The impact of human epidermal growth factor receptor-2 status of invasive breast tumors on thermography findings

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ABSTRACT

الأهداف: تحليل مدى تأثير ظهور مستقبل عامل النمو البشري–2 لدى الحالات المصابة بسرطان الثدي الاجتياحي وذلك على نتائج التخطيط الحراري.

الطريقة: أُجريت هذه الدراسة في قسم جراحة الأمراض السرطانية وقسم الباثولوجيا، مستشفى أخوات الرحمة، زغرب، كورواتيا وذلك بالتعاون مع خبراء في مجال التخطيط الحراري. شملت الدراسة 75 مريضة مصابة بسرطان الثدي الاجتياحي واللاتي شُخصن خلال الفترة من مايو إلى يوليو 2011م. ولقد قمنا بمقارنة نتائج التخطيط الحراري بين مختلف النتائج الكيميائية المناعية (ظهور وعدم ظهور مستقبل عامل النمو البشري–2).

النتائج: لقد قمنا بتسجيل درجات حرارة مرتفعة لدى الحالات المصابة بسرطان الثدي الاجتياحي مقارنة مع حالات الصدر الطبيعية. وكانت المناطق المصابة بالسرطان وكامل الصدر المصاب بالسرطان أكثر دفئاً من الناحية الإحصائية ($p<0.001$) مقارنةً مع حالات الصدر الطبيعية أو المناطق المعاكسة للأورام السرطانية. كما وكانت الحالات المصابة بالسرطان والتي ثبت ظهور مستقبل عامل النمو البشري–2 لديها أكثر دفئاً من تلك الحالات التي لم يظهر فيها مستقبل عامل النمو البشري–2 ($p=0.035$).

خاتمة: لقد قمنا بملاحظة زيادة درجات الحرارة لدى الحالات المصابة بالسرطان والتي ثبت ظهور مستقبل عامل النمو البشري–2 لديها. وتشير مثل هذه النتائج مدى تأثير حالة مستقبل عامل النمو البشري–2 على ارتفاع نتائج الحرارة أثناء التخطيط الحراري.

Objective: To analyze the impact of prognostic human epidermal growth factor receptor-2 (HER-2) in invasive breast cancers on the findings of thermography tests.

Methods: This study was carried out at the Department of Surgical Oncology and the Department of Pathology, University Clinical Hospital Center, "Sisters of Mercy", Zagreb, Croatia, in collaboration with licensed infrared (IR) thermography experts. The study involved 75 female patients diagnosed with invasive breast cancer from May to July 2011. Thermography findings were compared between different immune-histochemical (IHC) findings (HER-2 status: positive or negative).

Results: Significantly higher temperatures were recorded in invasive cancer breasts than in healthy breasts. The cancer sites and the entire cancer breasts were significantly warmer ($p<0.001$) than the healthy breasts and opposite tumors mirror sites. Considering the HER-2 status, HER-2 positive invasive cancers were significantly warmer in comparison with the HER-2 negative cancers ($p=0.035$).

Conclusion: The trend of increased temperature in HER-2+ tumors was noted. The findings clearly indicate that HER-2+ status has a higher impact on the increased thermographic temperature findings.

Saudi Med J 2012; Vol. 33 (10): 1118-1121

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Received 26th May 2012. Accepted 9th September 2012.

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The infrared (IR) thermography is a diagnostic method used for early detection, diagnostics, and prognosis of breast tumors.[1-6] Malignant breast cancers are the most important finding that can be detected by a thermographic method.[1-5] The practice had positively confirmed that thermal response is directly proportional to biological activity of cancers.[6] An intensified blood flow and increased vascularity in breast cancers are thermographically observed as hyperthermia, which is directly related to biological activity of tumors. Regardless whether it is an issue of metabolism or immunological reaction, a temperature is always increased. Cancer size is not directly related with a degree of hyperthermia. Hyperthermia varies in different cancer types compared to a normal breast.[1-6] Immunohistochemical reaction of invasive breast tumors to estrogen and progesterone receptor, Ki-67, human epidermal growth factor receptor-2 (HER-2) and other markers reflect cancer's biological aggression, which directly affects the disease prognosis.[6-8] Earlier thermography studies have shown that some immunohistochemistry (IHC) factors could determine a degree of aggression of invasive breast tumors by thermo-biological signs. The aim of this study is to analyze the impact of protein HER-2 status as a prognostic and predictive factor on thermography findings, which has so far not been the subject of research in thermography studies of invasive breast tumors.

Methods. The study was carried out at the Department of Surgical Oncology and the Department of Pathology, Clinical Hospital Centre "Sisters of Mercy", in collaboration with licensed infrared (IR) thermography experts from the Department of Thermodynamics, Faculty of Mechanical Engineering and Naval Architecture, Thermal and Process Engineering, University of Zagreb. The Ethics Committee of the School of Dental Medicine, University of Zagreb approved this study.

