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Evaluation of allergic response using dynamic thermography

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Skin dynamic termography supplemented by a mathematical model is presented as an objective and sensitive indicator of the skin prick test result. Termographic measurements were performed simultaneously with routine skin prick tests. The IR images were acquired every 70 s up to 910 s after skin prick. In the model histamine is treated as the principal mediator of the allergic reaction. Histamine produces vasolidation and the engorged vessels are responsible for an increase in skin temperature. The model parameters were determined by fitting the analytical solutions to the spatio-temporal distributions of the differences between measured and baseline temperatures. The model reproduces experimental data very well (coefficient of determination = 0.805÷0.995). The method offers a set of parameters to describe separately skin allergic reaction and skin reactivity. The release of histamine after allergen injection is the best indicator of allergic response. The diagnostic parameter better correlates with the standard evaluation of a skin prick test (correlation coefficient = 0.98) than the result of the thermographic planimetric method based on temperature and heated area determination (0.81). The high sensitivity of the method allows for determination of the allergic response in patients with the reduced skin reactivity.

Keywords: dynamic thermography, skin prick test, mathematical model.

1. Introduction

The skin prick test with a routine panel of allergens is a basic procedure in diagnosis of allergic diseases [1]. The usual method to quantify allergic skin reactions is to mark wheal and erythema regions and assess surfaces affected by reactions. Frequently, the results of skin tests are related to the size of histamine control and are reported in a 1 to 5 scale.

It is also possible to evaluate allergic skin reaction using thermographic (TH) camera [2–3]. The measurement is, however, a direct extension of the routine planimetry. Simply, the area of the elevated temperature region and/or the average rise in temperature are applied as diagnostic parameters. Sometimes the intensity of reaction is expressed in so called thermo-graphic units (area times average rise in temperature). Additionally, in cases of altered skin reactivity (elderly patients, sun-damaged skin with both hyper- and hypo-melanotic lesions, atrophy of subcutaneous tissue, hypertrophy of the epidermis), the quantification of the skin prick tests by TH planimetry delivers ambiguous results or is often impossible [4–6].

It should be noted that the application of infrared (IR) imaging starts in breast cancer diagnosis about 50 years ago [7]. Nowadays TH imaging is a well-established method for health monitoring and examinations, as well as for assisting diagnosis [8–22]. Recently, it was demonstrated that the TH measurements supplemented by the mathematical model offer a new approach to the quantification of allergen-induced skin reactions [23].

In the present studies the usefulness of TH measurements supplemented by the mathematical model for the skin prick test assessment, regardless of the skin reactivity, will be highlighted.

2. Materials and methods

Thermographic investigations were performed in a group of 24 patients aged from 18 to 65 years. The study has been approved by the ethics committee of the Jagiellonian University Medical College. All subjects gave informed consent before participation in the studies. Commonly accepted procedure was applied before examinations. The TH measurements were performed in a room controlled at 22°C. Patients were allowed to adapt to room temperature (testing area of forearms was disclosed) for 30 minutes. Next, the arms were placed on the special constructed table and fixed. TH camera (VIGO, Warsaw, Poland) was placed ~30 cm above the forearms. TH camera was calibrated to register temperatures in the range of (15÷49)°C. To perform the spatial calibration of thermal images six light emitting diodes were mounted in the table. It should be noted that the pixel size depends on the distance between the TH camera and the skin surface. In the study the pixel size ranged from 0.56 to 0.71 mm.

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The studies were performed using the commercial allergen panel (Allergopharma, Reinbek, Germany) and histamine (control). 8 inhalant allergens were tested (Dermatophagoides Pteronyssinus, Dermatophagoides Farinae, mixed grasses, cat hair, mixed trees, mixed weeds, mildews and feathers). A drop of the allergen was placed onto a marked area of the skin. Using a sterile lancet a small prick through the drop was vertically made. Additionally, two controls were applied in the tests. The positive control was the solution (9 mg NaCl, 4 mg phenol, 563 mg glycerin and injection water up to 3 ml volume) of 1.7 mg hydrochloride of histamine. A drop of the solution without histamine was used as the negative control to estimate the patient response to a prick.

