Echographic Evaluation with Image Analysis of Allergic and Irritant Reactions

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B Scanning Evaluation of Allergic Reactions with Binary Transformation and Image Analysis

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In order to characterize and quantify allergic skin reactions, 12 nickel sensitized women aged 18 to 45 underwent 5 patch tests with nickel sulfate in petrolatum at the following concentrations: 0.5, 1, 2, 3 and 5%. The tests, performed on volar forearm skin, were removed after a 24-h application. B scanning evaluations were carried out at the beginning and at 24, 48 and 72 h after patch test application using Dermascan C (Cortex Technology, Hadsund, Denmark). After obtaining echographic images, these were processed by a dedicated programme (Dermavision 2D, Cortex Technology, Hadsund, Denmark) enabling the selection of amplitudes of interest and the transformation into a binary colour system. By attributing one colour to a selected amplitude band, part of an image can be highlighted and assessed by a value corresponding to the number of pixels (picture elements). For the evaluation of the images, 2 bands were chosen. The first, ranging from 201 to 255 is able to highlight hyper-reflecting parts of the pictures (epidermis, lower part of the dermis); the second, ranging from 0 to 30, marks the hypo-echogenic part of the tissue, which is the site of inflammation. This method showed a progressive increase in the number of low reflecting pixels according to the nickel patch test concentration and to the elapsing of time. On the contrary, at 24 h there was a decrease in pixel values concerning the hyper-reflecting band. This evaluation method proved useful in assessing the intensity of patch test responses. ***Key words: Nickel patch tests; Echographic evaluation; Image analysis.***

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Besides clinical evaluation based on visual and palpatory examination many bioengineering methods have been employed to assess allergic patch test reactions. These techniques enable a quantification of the allergic reaction and an objective collection of data, as well as their elaboration and statistical evaluation (1–8). Echographic assessment of patch test reactions was first performed by Serup and co-workers who evaluated doubtful and positive patch test readings by A scan measurements of skin thickness as an expression of allergic edema, and found that this parameter increases according to the intensity of the reaction (9, 10). By B scanning assessment of positive patch test reactions we showed that, with the growth of the reaction, the extension of the hypo-echogenic area in the dermis increases and the mean amplitude value within it decreases. Therefore, these two measurements can be used to quantify the degree of positivity (11). This method, however, is based on the delimitation of an area of interest which is outlined by hand, thus partly implying the examiner's subjectivity. Recently, we described a new method for evaluating the intensity of patch test responses which employs a new software package to be used on images recorded by a 20 MHz B scanner (12). This method enables the determination of the number of pixels, forming areas reflecting with homogeneous echo-amplitudes. In a preliminary study, we demonstrated that the amount of pixels, reflecting within a pre-selected range, shows an increase that is proportional to the intensity of a positive patch test reaction (12).

In order to correlate the echographic assessments to clinical evaluation of patch test responses, recorded images, deriving from patch tests performed with various concentrations of the allergen and assessed at different times were processed by a special software package, after selection of two homogeneous echo-amplitude bands.

MATERIAL AND METHODS

Patients and patch tests

Twelve nickel sensitized women, aged 18 to 45 years, underwent patch tests with 50 mg of 0.5%, 1%, 2%, 3% and 5% nickel sulphate in petrolatum with a 24-h application time. Patch tests were applied on the flexural aspect of the right forearm. Two patch tests were placed, in pairs, 4 cm below the antecubital fossa, 2 more, 4 cm lower, and the fifth below these, at mid distance between the pairs. The positioning of the chambers was systematically varied, but blinded to the examiner. On the distal part of the forearm a patch test with petrolatum and an empty chamber without the paper disk were applied. Patch test sites were covered with large Finn Chambers 11 mm in diameter (Epitest, Ltd., Finland), fixed to the skin by Scanpor tape (Norgesplaster, Norway). After patch test removal and between assessments, patch test sites were covered by a light gauze dressing.

Clinical evaluation

Clinical scoring was carried out before the echographic evaluations by using a five-point intensity scale as follows: normal skin = 0; erythema with slight infiltration and papules = 1; erythema, infiltration, papules and vesiculation = 2–3; intense erythema, infiltration, and intense vesiculation = 4. Comparison between clinical scoring and echographic assessment was performed considering visual evaluations at 24, 48 and 72 h.

