Quantitation of Contact Allergy in Guinea Pigs by Measuring Changes in Skin Blood Flow and Skin Fold Thickness

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Skin blood flow determined by laser Doppler flowmetry (LDF) and skin fold thickness (SFT) have been used to quantitate allergic contact dermatitis in the guinea pig maximization test (GPMT) using chlorocresol as the allergen. The closed patch test procedure itself influenced both LDF and SFT measurements when determined in 12 sham-treated guinea pigs. The LDF was maximal at 24 hours and the SFT at 48 hours. Before correlating the quantitative measurements with the conventional visual scoring in test and control animals the value from a nearby control site was subtracted from the test site values. The correlations were highly significant (p<0.001-0.05) indicating that the quantitative methods were useful supplements to the visual scoring as a measure of interobserver and interlaboratory differences. Key words: Allergic contact dermatitis; Guinea pig maximization test; Laser Doppler flowmetry; Skin blood flow; Skin fold thickness.

The subjective visual grading scales traditionally used for the assessment of skin test reactions causes variations in test results between observers and laboratories. Weil & Scala (1) concluded in their study of intra- and interlaboratory variability in irritation tests in rabbits, that the primary reason for variability was in the scoring and rating.

Visible erythema and palpable edema are the main characteristics of the test reactions used for scoring. Laser-Doppler flowmetry (LDF) and skin fold thickness (SFT) measurements were found useful as objective methods to evaluate erythema and edema, respectively, in irritancy tests (2, 3). In allergic test reactions in humans both techniques yielded objective readings which correlated well with the subjective scores (4, 5).

This report compares the visual readings with the quantitation of changes in skin fold thickness and blood flow in challenge patch test reactions in the guinea pig maximization test, and finds the objective methods as a useful supplement to the scoring method.

MATERIALS AND METHODS
Guinea pig maximization test (GPMT)
Chlorocresol (BDH Chemicals Ltd., Poole, England) was the allergen. Magnusson & Kligman’s procedure was followed (6). For the comparison of the reading methods we chose at random 12 control animals and 36 test animals used in an investigation of the significance of the type of Freund’s complete adjuvant emulsion for the guinea pig sensitization rate (7). The induction concentrations used in test animals were chlorocresol 1% in propylene glycol intradermally day 0 and chlorocresol 10% in pet. topically day 7.

Chlorocresol 1% and 0.1% in pet. in Finn Chambers® (Epitest OY, Helsinki, Finland) on Scanpor® (Norgesplaster A/S, Oslo, Norway) were used for the 24 h closed challenge day 21. The patches were placed anterior-posterior on the mid-flank with 10 mm skin between the chambers. The order of the concentrations varied according to a rotation scheme.
Blind readings were done 24 and 48 h after removal of the patches, at 48 and 72 h. The scoring and measurements were performed independently of each other, and the results were compared later.

12 different sham treated animals were patch tested and bandaged in a similar way with an empty Finn Chamber® and one filled with the petrolatum used as vehicle. To examine the influence of the challenge procedure and the day to day variation these animals were read prior to patch testing and at 27 h (3 h after removal of the patches), 48, 72, and 96 h. They were shaved 3 h before reading at 48, 72 and 96 h.

**Visual scoring**

The scale of Magnusson & Kligman (6) was used: 0=no visible change, 1=slight or discrete erythema, 2=moderate and confluent erythema, 3=intense erythema and swelling. A grade 1 reaction was not regarded as evidence of sensitization.

**Quantitative readings**

**Laser Doppler flowmetry (LDF)**

The blood flow was quantitated by means of a laser-Doppler flowmeter (Periflux® microvascular flowmeter, Perimed, Stockholm, Sweden). The measuring technique has been described in detail previously (8, 9). The outputs of the procedure are related to fundamental skin perfusion characteristics, i.e., volume and flow rate, but do not quantitate skin blood perfusion as volume/weight/unit time. The instrument was adjusted to a bandwidth at 4 kHz and gain at 10.

The animals were immobilized by a helper, while the laser-Doppler probe was attached to the central part of each test site for about 30 sec. until a stable value was reached and the reading recorded. The blood flow value from a control site on the flank between the 2 test sites was subtracted from the readings of the test sites. This difference in blood flow was used as a measure of the challenge response (Fig. 1). The interjacent control site had remained covered with Scanpor® and Acrylastic® during the 24 h bandaging.