The distributions of temperature of both forearms were acquired every 70 s from 0 up to 910 s after skin pricks. The imaging frequency (1 image every 70 s) was selected to collect minimal data points necessary for proper determination of the parameter values. It should be noted that an increase of the capture frequency, even one order of magnitude, does not change markedly the coefficient of determination. Thermograms were evaluated using the software developed in our laboratory. The first step of calculations relies on the determination of the $\Delta T_{H/A}$ distributions. $\Delta T_{H/A}$ is described by

$$\Delta T_{H/A} = T_{H/A} - T_S, \tag{1}$$

where $T_{H/A}$ denoted the skin temperature after histamine/allergen injection and T_S is the skin temperature before examination. In some cases a correction for the forearm movement had to be made. For the correction of the forearm movement it was assumed that the positions of the maximum temperature points are fixed in space. Therefore, the correction is not influenced by the capture frequency. The error of ΔT was determined experimentally using a homogenously heated surface and equalled 0.3°C. Next, the heated region of the skin, correlated with particular allergen or histamine, was approximated by a circle and the radius of the circle (r) was determined. Error of the radius was estimated as the TH image pixel size.

To quantify the allergic response, the temperature difference distributions after histamine and allergen injection as a function of radius and time changes [$\Delta T_H(r,t)$ and $\Delta T_A(r,t)$, respectively] were described by the mathematical model. The model is presented, in details, elsewhere [23]. Briefly, the introduction of a histamine/allergen at site on the skin induces a complex sequence of events known as the local inflammatory response [24]. If a person is allergic, local mast cells de-granulate and release histamine (Fig. 1). In the model it was assumed that the reaction takes place in the thin skin layer and that histamine is the principal mediator of the allergic reaction.

Suppose histamine is introduced at the origin [$c_H(0,0)$] and it is transported across the skin. The histamine transport is treated as a complex process (hereby called migration) which is probably mostly influenced by the blood perfusion within skin. As histamine migrates we assume it is additionally eliminated via first-order kinetics. The equation governing the histamine concentration $c_H(r.t)$ is as follows

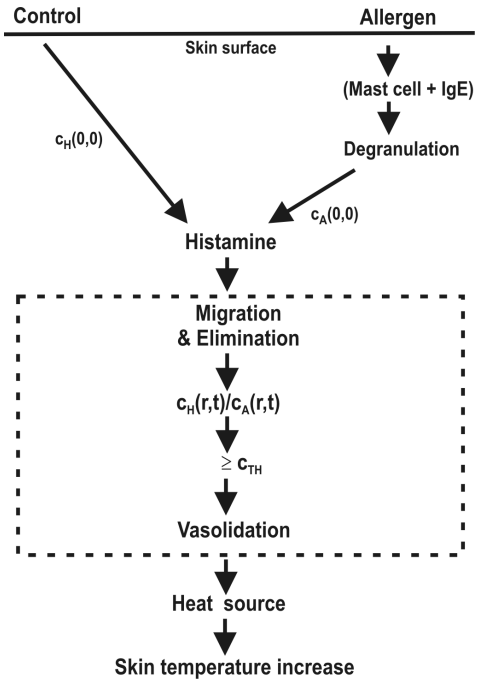

Fig. 1. Diagrammatic overview of events underlying a skin temperature increase. For details see text.