Echographic evaluation

Echographic evaluations were performed at the beginning of the experiment, after 24 h, 30 min after patch test removal, and after 48 and 72h. The echographic recordings were performed using a 20 MHz B scanner (Dermascan C, Cortex Technology, Hadsund, Denmark). Dermascan C is an instrument for echography which uses a 20 MHz transducer. This high frequency renders an image with high definition suitable for studying tissues close to the body surface (maximal depth = 3 cm). The instrument consists of 3 main parts: the probe, the elaboration and visualization system and the memorising and data-storing system. The probe with the 20 MHz transducer is placed on an articulated self-balanced arm allowing a specific fixed position on skin sites to be explored. It also has a computerized system for transverse movement to detect and record images of parallel sections. The instrument's central unit carries out elaboration of the data coming from the probe. The intensity of the reflection echoes is evaluated by a microprocessor and is visualized with a coloured bidimensional image.

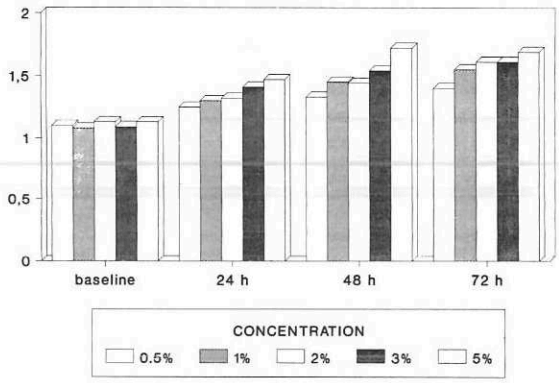


Fig. 1. Skin thickness values at sites of nickel patch tests performed with 0.5% to 5% nickel sulphate in petrolatum, assessed at different times. Skin thickness shows an increase in time that is more evident for high nickel concentrations.

In A scanning, the elaborator enables the visualization of data which are represented on the monitor by a peaked graph. B mode scanning presents a map-like picture consisting of coloured spots. It is possible to adjust the gain curve of the signal received in relation to the depth, to compensate for tissue attenuation and to optimize the instrument's operation according to the pathological aspects to be explored. Zooming can be made in both lateral and axial directions independently. There are 4 zoom levels within each direction: factors 1, 2, 4 and 8. For all recordings a standard swept gain curve at a constant level of 22 dB was used. Evaluations were performed by employing the zoom function in the axial direction at the first magnification (at factor 2) which enables exploration of the tissue to a depth of 6.71 mm. The automatic calculation of the thickness of epidermis and dermis was based on velocity of sound in skin of 1580 m/s. Assessment of skin thickness was performed in the B mode, outlining the image of the whole skin block on the screen and determining the extension by the region of interest (ROI) function. From this area a mean thickness, based on the values of 224 A scan lines (obtained by dividing the value of the area by 224) can be calculated (13).

Image analysis

After acquisition, the echographic images were processed by a dedicated programme (Dermavision 2D, Cortex Technology, Hadsund, Denmark), set up on an IBM compatible system (12). This software package ascribes an arbitrary numerical scale, ranging from 0 to 255, to the amplitude values of the echoes. Thus, each part of the picture or picture element (pixel) can be represented by a number. The programme enables the selection of one or more homogenous amplitude bands, whose width can be chosen, and which are reflected by steady structures or characterize transitory functional aspects of skin reactions. After the selection of an amplitude band, the original multicolour picture can be turned into a binary image, attributing one colour to the chosen amplitude levels, and a contrasting colour to the background, formed by not selected objects. Thus, it is possible to highlight on the screen only the areas in which the amplitude of the echoes is included within selected values and whose extension can be calculated in mm^2 or in number of pixels (the smallest elements in a visual display with controllable colour and brightness), on the whole picture, or within a selected portion of image, defined by hand by the ROI function. For the evaluation of the images two bands were chosen. The first, ranging from 0 to 30, marks hypo-reflecting parts of the tissue (corresponding to oedema and inflammatory infiltration), the second, ranging from 201 to 255, is able to highlight hyper-reflecting parts of the skin, like epidermis and the lower dermis. For every image, pixel values forming areas reflecting in these ranges were determined. The chosen regions of interest were, for the 0–30 band, the entire skin block appearing on the screen; for the 201–255 band, the hyper-reflecting band, corresponding to the epidermis and, separately, the lower part of the dermis.