The animals used to evaluate the day to day variation and the influence of the closed challenge procedure were measured twice and the mean is shown.

**Skin fold thickness (SFT)**

Skin fold thickness was measured by a Mitutoyo® skin fold caliper (Mitutoyo MFG. Co. Ltd., Tokyo, Japan), which used a pressure of 275 g/cm² between the 6 mm disks, in the skin fold thickness range of 0 to 4 mm.

The animals were immobilized by a helper who manually folded the skin over the test sites, and the thickness of the challenge reactions determined within few seconds to the nearest 0.05 mm. We attempted to measure at the same level each time, as close to the margin of the fold as possible (3, 10). Instant readings were made to avoid the effect of compression of soft tissue. An untreated site between the 2 challenge reactions served as a control skin fold, the thickness of which was subtracted from the thickness of the challenge reaction skin folds. Scanpor® and Acrylastic® had covered the test and control sites during bandaging. The sham treated animals were measured twice and the mean is given.

**Statistics**

Non-parametric distribution free methods were used (11).

The Spearman rank correlation coefficient was used to evaluate the relationship between the visual scoring system and the quantitative reading methods. The ability of the objective methods to differentiate between the visual scores was tested by the Mann-Whitney test. The effects of the 2 challenge doses on the LDF and SFT in animals judged to be sensitized were examined by the Wilcoxon test. The Friedman two-way analysis of variance was used to evaluate the day to day variation and the influence of the test procedure.

**RESULTS**

The visual scoring at 48 h showed that 28 of the 36 test animals were sensitized to chlorocresol (grade 2 or 3 reaction). Among the 12 control animals 5 had a grade 1 reaction not accepted as evidence of sensitization. Both the test and the control animals were included in the comparison between the visual scoring and the quantitative methods.
Fig. 1 and 2 show the differences in LDF readings and SFT at 48 h at the anterior challenge site correlated to the visual reading score \((p<0.001\) at 48 and 72 h). Similar results regarding the difference in LDF readings were obtained from the posterior challenge site at both readings \((p<0.001\). For the SFT measurements, the level of significance changed from \(p<0.001\) at 48 h to \(p<0.05\) at 72 h.

The readings from the interjacent control site gave similar results in control and test animals. Table 1 gives the median and interquartile range.

Fig. 3 shows the day to day variation during the test procedure in 12 sham treated guinea pigs. There was a significant influence on both LDF and SFT from the closed patch test procedure \((p<0.001\). The LDF was maximal at 27 h and increased again at 96 h probably due to skin irritation from the shavings. The SFT was increased by the bandaging \((\text{max. at } 48\ h)\).

When the 3 sites were compared (pet. filled chamber, interjacent site and empty chamber) there was no difference in the SFT, while the LDF was highest at the interjacent site \((p<0.001\), probably due to tape and shaving irritation.

None of the sham treated animals showed test reactions judged as positive \((\text{score } 2 \text{ or } 3)\), but 3 showed a score 1 reaction at 48 h interpreted as irritancy from the procedure. In case
Table I. Laser Doppler flowmetry (LDF) and skin fold thickness (SFT) at control sites interjacent to test sites

The LDF is in arbitrary units and the SFT in mm. The 48 h values are given with median and interquartile range.

<table>
<thead>
<tr>
<th></th>
<th>12 control animals</th>
<th>36 test animals</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>LDF</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3rd quartile</td>
<td>33</td>
<td>27</td>
</tr>
<tr>
<td>2nd quartile</td>
<td>24</td>
<td>23</td>
</tr>
<tr>
<td>1st quartile</td>
<td>18</td>
<td>21</td>
</tr>
<tr>
<td><strong>SFT</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3rd quartile</td>
<td>3.35</td>
<td>3.30</td>
</tr>
<tr>
<td>2nd quartile</td>
<td>3.00</td>
<td>3.15</td>
</tr>
<tr>
<td>1st quartile</td>
<td>2.90</td>
<td>3.00</td>
</tr>
</tbody>
</table>

We examined if the LDF readings could discriminate between sensitized and non-sensitized animals, determined by the visual score. Analysis showed a significantly higher blood flow value in the score 2 reactions compared to the score 1 reactions at both test sites at 48 h (Mann Whitney test, anterior site \( p<0.002 \), posterior site \( p<0.05 \)). The LDF readings did not discriminate between the scores 0–1, and 2–3.