$$\frac{\partial c_H}{\partial t} = -v\frac{\partial c_H}{\partial r} - \gamma c_H, \tag{2}$$

where v is the histamine migration rate and γ is the elimination rate constant. As histamine is moving across the skin, the vessel system reacts in response to the local histamine level. The engorged vessels are, in turn, responsible for skin redness and an increase in skin temperature. It should be pointed out that the mechanism(s) underlying histamine-induced vascular reaction(s) is unclear, and its relevance to the allergic reaction should be elucidated [24]. Several physiologically plausible mechanisms might be postulated. We assume that due to histamine-induced vasolidation a supplementary heat source (Q_H) is activated. The governing equation for the $Q_H - c_H$ relationship is

$$Q_H(r) = Q_0 c_H\left(r, \frac{r}{v}\right) = Q_0 c_{OH}\ \exp\left(-\gamma\frac{r}{v}\right), \tag{3}$$

where Q_0 is the positive constant. Intuitively, we can see how the mechanism of Eq. (3) generates a skin temperature distribution. The histamine "wave" propagates with the velocity of v and exhibits a biochemical switch behaviour. At the point r a supplementary heat source appears after the time $t = r/v$. The increase of heating is constant in time and is linearly related to the maximal histamine concentration at the point r. Of course, there is a threshold histamine concentration (c_{TH}) to produce the heating effect. If over the region of the skin $c_H \geq c_{TH}$, then a domain of permanently nonzero values of heating is generated.

The temperature distribution of the skin is given by the solution of Pennes bio-heat equation [25]. In the model we made additional approximations that the thermal diffusivity in the skin may be neglected and the metabolic heat generation rate is equal to 0. The further justification for this approximation comes from the calculation of the Biot's number [26] and from the numerical simulations of heat transport in the skin [23]. Finally, it is possible to rewrite the bio-heat equation as

$$\frac{d(\Delta T_H)}{dt} + \tau \Delta T_H = S_H(r), \tag{4}$$

with initial condition $\Delta T_H(r,0) = 0$ and parameters

$$\tau = \frac{1}{\rho_s c_{shs}} (\omega \rho_b c_{shb} + \alpha), \tag{5}$$

$$\begin{aligned} S_H(r) &= \frac{1}{\rho_s c_{shs}} [\omega \rho_b c_{shb}(T_b - T_a) - \alpha(T_{s0} - T_a) + Q_H(r)] \\ &= S_0 + \frac{Q_H(r)}{\rho_s c_{shs}} = S_0 + \frac{Q_0 c_{0H}}{\rho_s c_{shs}} \exp\left(-\gamma \frac{r}{v}\right) \\ &= S_0 + Q_{0H} \exp\left(-\gamma \frac{r}{v}\right), \end{aligned} \tag{6}$$

where ρ_s is the density of the skin, c_{shs} is the specific heat of the skin, ω is the blood perfusion, ρ_b is the density of blood, c_{shb} is the specific heat of blood, α is the heat transfer coefficient, T_b is the blood temperature (assumed to be constant), T_a is the ambient temperature (assumed to be constant) and Q_H is the heat generation rate due to histamine action of Eq. (3). The solution of Eq. (4) is

$$\Delta T_H(r,t) = \frac{S_H(r)}{\tau}(1 - e^{-\tau t}). \tag{7}$$

In the modelling of the allergen action immunoglobulin E (IgE) mediated hypersensitivity is assumed [24]. The exposure to the allergen (Fig. 1) leads to cross-linking of the IgE molecules on skin mast cells and the cell releases histamine directly generating allergic symptoms. The problem arises how to treat the allergen transport in the skin. The description is based on the observation that most of the allergens are proteins or protein-bound substance having a molecular weight between 15000 and 40000 [24]. This allows us to consider an assumption that the allergen migration can be ignored, while histamine migration is viewed as fast. In this approximation allergen molecules are injected to the skin but they do not move comparing to histamine motion.

The mechanism of an allergen conversion, via a mast cell, into histamine consists of two steps. The first step relies on activation of mast cells. The activation means that an allergen cross-links two receptors with bound IgE on the surface of a mast cell. Assuming that activation of the mast cells is a very fast process, it follows that the total number of the activated mast cells $M_0 \approx$ constant. The second step of the histamine production relies on the de-granulation of the activated mast cells. The rate of de-granulation of a mast cell is also treated in the model as a fast process. Finally, the concentration of histamine (c_{0A}) at the origin after allergen injection is described by

$$c_{0A} = M_0 H_{0A}, \tag{8}$$

where H_{0A} is the amount of histamine accumulated in one mast cell.