Statistics

For each nickel concentration, thickness and pixel values referring to baseline skin and to 24, 48 and 72h assessments were compared using the ANOVA test for repeated values and the Student-Newman-Keuls (SNK) test. Moreover, thickness values referring to patch tests performed with different nickel concentrations, at the same time of assessment, were compared by the one-way ANOVA test and the SNK test. Values of pixels reflecting in the 0–30 range, at the same time of assessment, referring to 0.5% nickel patch test, were compared to values recorded with 3% and 5% patch tests, employing the Student's *t*-test. The chosen level of significance was 0.05. Comparability of

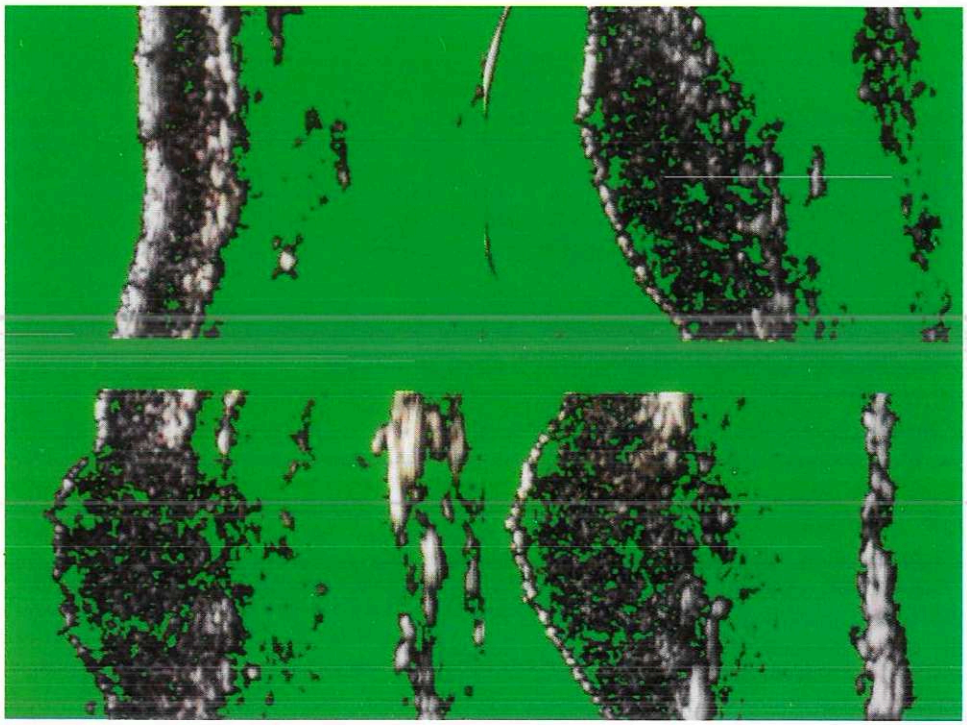


Fig. 2. Images of normal skin of the volar forearm (left upper corner) and of patch test reactions of different degrees of positivity, after selection of the 0–30 amplitude band and binary transformation. The area reflecting in the selected interval appears green in the dermis and its extension increases according to positivity degree.

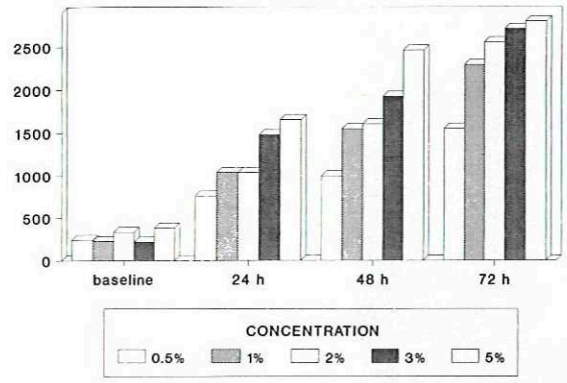


Fig. 3. Number of pixels forming areas reflecting in the 0–30 amplitude interval at nickel patch test sites, performed with 0.5% to 5% nickel sulphate in petrolatum, assessed at different times. Number of pixels increases in time and is more evident for high nickel concentrations.

values of pixels reflecting in the 0–30 range and of those comprised in the 201–255 interval was analyzed by the Spearman test. The same test was used to study the relationship between the visual grading system and the echographic method.

