ABSTRACT:
In the present study we aimed to optimize the thermal imaging method for evaluation of skin patch test reactions in dental clinical allergology. A total of 30 patients without a history of allergic diseases were included in the study - 12 men and 18 women, age 21 - 32 years. Skin patch testing was performed with a set of 20 standardized allergens. The thermographic investigations were performed with the FlirT620 infrared camera with a temperature resolution of 0.06°C. Thermograms were analysed using the Flir Reporter Professional software 2013. The statistical analysis of the results revealed an average correlation between the clinical evaluation of the results according to the cheme of ICDRG and the thermal image diagnostic (rphi = 0.538, p = 0.001). Absolute matching of clinical and thermal image results was observed only for the negative ones. For the clinically positive skin patch results matching with thermal image method was observed in 60.7% of the cases. Optimization of thermal imaging as a method for evaluation of skin patch test results could serve as a safe, accurate and non-invasive method, especially to distinguish weak (+), doubtful and irritant reactions, although not standardized criteria to distinguish these reactions have been elaborated yet. Crucial factor for the accurate interpretation of results is the precise diagnosing performed by well-trained physicians in this area, with clinical relevance, to minimize the role of subjective factors.

Key words: thermal imaging, dental allergology, skin patch testing,

INTRODUCTION
Dental clinical allergology is a comparatively new academic subject in Bulgaria. It may be determined as boundary specialty, closely related with several medical disciplines. The methods of diagnosis and treatment of dental clinical allergology are a combination of the routine immunological and allergological tests and treatment regimens with a completely consistent with the allergological status of the patient dental care. In diagnostic term, using advanced and non-invasive techniques is a priority for most scientific teams working in this area of dental medicine.

Infrared thermal imaging – thermography, is an innovative diagnostic method, evaluated as an objective and sensitive indicator of the skin prick test result. It is widely used in various areas of science and practice - military science, construction, printing, geology, biology and medicine [1-7]. The survey of scientific literature revealed that one of its key applications is for diagnostics in dermatology and allergology [8-10].

Allergic reactions to dental materials and alloys are manifested as delayed type hypersensitivity (classified as Gell and Coombs type IV reaction) - the antigen binds to specifically sensitized T lymphocytes with subsequent synthesis of series of cytokines. The main clinical presentation of reactions to dental materials is allergic contact dermatitis. Contact dermatitis could be classified as irritant and allergic. The irritant is manifested as an acute inflammatory response after exposure to primary irritants. The pathogenesis is most often toxic in nature.

Jadassohn was the very first who performed patch tests for diagnosis of allergic contact dermatitis in 1894, while Bloch prepared the first kit of allergens. Since that time various allergens and allergen concentrations had been tested. In patch skin tests a certain amount of the allergen is applied using fragments of blotting paper, filter, aluminum foil or gel. Reactions such as weals and erythema are evaluated, but the subjective interpretation of the results may result in errors [11, 12]. Thermal imaging is considered as an objective and sensitive method for evaluation of the skin patch test results.

The purpose of the present study is to optimize the thermal imaging method for evaluation of skin patch test reactions in dental clinical allergology.

MATERIAL AND METHODS
A total of 30 patients without a history of allergic diseases were included in the study -12 men and 18 women, at age 21 - 32 years. The present investigation was carried out within the framework of a scientific project on investigation of the conditions for occurrence of occupational allergy in exposure to methacrylates among dental students, dental technicians, assistants and medical personnel, involved in the process of education and was granted by the Medical University of Sofia - Grant No 5-C/2013.

The study was approved by Medical Ethics Board at Medical University of Sofia. All participants were informed about the purpose of the study and gave their written informed consent.

Skin patch testing
Skin patch testing was performed with a set of 20 standardized allergens (Chemotechnique Diagnostics) presented in Table. 1, by placing them in IQ-Ultra
hypoallergenic patches (IQ Chambers®, Vellinge, Sweden) - two patches with 10 allergen. To each patch an empty chamber serving as a reference and a negative control was added.