Under the above described assumptions, the temperature distribution after allergen injection is described by Eq. (4) with the new value (S_A) of the S_H constant of Eq. (6)

$$S_A(r) = S_0 + \frac{Q_0 c_{0A}}{\rho_s c_{shs}} \exp\left(-\gamma \frac{r}{v}\right) = S_0 + Q_{0A} \exp\left(-\gamma \frac{r}{v}\right). \tag{9}$$

The solution of Eq. (4) with $S_H = S_A$ and $\Delta T_H = \Delta T_A$ is

$$\Delta T_A(r,t) = \frac{S_A(r)}{\tau}(1 - e^{-\tau t}), \tag{10}$$

with the value of τ given by Eq. (5). The histamine production after allergen injection, i.e., the allergic response, may be quantified by the c_{0A} value or the $Q_{0A}/Q_{0H} = c_{0A}/c_{0H}$ ratio.

The $\Delta T_H(r,t)$ distribution was used to determine the model parameters describing the increase of the skin temperature after histamine injection. Parameters τ, v, γ, S_0 and Q_{0H} were obtained by fitting Eq. (7) to the experimental data. The parameters characterizing the temperature distribution after allergen injection were determined by fitting Eq. (10) to the $\Delta T_A(r,t)$ data. It should be pointed out that for each patient the values of τ, v, γ, S_0 and Q_{0H} were extracted from analysis of the histamine data. Therefore, the evaluation of the allergen data relied on the determination of the Q_{0A} value of Eq. (9).

For comparative purposes the routine method was used to assess allergic reaction. The method relied on the evaluation of weal and erythema areas 10 min after the allergen injection. Next, the areas were related to the dimension of the histamine control and were expressed in a 1 to 5 scale. Moreover, the skin reaction was evaluated using TH planimetric method [2–3]. The area of the elevated temperature region times average rise in temperature was applied as the diagnostic parameter.

3. Results

An example of rough histamine results is presented in Fig. 2. It should be noted that all patients showed positive skin reactions to histamine. The maximum increase of temperature was patient-dependent and ranged from 1.5°C to 4.0°C despite of the identical dose of histamine in all cases. The quantitative assessment of the allergic response was obtained by fitting the model equations to the $\Delta T_{H/A}(r,t)$ distributions. In the study 192 allergic reactions were examined (24 patients times 8 allergens). In most cases (132) the allergic responses were not observed. A wheal-and-erythema did not develop, as well as an increase of the skin temperature was not detected. The same pertained to the negative con-

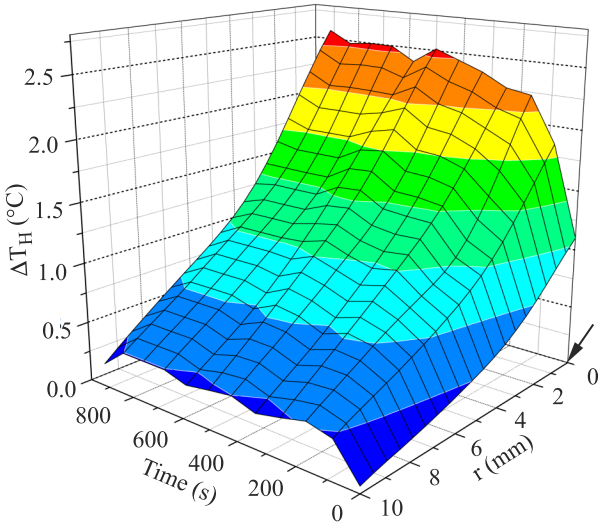

Fig. 2. The increase of skin temperature after histamine injection (ΔT_H) versus time and radius (r) of the heated region. Histamine was injected at point marked by the arrow.

trol, i.e., the patient did not respond to the prick. In the case of a positive reaction (60 cases), the allergen data have been fitted.

