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# Specific thermographic changes during Walker 256 carcinoma development: Differential infrared imaging of tumour, inflammation and haematoma

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**Abstract**

*Background*: Infrared imaging measures spatial variations in the skin temperature aiming to determine pathological processes; hence possible use of this non-invasive analytical method in cancer detection is emerging. *Methods*: Infrared thermal imaging was used to detect changes in rat skin surface temperature associated with experimental cancer development (Walker 256 carcinoma), inflammation (upon s.c. Sephadex injection) and haematoma (provoked by s.c. blood coagulate injection). Infrared camera with a geometric resolution of 76,800 pixels, spectral range of 8–14 μm and the minimal detectable temperature resolution of 0.07 °C with spatial resolution of 0.48 mm at measuring distance of 30 cm was used to obtain computerised thermal scans. Genuine *ThermoWEB* software developed for remote internet control as open source software was used. *Results*: The raise of peripheral temperature was observed after induction of local inflammation or haematoma. Opposite to that, transient decrease of the skin surface temperature was observed after tumour transplantation. Progressive growth of tumour was associated with the raise of the skin surface temperature from the 10th day after tumour inoculation, when the tumours developed supportive neoangiogenic blood supply, as verified by histology. *Conclusion*: While the raise of peripheral temperature in advanced tumour was caused by neoangiogenesis, the reduction in skin surface temperature in an early period after tumour cell inoculation indicated a decay of transplanted tumour cells due to the immune response and the lack of blood supply. Thus, infrared thermal imaging may have considerable value in evaluation of the tumour development and discrimination of cancer from inflammation and haematoma.


*Keywords:* Infrared thermal imaging; Walker 256 carcinoma; Haematoma; Inflammation

## 1. Introduction

Infrared imaging is a non-invasive method that measures spatial variations in the skin temperature aiming to determine pathological processes. The diagnosis of neurological, musculoskeletal or other tissue abnormalities by thermal imaging is based on comparative temperature asymmetry determined between healthy and diseased regions of the body. A change in normal temperature outside the known standard range of temperature is considered to be a sign of abnormal function, i.e. the development of various disorders [1].

The fundamental basis of thermography in case of cancer is detection of heat generated by the metabolic activity of the proliferating tumour cells and of heat generated by new blood vessels supporting the growth of tumour [1,2]. Thus, the process of carcinogenesis associated with neoangiogenesis results in an increase of the skin temperature above developing tumour.

*Abbreviations:* PBS, phosphate buffered saline.

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A typical infrared image of a breast tumour reveals a 1–3 °C elevation in skin surface temperature at the periphery of the tumour, while the tumour mass itself sometimes shows reduced temperature. The cause of these temperature changes is not entirely understood, although it is assumed that the elevation in temperature at the tumour periphery is due to hypervascularity around the tumour as a result of tumour-induced neoangiogenesis [2].

The infrared imaging has been rarely used to monitor tumour growth in experimental animals, therefore we lack basic knowledge based on comparative studies evaluating thermal imaging of experimental malignancies and other pathologies causing local tissue swelling (Latin – *tumour*) and the heat (Latin – *calor*) associated with the tissue damage.

The most common feature of such tissue damage is inappropriate function of the blood vessels, often manifested by the damage of their integrity which cause haematoma, a local accumulation of blood coagulate in tissue [3]. The swelling often starts instantly, while the change of the tissue colour may be pronounced within a few hours after injury. In response to tissue damage the repair process is initiated immediately by the release of cytokines and various chemo-attractants by the affected tissue and blood cells, in particular platelets in the blood coagulate. These factors attract and stimulate leukocytes to the site of injury. Neutrophils arrive within a few minutes after injury and contribute to the defence against micro-organism (if present) by phagocytosis and production of proteinases and reactive oxygen species. Monocytes/macrophages reach maximal numbers when the neutrophil number is declining again. Together these cells then orchestrate the inflammatory tissue response that is essential for the induction of angiogenesis and the healing/closure of the wound.

Therefore, inflammation is a physiological process which could turn into pathology. Acute inflammation is a rapid self-limiting process while chronic inflammatory disorders are mostly considered to be autoimmune or age-associated pathologies. The pathology of interactions between inflammation and cancer is not understood well although the association of inflammation and cancer was discovered already by Virchow in 1863, who described the presence of a leukocyte infiltrate in tumour tissues and concluded that there should be some pathological connections between inflammation and cancer [4].

Hence, although inflammation, cancer and haematoma are very different processes they could overlap and interfere with each other as well as they could resemble each other exerting some similar symptoms known as *rubor*, *tumour*, *calor*, *dolor* and *functio laesa* (red colour change, swelling, heat, pain and tissue dysfunction). Therefore, the aim of our study was to use infrared thermography to monitor changes in skin surface temperature associated with haematoma, inflammation and cancer development in the right hind limb of rats to see if such sensitive non-invasive analytical method could discriminate these processes.

## 2. Materials and methods

### 2.1. Animals and experimental procedure

All experiments were performed on male, 5 months old Sprague–Dawley rats with average body weight of 450 g. Animals were intramuscularly injected in the shaved right hind limb with: $2 \times 10^7$ Walker 256 carcinoma cells dispersed in 200 μL of PBS (group I), 200 μL of 2% solution of Sephadex in 0.9% sterile NaCl solution (Sephadex G-200, Pharmacia Fine Chemicals, Sweden) (group II) or 200 μL of syngeneic blood coagulate (group III). There were four animals per experimental group while additional four animals were treated with PBS only (control group). The skin surface temperature was monitored daily from day 0 (5 h after treatment) to 4th day after treatment and again from day 7 to day 11 after tumour inoculation.

The animals were kept in individual cages under standardised room temperature and humidity with 12 h light/dark daily rhythm with water and food given *ad libitum* while the room temperature in which the measuring took place was 21 ± 1 °C.

Tumour growth was evaluated by the use of calliper measuring three times a week the three diameters of the tumours formed at the site of tumour cells inoculation.

To study the tumour histology changes, the additional nine rats were ether anaesthetised and sacrificed on days, 1, 7 and 10 after tumour inoculation. Tumour mass was surgically removed, fixed in 10% buffered formalin and embedded in paraffin. Sections made from paraffin blocks were stained with haematoxylin and eosin and examined by a pathologist well experienced in the Walker 256 tumour histology.

The experiments were performed in accordance with the ILAR Guide for the Care and Use of Laboratory Animals, Council Directive (86/609/EEC) and Croatian animal welfare law (NN 19/99).

### 2.2. Infrared imaging

Infrared camera Thermo Tracer TH7102WL (NEC San-ei Instruments, Ltd., Japan) was used during all measurements. This infrared measurement system contains an uncooled focal plane array detector (micro bolometer) with geometric resolution of 76,800 pixels per picture (320 × 240). Spectral range is from 8 μm to 14 μm and the temperature range lies between −40 °C and 120 °C (optional 500 °C). The minimum detectable temperature resolution (difference) is 0.07 °C at 30 °C (Normal mode) and spatial resolution is 0.48 mm at measuring distance of 30 cm (IFOV 1.58 mrad).

For experimental purposes we developed the *Thermo-WEB* software for remote control and transferring data from infrared camera TH7102WL to a computer, which is designed to be used further as an open source thermoscan analyses software [5]. This software supports thermal