<table>
<thead>
<tr>
<th>1. Potassium dichromate</th>
<th>1. Methyl methacrylate</th>
</tr>
</thead>
<tbody>
<tr>
<td>2. Cobalt(II)chloride hexahydrate</td>
<td>2. Triethylene glycol dimethacrylate</td>
</tr>
<tr>
<td>3. Copper(II)sulfate pentahydrate</td>
<td>3. Ethyleneglycol dimethacrylate</td>
</tr>
<tr>
<td>5. Aluminiumchloride hexahydrate</td>
<td>5. 2-Hydroxyethyl methacrylate</td>
</tr>
<tr>
<td>6. Gold(I)sodium thiosulfate dihydrate</td>
<td>6. Tetrahydrofurfuryl methacrylate</td>
</tr>
<tr>
<td>7. Tin</td>
<td>7. 1,4-Butanediol dimethacrylate</td>
</tr>
<tr>
<td>8. BENZOPHENONE-3</td>
<td>8. 1,6-Hexanediol diacrylate</td>
</tr>
<tr>
<td>10. Nickel(II)sulfate hexahydrate</td>
<td>10. GLUTARAL</td>
</tr>
</tbody>
</table>

Table 1. Set of 20 standardized allergens for skin patch testing.

Skin patch testing was performed according to the Jadassohn & Bloch classical methods for diagnosis of contact allergy. A standard patch is composed of 10 chambers. Patches with allergens were applied on the back of the tested individuals both on left and right sides. Prior to patch application the skin of the back was carefully examined to exclude skin lesions, folliculitis or nevi. The application time was 48 hours and the reading is carried out on 48 h, several hours after removing of the patches, with control revision on 72 h. Obligatory condition was lack of anti-allergic medication before placing the patches and during the study. For the interpretation of the test result the following scheme was used (International Contact Dermatitis Research Group - ICDRG):

| (-) | Negative reaction |
| ? | Doubtful reaction |
| + | Weak reaction (non-vesicular) |
| ++ | Strong reaction (oedematous or vesicular) |
| +++ | Extreme reaction (ulcerative or bullous) |
| IR | Irritant reaction |

Thermal imaging

The thermographic investigations were performed with the FlirT620 infrared camera with a temperature resolution of 0.06°C. During the whole procedure the following conditions were maintained: a closed room with white walls providing a constant level of emission, a constant air temperature of 20-22°C and relative humidity - 40-50%, no more than 2 people presenting of in the room during the test. The time for adaptation of the patient in the room was approximately 10 min. The distance between skin of the back and the camera was 1.5 m. The analyses of thermograms were performed using the Flir Reporter Professional software 2013.

In order to determine precisely the temperature inside the patch chambers, were the test allergens were applied, we tested use of plastic patterns - Reading Plate, cut by exact analogy with the location of the patch chambers (image 1).

- Analyzing further the latter images, we observed that an overall thermal visualization of an allergic reaction, if such, was no available.
- Due to the compression of the skin during the test and the lower temperature of the plastic template the tested areas were cooled.

In order to optimize the test protocol, we switch to the regimen of precise focusing and a shooting mode “picture in picture” and / or “image fusion” (image 2).
Image 2. Thermal imaging in the regimen of precise focusing and a shooting mode “picture in picture” and / or “image fusion”

Statistical methods
The statistics were calculated with SPSS 19.0. Available for cross-tabulation statistics were used: chi-square test and phi-coefficient for testing hypothesis of a link between categorical variables.

RESULTS
The results from the clinical and the thermographic evaluations of the patch tests are presented in table 2.

No positive skin patch test reaction to any of the tested allergens was observed among ten of the studied a total of thirty patients. Using the method of thermal imaging, no temperature peaks in the areas corresponding to patch chambers were in this group of patients.

Regarding the remaining twenty patients (n = 20), a total of 28 positive reactions were diagnosed. Four of them (n=4) manifested strong allergic reactions simultaneously to two allergens from the group of metals. This is in accordance with the results of Stoeva et al. (2010), indicating highest incidence of sensitization to nickel, palladium, gold, cobalt, chromium (18). According to the criteria of the ICDRG, eight of the reactions were evaluated as strong (++) and extreme strong (+++) positive. Concerning the latter eight reactions, an absolute matching between the recorded with the infrared camera thermal areas and the clinical interpretation was observed.