The proposed approach of the skin prick test quantification was validated by the comparison to a routine diagnostic method. It should be noted that the model reproduces experimental data very well. The coefficient of determination ranged from 0.805 to 0.995. The comparison of routine (1 to 5 scale), proposed and TH planimetric methods for the skin prick test evaluation is presented in Fig. 3. The correlation coefficient values confirm the advantage of the proposed method (0.98) in comparison to the TH planimetric technique (0.81). Assuming, in agreement with commonly

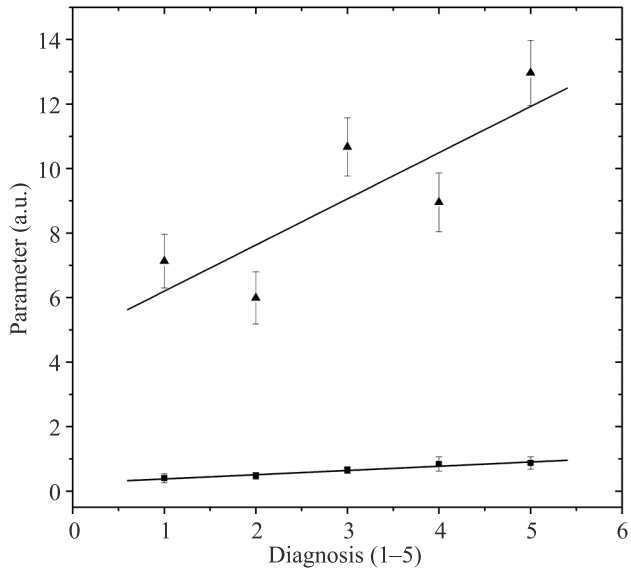

Fig. 3. Comparison of routine (1 to 5 scale), proposed (■) and planimetrie thermographic (▲) methods of the skin prick test assessment (mean ±SD).

accepted opinion, that the routine evaluation results of the skin prick constitutes a gold standard it was possible to calculate sensitivity and specificity of the proposed method. The sensitivity value equals 0.86 with 95% confidence interval (0.83,0.89) while specificity is 0.94 with 95% confidence interval (0.92,0.96). The accuracy (the proportion of cases, considering both positive and negative test results, for which the test results are correct) is 0.96.

Additional advantage of the proposed method relies on the possibility to distinguish subtle alterations of the allergic response. In Fig. 4 the time changes of the temperature after injection of histamine and 2 allergens are given. Assuming that the response to histamine is equal to 1.0, the responses of allergens are 0.67 (mixed weeds) and 0.54 (D. Pteronyssinus), i.e., the differences in the responses to both allergens were observed. It was impossible to observe differences between allergen responses using the TH planimetric or routine diagnostic methods for the evaluation of the skin prick test.