The difference in SFT could not discriminate between sensitized and non-sensitized animals (based on the visual score), but it discriminated between the scores 2 and 3 at the anterior challenge site at 48 h (Mann Whitney test, \( p<0.002 \)), as the score 3 reaction is characterized by the presence of edema.

In 28 animals judged to be chlorocresol sensitized at 48 h by showing a visual score of 2 the quantitative measurements showed responses which significantly discriminated between the 2 challenge concentrations (Wilcoxon test \( p<0.001 \)) (Table II).

**DISCUSSION**

Both methods were suitable as supplements to the visual scoring. The laser-Doppler flowmetry statistically separated sensitized (grade 2 or 3) from non-sensitized animals.

Table III. Maximal agreement between scoring and increase in LDF and SFT at the anterior test site at 48 h. The number of animals is given

<table>
<thead>
<tr>
<th>Increase in LDF</th>
<th>Increase in SFT</th>
</tr>
</thead>
<tbody>
<tr>
<td>( &lt;15 )</td>
<td>( \geq 15 )</td>
</tr>
<tr>
<td>0 and 1 (negative)</td>
<td>21</td>
</tr>
<tr>
<td>Score</td>
<td></td>
</tr>
<tr>
<td>2 and 3 (positive)</td>
<td>2</td>
</tr>
</tbody>
</table>
Objective reading of guinea pig allergy

The maximal agreement between the visual scoring and the quantitative methods was determined for the anterior challenge site at the 48 h reading (Table III). For the LDF an increase of 15 units minimum (about 100%) corresponded to a grade 2 or 3 reaction. For the SFT an increase of 0.6 mm minimum (about 20%) corresponded to a grade 3 (edematous) reaction.

The closed patch test procedure as such increased both quantitative measurements in the guinea pigs (Fig. 3), supporting the choice of the difference value between test and interjacent control site as a measure of the challenge response. Wahlberg & Nilsson (12) found occlusion to increase the propylene glycol induced skin blood flow in man, but no increase was seen in closed patch tests with destilled water.

In some cases a negative difference in LDF and SFT was seen (see Figs. 1 and 2), because tape and shaving irritation appeared at the control site and not at the test sites, which had been covered by the chambers. This finding limits the use of the quantitative methods. Comparative studies using an open challenge technique are desirable. The open epicutaneous test (OET) utilizes open applications, thus avoiding the influence of tape and bandaging (13).

A regional variation in LDF and SFT at the anterior, posterior and interjacent control site prior to testing was not found. In humans the regional variation in skin blood flow has been reported (14).

The skin fold thickness obtained instantly to the nearest 0.05 mm decreased over the following seconds due to compression of the soft tissue. Larger patches and subsequently larger test reactions might decrease the variability. Wahlberg (3) found the skin fold thickness measurement reproducible in man and animal when studying skin irritancy. He used a Harpenden skin fold caliper, as did Friedman et al. (5) when examining the dinitrochlorobenzene skin hypersensitivity in normal subjects. They found the skin fold thickness increase to be the best method of assessing the challenge dose related response, when compared to clinical grading and induration diameter of the reaction. High frequency pulsed ultrasound is an alternative method for skin thickness measurements. It was found suitable for assessment of human patch test reactions (15).

The visual judgement of a trained observer may still be the best method of reading, but the objective measurements of LDF and SFT may be useful supplements as a measure of interobserver and the same animal source and the same test sites on the animals; these are other sources of variation between test results.

ACKNOWLEDGEMENTS

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REFERENCES

**Erratum**


Table III, p. 40, should read:

Table III. *Maximal agreement between scoring and increase in LDF and SFT at the anterior test site at 48 h. The number of animals is given*.

<table>
<thead>
<tr>
<th>Score (negative)</th>
<th>Increase in LDF</th>
<th>Increase in SFT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;15</td>
<td>≥15</td>
</tr>
<tr>
<td>0 and 1</td>
<td>21</td>
<td>3</td>
</tr>
<tr>
<td>2 and 3 (positive)</td>
<td>2</td>
<td>22</td>
</tr>
</tbody>
</table>