

The Quantification of Patch Test Responses: A Comparison Between Echographic and Colorimetric Methods

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Patch testing is widely used both for clinical and experimental purposes. Although the clinical grading employed routinely is of practical value, the lack of objectivity makes it unsuitable for research purposes and dose-response analysis studies. Instrumental measuring techniques have been applied to patch test evaluation, because they enable objective quantification of different biophysical aspects of the inflammatory reaction by means of a continuous assessment scale, providing data suitable for statistical analysis.

In order to compare the colorimetric and echographic methods for the evaluation of reactions of different intensity, we performed patch tests with 5% nickel sulfate on the flexor aspect of the forearm in 120 nickel-sensitive patients. Clinical and instrumental measurements were performed at 72 h. Numerical values corresponding to instrumental measurements were compared to the positivity degree, as assessed clinically. Whereas echographic parameters, expressing the intensity of oedema and inflammatory infiltration, enabled a distinction between +, ++ and +++ reactions, colorimetric a^* values, describing erythema, failed to distinguish between ++ and +++ reactions. Thus, the use of ultrasound is advisable for the quantification of skin reactions of great intensity, whereas the colorimetric method could be usefully employed for dose-response studies assessing minimal eliciting concentrations of allergens, and for the evaluation of clinically undetectable reactions. *Key words: contact dermatitis; bioengineering methods; biophysical parameters; ultrasound; erythema index.*

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Patch testing is widely used both to identify subjects with delayed allergic reactions to contact sensitizers and in experimental conditions for the study of the sensitizing properties of chemicals or the anti-inflammatory effects of drugs. Although the clinical grading employed routinely is of practical value, the lack of objectivity makes it unsuitable for research purposes and dose-response analysis studies. Instrumental measuring techniques have been applied to patch test evaluation, since they enable objective quantification of different biophysical aspects of the inflammatory reaction by means of a continuous assessment scale, providing data suitable for statistical analysis (1).

The choice of the instrument for evaluation of patch test reactions is important because different methods highlight only one aspect of the eczematous reaction: the evaporimeter measures the barrier disruption (2); the flowmeter and the colorimeter measure the component of the inflammatory reaction secondary to increase in blood flow (3–10); and the ultrasound

scanner evaluates oedema due to vasodilation, increase in blood vessel permeability and extravasation of water within the tissue (11–15).

Both colorimetric and echographic procedures seem to provide parameters corresponding to numerical values which increase proportionally to the intensity of the patch test response.

The aims of our study were to compare the employment of colorimetry and ultrasound for the evaluation of patch test reactions of different intensity, and to highlight the pros and cons of these different evaluation methods.

MATERIALS AND METHODS

Patients and patch tests

One hundred and twenty nickel-sensitive patients aged 19–48 participated in the study, after giving their informed consent. Subjects underwent a patch test on the flexor aspect of the left forearm, 6 cm below the elbow crease. Forty mg of 5% nickel sulfate in white petrolatum (Trolab, Hermal Chemie, Germany) were applied to the test area by means of large aluminium Finn Chambers (11 mm in diameter). These were fixed to the skin by Scanpor tape (Norgesplaster, Finland) and removed after 24 h. After removal of the patches, the skin was gently cleaned with cotton wool in order to remove the residual ointment, and the test areas were covered with an empty chamber in order to protect the test site until the final assessment. Evaluations were performed immediately before the application and at 72 h, 48 h after removal of the nickel sulfate preparation.

Clinical evaluation

Scoring was attributed according to the International Contact Dermatitis Research Group, as follows: normal skin = 0; erythema with slight infiltration and papules = +; erythema, infiltration, papules and vesiculation = ++; intense erythema, infiltration, intense vesiculation and/or bullae = +++.

Instrumental assessments

Instrumental assessments were carried out after a 30-min acclimatization period on relaxed reclining subjects in a room with temperature set at 21–22°C and relative humidity at 45–50%.

Echographic evaluations were performed using a 20 MHz B-scanner (Dermascan C, Cortex Technology, Denmark) which produces images representing a cross-section of the skin. Equipment, calibration methods and recording conditions have already been described in detail elsewhere (13). The echographic images were processed by a program (Dermavision 2D, Cortex Technology, Denmark) enabling numerical representation of the picture data based on segmentation procedures. Evaluation of the reflectivity of the dermis was performed employing a 0–30 amplitude interval, marking the hypo-reflecting parts of the dermis. The increase in the extension of the 0–30 (hypo-reflecting) areas is proportional to the inflammatory component of the reaction (12, 13).