The other 20 positive reactions were clinically evaluated as “weak reaction” (+), and matching with thermal imaging was observed only in 9 of them. In conclusion, matching between the clinical and thermal imaging evaluations was observed for a total of 17 reactions. For the remaining 11 clinically positive patch test reactions, the skin temperature was comparable to the temperature of the negative controls (empty chamber).

The statistical analysis of the results revealed an average correlation between the clinical evaluation of the results according to the cheme of ICDRG and the thermal image diagnostic (rphi = 0.538, p = 0.001).

Absolute matching of clinical and thermal image results was observed only for the negative ones. For the clinically positive skin patch results matching with thermal

Table 2. Results from the clinical and by thermo vision evaluations of skin patch tests.

<table>
<thead>
<tr>
<th>Patients with clinical positive/negative skin patch test reactions</th>
<th>Physicians with thermo graphically diagnosed positive/negative skin patch test reactions</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative N / (%)</td>
<td>Positive N / (%)</td>
<td>N / (%)</td>
</tr>
<tr>
<td>Negative N / (%)</td>
<td>10 / (26,3%)</td>
<td>0 / (0,0%)</td>
</tr>
<tr>
<td>Positive N / (%)</td>
<td>11 / (28,9%)</td>
<td>17 / (44,7%)</td>
</tr>
<tr>
<td>Total</td>
<td>21 / (55,3%)</td>
<td>17 / (44,7%)</td>
</tr>
</tbody>
</table>
image method was observed in 60.7% of the cases.

**DISCUSSION**

Medical thermography originates from 1957, when the surgeon Dr. R. Lawson discovered that patients with breast cancer have an increased skin temperature in the area of cancer. Since 1970 thermography has been applied in many fields of medicine. Until 1990, there were some problems such as low sensitivity of the detector and the lack of trained professionals. Since then thermographing equipment has evolved significantly.

The modern thermal imagers systems include technically perfected thermal cameras and with sophisticated software. The images are of good quality and could be further processed to obtain reliable information and reproducible results [13]. Basing on our experience we could recommend to perform the investigations applying extremely accurate focusing, preferably using the infrared’s camera function “Image fusion”, outlining the patches and the chambers with a permanent marker.

Thermography may be applied as a diagnostic tool in many fields of medicine such as oncology, allergology, angiology, rheumatology, etc. Jung et al. applied thermal infrared imaging for assessment of skin prick tests and demonstrated the its applicability as an objective and repeatable technique in the evaluation of skin prick tests [6, 14].

During the classical reading of skin patch test reactions greatest difficulties are encountered to distinguish weak (+), doubtful and irritant reactions. In these cases the application of thermal imaging could be especially beneficial, although not standardized criteria to distinguish these reactions have been elaborated yet.

Clinically, the allergic reactions are associated with erythema, vesicles, infiltration throughout the surface of the patch chamber, while the irritant reactions include sweat rash, follicular pustules and burn-like reactions.

Our findings confirm the pattern found for strong and extreme allergic reactions and described by Mikulska D. et al. (2005) and Laino L. et al. (2010) [7, 15] and characterized by a hot area that extended beyond the borders of the patch site and oriented towards the nearest lymph-node(s), spreading as a hyperthermic line or lines. For the irritant reactions, the temperature didn’t differ from the temperature of the skin area, serving as a control.

It should be noted that from a practical point of view, skin patch test reaction to allergens from the dental series is not indicative for the diagnosis of allergic contact dermatitis or allergic oral manifestations. Some patients with positive reaction have never experienced symptoms when exposed to the investigated haptens/allergens. In such cases of particular importance for interpretation of the results from skin patch tests is the COADEX system for assessing the clinical relevance of positive reactions, in which the result is associated with past, current or active sensitization with the investigated allergen [16].

**CONCLUSION**

Optimization of thermal imaging as a method for evaluation of skin patch test results could serve as a safe, accurate and non-invasive method, especially to distinguish weak (+), doubtful and irritant reactions. Crucial factor for the accurate interpretation of results is the precise diagnosing performed by well-trained physicians in this area, with clinical relevance, to minimize the role of subjective factors.

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