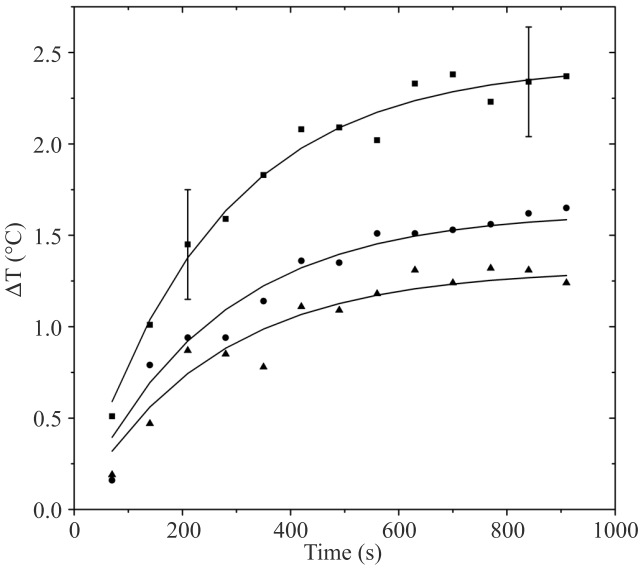

Fig. 4. The increase of skin temperature (ΔT) after injection of histamine (■) and 2 allergens (mixed weeds ● – and D. Pteronyssinus – ▲) versus time. The solid lines present the fits of the model curves. The distance from the histamine/allergen injection point equals to 6 mm in all cases. Error bars mark the experimental errors.

4. Discussion

The collected results confirm that TH imaging is a non-invasive technique that allows for visualization and quantification of changes in a skin surface temperature caused by allergic reactions. The rough TH data are interpreted using a mathematical model based on the pathophysiology of heat generation. It should be noted that the model description enables separation of 2 processes responsible for the temperature increase. The mediator production as a result of allergic reaction (parameter c_{0A}/c_{0H} describes directly the amount of histamine released from mast cell) and the media-

tor transport in the skin are described independently. The later factor is mostly correlated with the skin reactivity. Although, the proposed model is very simple, it reproduces very well the experiment (Fig. 4). It was also confirmed that the proposed method is superior to the TH planimetric method (Fig. 3).

Probably, the main drawback of the TH planimetric method is the use of the heated region (HR) radius for the quantification of the allergic reaction. The model calculations reveal that the histamine transport in the skin is responsible for the dimension of the HR. The HR radius is not influenced by the histamine concentration. Therefore, planimetric data have a limited value in the quantification of allergic response.

Next problem of the TH planimetric method is connected with the maximal radius of the HR. In Fig. 5 time changes of the HR radius after histamine injection are presented for 2 patients. In most cases the HR radius reaches saturation after ~400 s. There are cases, however, for which the HR radius increases during measurement and the saturation value is not reached. The question arises how to determine the area of the HR in such case. It should be noted that application of dynamic thermography supplemented by the mathematical model eliminates this problem.

It should be pointed out that two main simplifications concerning the heat generation after allergen/histamine injection have been used in the model. The first one deals with heat transport in the skin, the second simplification is the treatment of histamine release by the mast cells after the allergen injection. The heat transfer in the skin has been traditionally addressed using Pennes bio-heat equation [25] which accounts for the ability of tissue to transfer heat by both passive conduction and blood perfusion. In the model the core of the body constitutes a homogenous heat source with constant temperature. The histamine injection changes thermal insulation property of the skin. The process is approximated as formation of a supplementary heat source. Hence, the simplification that skin temperature distribution is governed by the spatially distributed heat generation rate [Eq. (3)] and the surface heat loss while the heat conduction in skin may be neglected seems to be reasonable.

The assumption concerning rapid release of histamine after allergen provocation agrees well with the previously reported data. The investigations of changes in local blood flow after prick tests with histamine and allergen injections using laser Doppler flowmetry [27] revealed that the initial rapid increase in blood flow has been observed after both provocations. The same conclusion may be drawn from the measurements of the exudation and vasodilation responses after skin prick test using a radioactive tracer [28]. The largest responses to histamine and allergens occurred immediately after provocation. Probably, there are two time scales in the model. The histamine release depends instantaneously on the allergen concentration, while the temperature distribution in controlled by the histamine concentration on a slower time scale.