One hundred and thirty female patients with an indication for surgical treatment of suspected breast changes were preoperatively examined using thermography during the period from May to July, 2011. Only those with the confirmed diagnosis of the invasive breast cancer remained in the study (75 women). The study was conducted in accordance with the principles of Helsinki Declaration. The thermography was carried out using the thermography system: Therma CAM 2000 (FLIR Systems, Inc. North Billerica, MA, USA) under ambulatory conditions, in an air-conditioned room with constant humidity and temperature between 22^0C and 23^0C. The imaging was carried out with patients in sitting position with their arms resting on the back of the head from a distance of 80 cm. The patients inspired maximally during recordings. A front image was made of thorax with axilla, both in right and left oblique projections.

The HER-2 expression determination (Kit HER-2, DAKO, Denmark, K 5207; ready to use) was carried out routinely using Hercep-Test® according to the manufacturers' protocol on a 0 to 3+ scale using the United States Food and Drug Administration (FDA)-approved grading system. Positive findings for HER-2 receptors were considered 3+ or 2+, and were clearly confirmed by a chromogenic in situ hybridization (CISH) or, when the CISH finding was not clear, by fluorescence in situ hybridization (FISH).

The IR image (thermogram), namely, the measurement results was analyzed using a computer program 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 the temperature of tumors sites, entire tumor breasts, healthy breasts, and mirror tumors sites on healthy breasts, and difference between the average temperature of tumors site and mirror site of healthy breast, and difference between the entire breast with tumor and entire healthy breast. Thermography findings were compared between different HER-2 status (positive or negative).

A statistical analysis of the data was carried out using the SPSS Windows 17 (SPSS Inc., Chicago, IL, USA). The normality of the distribution was tested with the one-way Kolmogorov-Smirnov test. The statistical analysis included descriptive statistics and Student t-test for independent samples, paired t-test and Mann Whitney test. The level of significance was set at 95% probability (p=0.05). We hypothesized that there would be no significant differences between the HER-2 positive and the HER-2 negative tumors considering the thermographically measured temperature.

Results. The study included 75 patients with invasive breast tumors, ages 36-86 years. The mean age was 64 ±11.36 years. A total of 30 patients (40%) were 60 years or younger, and 45 patients (60%) were 60 years or older. Thirty patients (40%) had tumors in the right breast, and 45 (60%) in the left breast. Most of the patients (n=58) had ductal invasive tumors, 5 patients had lobular invasive tumors, and 12 patients had some other histological type of cancer: papillary, mucinous, tubular, medullar, malignant phyllodes or neuroendocrine tumors. With regard to the histological grade, 13 patients had grade I, 38 had grade II, and 22 patients had grade III tumors. Out of the total 75 patients, 50 had no positive axillary metastases (68%), while 25 patients had positive axillary lymph nodes (32%). Distant metastases were found in 3 patients

(4%) only. Among all the patients with invasive breast tumors, 56 had estrogen positive receptors (77%), and 17 (23%) had estrogen negative receptors, 44 patients (60%) had positive progesterone receptors, and 29 had negative progesterone receptors (40%). Fourteen patients (19%) had HER-2 positive receptors, and 59 patients (81%) had HER-2 negative receptors.

A statistically significant difference in the measured temperatures was recorded in all cases between the breast with cancer and the healthy breast, as well as between the cancer site and the mirror site in a healthy breast. Cancer breasts and cancer sites were significantly warmer than the healthy breasts (p<0.001) (**Table 1**). Maximum and average temperatures were higher in the patients with HER-2 positive tumors compared to the patients with HER-2 negative tumors. A significantly higher temperatures were found in patients with HER-2 positive tumors (N=14) compared to the patients with HER-2 negative tumors (N=59) for the following variables: average cancers temperature (p=0.035), maximum temperature of the entire breast with cancer (p=0.012), maximum temperature of entire healthy breast (p=0.029), and for the difference between the average temperature of tumor site and the mirror site on healthy breast (p=0.030). The arithmetic means of average temperatures of tumors and maximum temperatures of entire breasts with tumors, and/or the differences between the average temperature of tumor site and the mirror site on healthy breast were significantly higher for HER-2 positive patients (p<0.05) (**Table 2**).

Table 1 - The significance of differences between arithmetic means of temperatures measured in healthy breasts and in breasts with tumors (paired t-test).

Variable	x^2	SD	dif	SD	t test	df	*P*-value
Maximum temperature of tumor site	35.75	1.05	0.83	0.79	9.17	74	<0.001
Maximum temperature in healthy breast tumors mirror site	34.92	1.36					
Average temperature of tumor site	34.96	1.16	0.76	0.75	8.94	74	<0.001
Average temperature in healthy breast tumors mirror site	34.2	1.3					
Maximum temperature of entire breast with tumor	36.09	0.94	0.24	0.56	3.7	74	<0.001
Maximum temperature of healthy breast	35.85	1.08					
Average temperature of breast with tumor	34.39	1.51	0.45	0.96	4.04	74	<0.001
Average temperature of healthy breast	33.94	1.37					

SD - standard deviation, df - degrees of freedom, dif - mean of the differences

Table 2 - The significance of difference in temperature findings between patients with human epidermal growth factor receptor-2 (HER-2) positive and HER-2 negative tumors (Student t-test for independent samples and Mann Whitney test).