For colour evaluations, the Minolta Chroma Meter CR 200 (Osaka, Japan) was used, employing the $L^*a^*b^*$ system recommended by the CIE (Commission Internationale de l'Éclairage). For evaluating erythema, the colour coordinate a^* representing the colour range from green (negative values) to red (positive values) was used.

Table I. Echographic and colorimetric values at positive patch test sites according to clinical scoring

Positivity degree (number of cases)		+	++	+++
		(40)	(65)	(15)
0–30 pixel areas	baseline	1322 ± 603	1604 ± 855	1453 ± 748
	72 h	2269 ± 875 (a)	7011 ± 2489 (a) (b)	14944 ± 2616 (a) (b) (c)
a*	baseline	3.55 ± 2.03	3.95 ± 1.83	4.61 ± 1.18
	72 h	7.45 ± 4.20 (a)	10.12 ± 3.56 (a) (b)	10.18 ± 2.88 (a) (b)

a = significant in respect to baseline. b = significant in respect to + reactions. c = significant in respect to +++ reactions.

Statistics

Student's *t*-test for paired data was used to check differences between baseline and values after 72 h, whereas Student's *t*-test for unpaired values was employed for the comparison of reactions of different intensity. For correlating echographic and colorimetric values, Pearson's correlation coefficient was employed. A *p*-value of ≤ 0.05 was considered statistically significant.

RESULTS

Forty patch test reactions were scored +, 65 were scored ++ and 15 were scored +++. Table I shows echographic and colorimetric values at baseline and after 72 h, according to the intensity of the patch test reaction as evaluated clinically, whereas Fig. 1 illustrates echographic and colorimetric Δ values (72 h values – baseline values).

72-h 0–30 pixel values were significantly higher compared to baseline both for + reactions and for ++ and +++ reactions. As already described, 0–30 pixel values showed a linear increase according to the intensity of the reaction, as evaluated by clinical judgement (13). Moreover, significant differences were present between values referring to +, ++ and +++ reactions.

Colorimetric a* values showed an increase according to the increase in clinical scoring. However, whereas significant differences were observable between baseline and 72-h values for

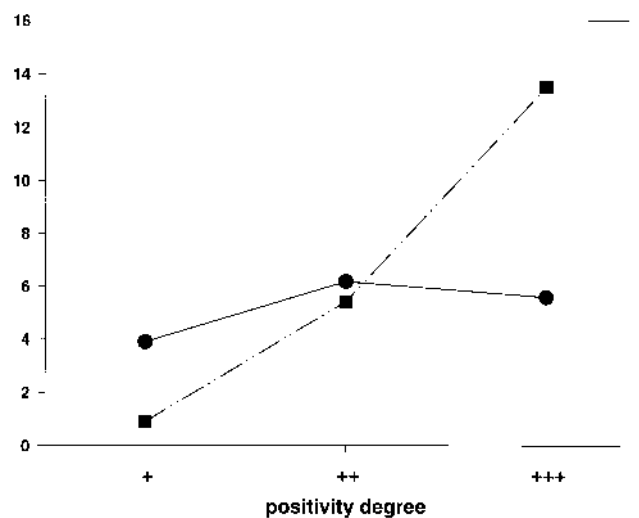


Fig. 1. Echographic (■) and colorimetric (●) values (72-h values – baseline Δ values) at positive patch test sites according to clinical scoring. Extension of 0–30 pixel (hypo-echogenic) areas corresponds to oedema and inflammatory infiltration, whereas a* values measure erythema.

all three classes of reactions, 72-h values referring to ++ and +++ reactions did not differ.

Differences with respect to baseline values (Fig. 1) increased from + to +++ reactions for echographic measurements, whereas no such trend was observable for colorimetric a* values.

Correlation coefficients between colorimetric and echographic values calculated for +, ++ and +++ reactions were +0.4026, +0.1432 and –0.0709, respectively.

DISCUSSION

In order to understand better the functional aspects of contact hypersensitivity reactions and to study different factors that modify the response, objective measurement methods have been applied to patch test reactions. In contrast to visual grading, instrumental measurements enable a precise non-invasive evaluation, are reproducible, and provide continuous data grading of disease intensity suitable for dose-response studies.

Recently, the erythema index value, provided by the Cortex Dermaspectrometer, values of which appear proportional to the amount of blood in the upper papillary dermis, was described as being related to the intensity of eczematous reactions (9). The erythema index showed a significant positive trend towards higher values for increasing the magnitude of the clinical reading, but when the intensity of the reactions was compared, there were only significant differences between 0 and ?+ and + and ++ reactions, whereas no significant differences between ?+ and +, or between ++ and +++ reactions were observed.