RESULTS

Clinical evaluation

At all control sites, skin appeared normal. Performing patch tests with a 24 h application time gave rise to positive reactions which, at 72 h, were at their maximum intensity (score 3–4). One patient, however, showed reactions which were fully developed at 48 h, 24 h after patch test removal, and presented a lessening of intensity at the 72 h assessment (score 2). The force of the reaction, as assessed by clinical examination, was proportional to nickel concentration in 7 patients, whereas in 5, patch test responses were equivalent for all 5 concentrations, and response to the 0.5% nickel sulphate patch test, as assessed by visual scoring, was not different from that induced by a ten times higher nickel concentration (score 3–4). In spite of this, the total score per area was higher where higher nickel concentrations had been applied and increased with the elapsing of time.

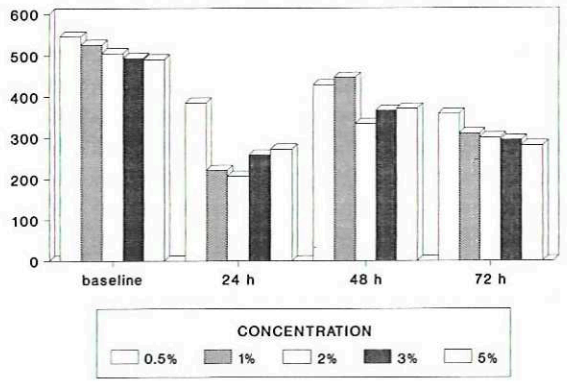


Fig. 4. Number of pixels forming areas reflecting in the 201–255 amplitude interval, determined in the upper part of the skin, at sites of nickel patch tests, performed with 0.5% to 5% nickel sulphate in petrolatum, assessed at different times. Pixel number decreases at 24-h determinations.

Echographic evaluation

Skin thickness, as assessed in B mode, showed an increase in time, with the intensification of the reaction, that was more evident at high nickel concentrations (Fig. 1). However, for the 5% patch test, the skin thickness mean value reached its peak at 48 h assessment and was constant at 72 h. Skin thickness values at patch test reactions, performed with the same nickel concentration, assessed at different times, showed statistical differences as evaluated by the ANOVA test for repeated values. At a limit of significance of 0.05, it was possible to distinguish between baseline and 24, 48 and 72 h assessments and between 24 h values and 72 h values, for all nickel concentrations. Differences in thickness values of patch tests at 48 h were significant for 5% patches, compared to 0.5, 1 and 2% patch tests.

Assessment by the 0–30 band, evidenced a progressive increase in the number of pixels corresponding to the higher extension of the hypo-reflecting area in the tissue, site of inflammation, in relation to the nickel patch test concentration and the elapsing of time (Fig. 2). The highest values were observed for 5% nickel patch tests at 72 h evaluation (Fig. 3). Values of pixels reflecting in the 0–30 range, calculated at different times, for the same nickel concentration, showed statistical differences according to the ANOVA test for repeated values. At a level of significance of 0.05, it was possible to distinguish between baseline assessments and 48 h and 72 h assessments for all nickel concentrations. Differences between pixel values of patch tests performed with 0.5% nickel sulfate and pixel values recorded from 5% patch tests were significant at 24 and 48 h.

Assessment of the upper part of the skin with the 201–255 band showed a slight decrease in the number of pixels, reaching its maximum fall at 24 h (Fig. 4). For 1%, 2%, 3% and 5% concentrations, statistical differences were observed between 72 h and baseline and between 24 h and baseline assessments.

With an increasing reaction, the hyper-reflecting part of the lower dermis gradually disappeared (Fig. 5). This aspect was also confirmed by the echographic quantification: in fact, the evaluation of the lower dermis using the 201–255 band, showed a marked decrease in the number of pixels, more pronounced at high nickel concentrations and at 72-h evaluations (Fig. 6). Statistical assessment of values of pixels reflecting in the 201–255 range, determined in the lower dermis at different times, for the same nickel concentration, enabled us to distinguish between baseline and 24-, 48- and 72-h values.

No significant variations were observed for values of echographic measurements at control tests sites, although a slight increase in pixel values, reflecting in the 201–255 range, was detected at petrolatum patch test areas.

The values of pixels reflecting in the 0–30 range showed a significant correlation to clinical scoring ($r = 0{,}901$). Moreover, an inverse correlation between pixel values reflecting within the 0–30 band and pixel values reflecting in the dermis in the 201–255 range was evident ($r = -0{,}641$).