In addition to the excellent quantification of the allergic reaction for diagnostic purposes, the continuous recording of the skin temperature might yield additional information on the skin reactivity. To determine whether the histamine migration velocity (v) and age contribute to the skin sensitivity v was plotted as a function of age (Fig. 6). We observed a trend towards reduced skin reactivity in a higher age group in agreement with previously reported data [29–30]. It should be also noted that two groups of

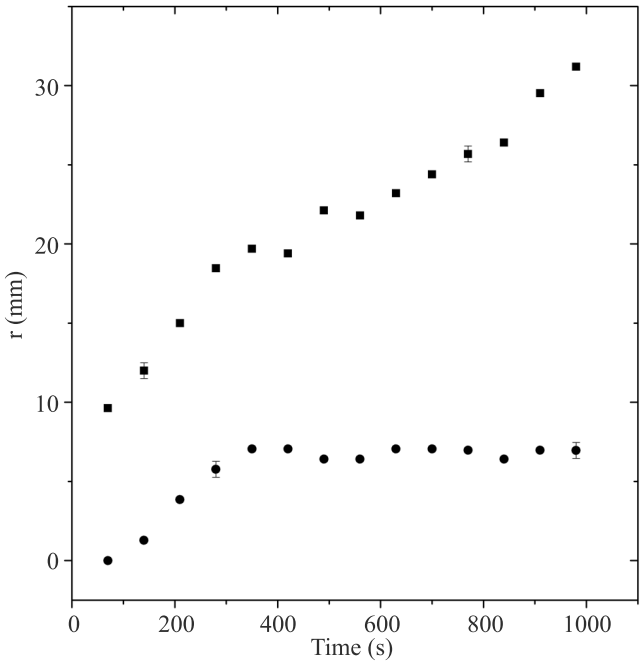

Fig. 5. The radius of heated region (r) versus time after histamine injection for 2 patients. For first case (●) the saturation is observed for the second case (■) does not.

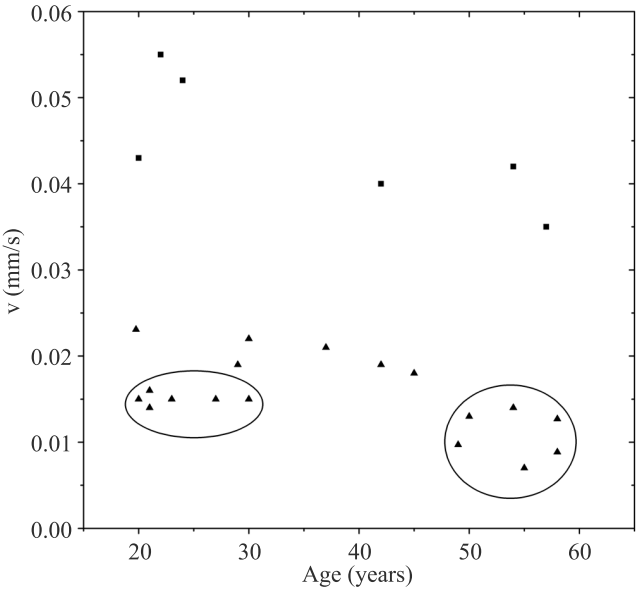

Fig. 6. Histamine migration velocity (v) in the skin versus age of the patients, 2 groups of v values (■ – high and ▲ – low) may be distinguished. Circles mark the values < 0.02 mm/s for young (age ≤ 35 y) and old (age > 50 y) patients.

different v values might be distinguished. Moreover, the low v value (< 0.02 mm/s) is observed both for "young" and "old" patients. The explanation of the observed relation awaits further study.

Methods based on registration of the emitted infrared radiation through the body are currently being implemented in many branches of medicine. The possible range of applications of thermography in medicine is not clearly defined and the proposed method will also make a contribution in this field.

5. Conclusions

It was demonstrated that the thermographic measurements supplemented by the mathematical model offer a new approach to quantification of allergen-induced skin reactions. The rough TH data are interpreted using a mathematical model based on the pathophysiology of heat generation. Although, the proposed model is very simple, it reproduces very well the experiment. Moreover, the continuous recording of the skin temperature represents an additional possibility to investigate skin reactivity. The main drawback of the TH quantification based on temperature and heated region area determination relies on the uncertainty of the heated region area measurement. The collected results confirm also that pathophysiological-based interpretation of TH images creates a new possibility for medical applications of thermography.

Acknowledgements

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