Variables	HER-2	N	x^2	SD	t test	*P*-value
Maximum temperature of tumor site	Negative	59	35.60	0.97	-1.95	0.055†
	Positive	14	36.17	1.10		
Average temperature of tumor site	Negative	59	34.78	1.15	-2.15	0.035*
	Positive	14	35.49	0.97		
Maximum temperature of entire breast with tumor	Negative	59	35.92	0.91	-2.60	0.012*
	Positive	14	36.59	0.68		
Average temperature of entire breast with tumor	Negative	59	34.22	1.60	-1.60	0.11†
	Positive	14	34.94	0.89		
Maximum temperature of mirror cancers site in healthy breast	Negative	59	34.80	1.36	-0.91	0.365†
	Positive	14	35.16	1.28		
Average temperature of mirror cancers site in healthy breast	Negative	59	34.08	1.31	-1.08	0.284†
	Positive	14	34.49	1.15		
Maximum temperature of entire healthy breast	Negative	59	35.68	1.09	-2.23	0.029*
	Positive	14	36.36	0.74		
Average temperature of entire healthy breast	Negative	59	33.76	1.42	-1.195	0.06†
	Positive	14	34.52	0.92		
Difference between the average temperature of tumor site and mirror site of healthy breast‡	Negative	59	0.71	0.79		0.030*
	Positive	14	1.01	0.44		
Difference between average temperature of entire breast with tumor and entire healthy breast‡	Negative	59	0.46	1.08		0.206†

*Significant; †non-significant; ‡the significance of difference in temperature findings between patients with HER-2 positive and HER-2 negative tumors (Student t-test for independent samples and Mann Whitney test). SD - standard deviation

Discussion. All previous thermographic studies indicated that larger tumors with metastases in regional lymph nodes (compared to smaller tumors), fast proliferating tumors (compared to slowly proliferating), and less differentiated tumors (compared to well differentiated) have evidently more pathological thermo-biological indicators, which are a characteristic of more aggressive invasive tumors. It has also been observed that more aggressive invasive tumors are classified into the group of "so called warmer tumors" according to their thermography findings, and they directly affect the shorter disease-free period, and total survival of patients compared to the so called "colder tumors".[6] The clinical value of IR thermography as a prognostic factor in patients with invasive breast tumors has been evaluated based on individual impact of clinical, histopathological, and IHC parameters dependent on their number of thermo-biological pathological signs.[1-8]

The fact that the aggressive growth of invasive breast tumors causes an increase of temperature reflected on the body surface (skin), which can be measured by IR thermography was confirmed also by the results of this study, as we found the significantly higher temperatures in the breasts with tumors and the tumors sites in comparison with the healthy breast. The maximum and the average temperatures of both tumors and the entire affected breast were significantly higher than the maximum and the average temperatures of the mirror site of the healthy breast, and the entire healthy breast in all patients who participated in this study.

Several studies have reported an association with HER-2 overexpression and other adverse prognostic factors, such as positive lymph nodes, larger tumor size, high histological grade, high proliferation rate, and lack of expression of estrogen and progesterone receptors.[9] The importance of the HER-2 is higher as a predictive than a prognostic factor.[10] TheHER-2 amplification or protein overexpression is seen in approximately 20-30% of invasive breast cancers in humans, commonly in high-grade invasive ductal carcinomas. The American Society of Clinical Oncology (ASCO) Tumor Marker Guidelines Panel newly has recommended routine testing of HER-2 in diagnosed and metastatic breast cancer since 2001.[11] Furthermore, no thermography researches have been published evaluating the protein HER-2 status in patients with invasive breast tumors. The results of this study point out the significant impact of a temperature increase in positive HER-2 tumors. Several temperature findings were increased in this study in HER-2 positive patients: average temperature of tumors site, maximum, and average temperature of entire breast with tumors, a difference between the average tumors site temperature and the mirror site of the healthy breast The increased temperature trend was evident in the rest of the measured temperature in the HER-2 positive tumors. The findings clearly indicate that HER-2+ status has a higher impact on the increased thermographic temperature findings. Potential limitation of the study includes a small number of the HER-2+ patients (n=14). Further, studies of a larger sample of the HER-2 positive patients are warranted. Acknowledging the limitation of this study, our findings indicate that positive HER-2 status has a high impact on the increased temperature measurement findings.

In conclusion, the results of this study indicate a good prognostic value of the thermography in a clinical use as thermographically warmer tumors were HER-2 positive, which are also classified as aggressive invasive tumors with adverse prognosis according to other IHC tests.

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