters in invasive breast tumors has not been established yet. Previous studies showed that small tumors did not generate sufficient metabolic heat to produce significant increase of skin temperature above a tumor site of affected breast [7–9]. However, before mentioned study cannot explain those large tumors, without axillary

Table 4
Average temperatures and average tumor sizes of affected breast based on the IHC profile.

n	ER	PR	HER-2	Average tumor size (mm^3)	Average temperature of the affected breast (°C)	Average temperature of the tumor (°C)
41	+	+	–	**16,254**	**34.09**	**34.58**
11	–	–	–	5418	34.15	34.95
6	–	–	+	18,577	34.53	35.18
7	+	–	–	3396	35.11	35.70
5	+	–	+	**8863**	**35.24**	**35.74**

metastases, of low grade and low proliferation rate which do not increase temperature or those small tumors with axillary metastases, high grade and a high proliferation rate which increase breast temperature [10]. Therefore we tried to reveal the processes that generate tumor thermal energy.

The results in this study showed that clinical and HP prognostic factors of breast cancer, such as age of a patient, histological type, dissemination of a disease, nodal status and histological grade had no significant impact on the thermographic findings of the tested patients. A significant impact on increased temperature was recorded for the HER-2+, PR– and a high proliferative activity (Ki-67 > 30%). Since there has been none of the thermographic studies concerning the impact of HER-2 status on the affected breast thermographic findings in the available literature [9,11], this paper aimed to study, besides other HP and some IHC parameters, the role of the HER-2 receptor. The results revealed that positive HER-2 tumors had the highest impact on the increased temperature. The HER-2 belongs to a family of the 4 transmembrane receptor tyrosine kinases that mediate the growth, differentiation, and survival rate of cells [11]. It is overexpressed or amplified in approximately 15–25% of breast cancers and it has been associated with aggressive tumor behavior [12,13]. Some studies reported association of HER-2 overexpression and other adverse prognostic factors, such as positive lymph nodes, larger tumor size, high histological grade, high proliferation rate, and a lack of expression of ER and PR [12,13].

Except for the HER-2+, this study also revealed PR– influence on the increase of the affected breast temperature. Progesterone is supposed to be an indicator of tumor aggressiveness. Elevated PR+ levels indicate less aggressive tumors, associated with a longer time to treatment failure and a longer overall survival rate, whereas PR– tumors are supposed to be more aggressive [14–16]. Some studies reported association of HER-2 overexpression and a lack of expression of progesterone receptors [15–20]. Other thermographic studies found no association between the PR– status and the increased temperature of affected site [6–9].

This study showed a significant impact on the temperature increase of the affected sites for high proliferative activity (Ki-67 > 30%) (Table 3) and a summed effect of a positive HER-2 with high Ki-67 (multifactorial analysis). Some studies showed that the proliferation-associated antigen Ki-67 and their metabolic heat pattern were higher in tumors with abnormal asymmetric breast infrared images [7–9]. Only one study reported significant Ki-67 association with the abnormal thermogram (increased temperature) [6]. The high Ki-67 prognostic indicator suggest that breast cancer patients have faster-growing tumors which are more likely to metastase or have a shorter disease-free interval [21,22]. Gene expression profiling has greatly contributed to our knowledge of the biology of breast cancer by identifying distinct prognostic phenotypes. There are many genetic and epigenetic factors involved in breast cancer initiation, local progression and metastasis. Compared to gene expression profiling, IHC staining provides a more rapid diagnosis and classification of breast tumors. Analysis of ER, PR, and HER-2 expressions has been a reliable surrogate marker to the gene expression based tumors classifications [23]. It has been reported that patients with ER+ and PR+ tumors may have a better survival rate than those with hormone negative tumors [21–23]. The ER and the PR are supposed to be a biological gradient for cancer survival, with worse prognosis from ER+PR+, towards ER+PR–, ER–PR+ and ER–PR– [21–23].

So far, no thermographic study analysed impact of different IHC phenotypes of breast tumors on thermographic findings. This study showed significant influence of ICH findings on the increased temperature of the affected breast with a contribution of a proliferation rate and a higher tumors grade. However, tumors sizes had no significant impact. The coldest tumors had the highest size, the lowest histologic grade, the lowest proliferative activity and the IHC phenotype with no significant impact. The warmest tumors showed significant influence of all IHC parameters on the thermographic findings. The warmest tumors group of patients had much smaller tumors in relation to the cold group, but the highest histologic grade and the highest proliferative activity. However, the group with the poorest IHC prognostic parameters, i.e. the triple-negative phenotype (ER–, PR–, HER-2–) also belonged to a group of cold tumors. Cold tumors were found both in those with the best and in those with the worst IHC prognostic parameters, contrary to observations from earlier thermographic studies [6–9]. All other tumors followed the principle that the poorer IHC prognostic parameters (except triple-negative tumors) corresponded to the more increased temperature. According to the results of the present study, patients with ER and PR negative tumors, were classified into the warm tumors, conversely, a group of patients with hormone positive tumors were classified into the cold tumors, which is not in agreement with some other thermographic studies [6–9].

T component of TNM classification system of malignant breast tumors, whether it is clinical or pathological classification, is used as a prognostic indicator in a way that more negative prognosis is in correlation with increased T value, thus with the later stage of the disease. In this research it was shown that thermography can be prognostic method (with other prognostic methods) since the production of visible warmer areas was seen with very small tumors (as seen in Tables 3 and 4), and that heat production is linked to immunohistochemical phenotype which represents aggressive biological character of malignant tumor. It is also seen that large tumors of certain immunohistochemical phenotype produce less heat since they are biologically more inert, i.e. less aggressive. This paper clearly shows that heat production by malignant tumor is in correlation with biologically more aggressive tumors (i.e. IHC tumor phenotypes), not their size.

5. Conclusions

Based on the results of the present study, the highest increase of temperature in the affected breast was found in the positive HER-2, negative PR and the high Ki-67. The tumor size and other histopathological and clinical factors had no significant influence on the temperature findings.

Conflict of interest

Authors deny any conflict of interest.

Financial relationship

Authors have no relevant financial interests in this manuscript.

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