B-scanning echography enables real time visualization of cross-sections of the skin. As evaluated by ultrasound, the typical echographic aspect of a positive patch test reaction is characterized by increased skin thickness and homogeneity of the tissue, by expansion of hypo-echogenic dermal areas and attenuation of hyper-reflecting areas in the lower dermis (12) (Fig. 2). Image analysis procedures enable the determination of the extension of hypo-echogenic dermal areas, values of which are proportional to the positivity degree (12–18). Moreover, the echographic method has been employed for the quantification of the intensity of a wide range of clinical grades of responses, including those induced by dilutions of allergens producing a subthreshold response (15). Therefore, correlation with clinical scoring covers the whole range of positivities, including subclinical responses.

In this study, an increase in 0–30 pixel area values, corresponding to inflammation-induced dermal hypo-reflectivity, correlating with the clinically assigned positivity degree, was observed. In direct comparison, hypo-echogenicity values were able to distinguish between +, ++ and +++ reactions, and

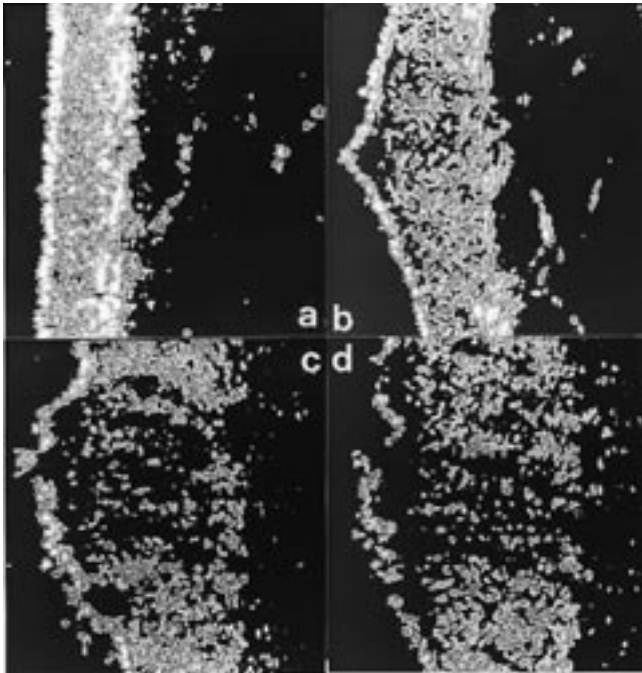


Fig. 2. Echographic images of normal volar forearm skin (a) and of positive patch test reactions of different positivity degrees. b, c and d represent +, ++ and +++ reactions, respectively.

values referring to +++ reactions were almost double compared to those of ++ reactions.

The colour parameter a^* , describing the colour range from green to red, provides a suitable supplement to clinical scoring in the evaluation of skin responses to irritant substances (1, 19). Our study showed an increasing trend in a^* values, describing erythema, according to the increase in clinical scoring. However, ++ reactions were not distinguishable from +++ responses; with respect to the former, no increase in values referring to the a^* parameter was observable at +++ reaction test sites. In fact, considering correlations between echographic and colorimetric values, coefficients were high for + reactions, whereas for +++ reactions a negative trend was observable, reflecting the pathogenetic sequence of the allergic inflammatory response, showing an initial stage dominated by vasodilation, and a more advanced stage dominated by the formation of oedema compressing the vessels and preventing erythema from appearing on the skin surface.

Bioengineering devices are typically based on a single physical modality and only measure a specific feature of the inflammatory response. Therefore, for the description of a biological phenomenon, it is generally much more convenient to use several techniques in combination. The usefulness of any method depends on sensitivity, measurement error, slope of the curve describing the intensity of the response and ease of use. If the purpose of the study, however, is quantification and dose ranging, one selected method may suffice, provided that a correlation between clinical evaluation and instrumental grading is demonstrated. In the case of ultrasound and colorimetry, the differences in the shape of the grading curves reflect variations in the relative importance of vascular changes and oedema/cellular infiltration as the intensity of the allergic reaction increases. Our data show that colorimetry is not suitable for the grading of reactions of great inten-

sity. In fact, a maximum response with colorimetry is already observable for ++ reactions, whereas a linear increase from + to +++ reactions is demonstrated with ultrasound.

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