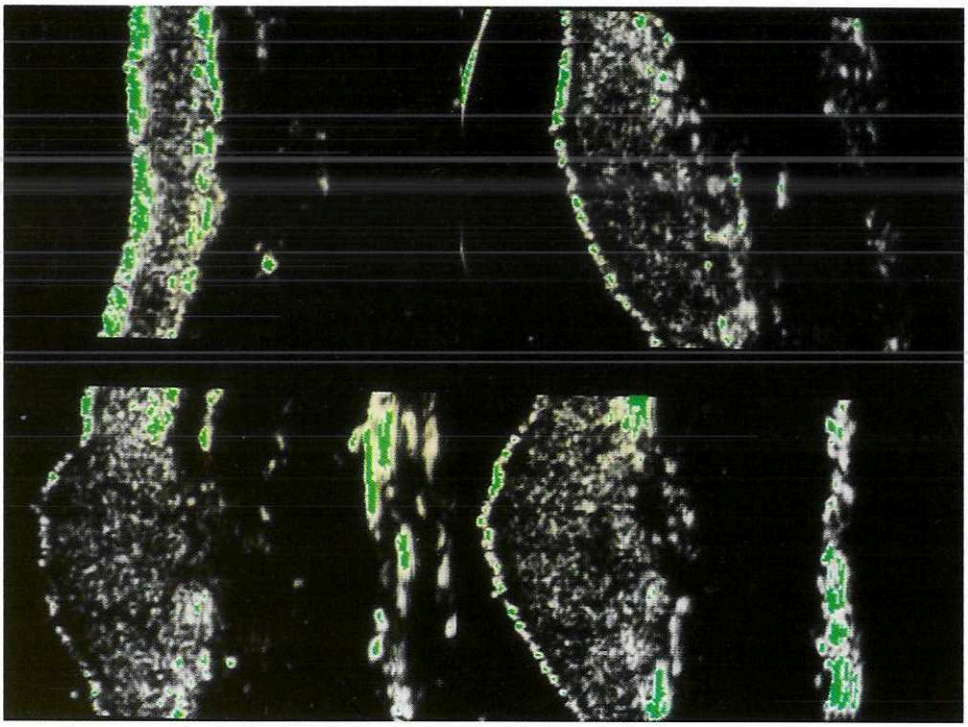


Fig. 5. Images of normal skin of the volar forearm (left upper corner) and of patch test reactions of different degrees of positivity, after selection of the 201–255 amplitude band and binary transformation. The area reflecting in the selected interval appears green. Epidermis echo and echogenicity of the lower dermis have clearly decreased at positive patch test sites compared to normal skin.

DISCUSSION

From a clinical point of view, this study demonstrates that most nickel sensitized subjects who react to a 5% nickel patch test with a clear response also react to lower nickel concentrations. In fact, all our patients reacted to a 0.5% nickel patch test too. Moreover, in well sensitized subjects, a 24-h application time with assessment at 48 h could be sufficient in evidencing the reaction.

Our results referring to skin thickness confirm previous observations of Serup and co-workers (9–10) who, using a 15 MHz A scanner, first claimed that thickening of the skin in patch test reactions is proportional to the intensity of the response. However, B scanning assessment of skin thickness is based on a more precise procedure, enabling a measurement corresponding to the average value of 224 A scan assessments.

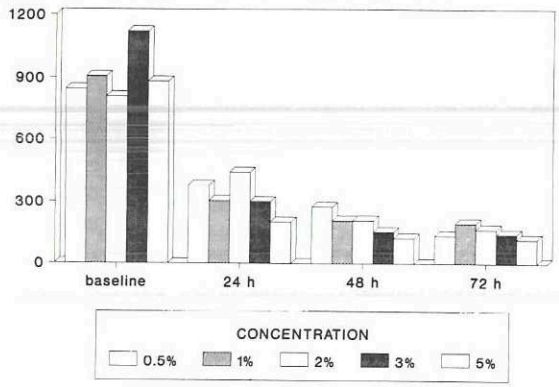


Fig. 6. Number of pixels forming areas reflecting in the 201–255 amplitude interval, determined in the lower dermis, at nickel patch test sites performed with 0.5% to 5% nickel sulphate in petrolatum, assessed at different times. The number of pixels decreases especially at high nickel concentrations and at 72-h evaluations.

Thus B mode assessment of skin thickness, which can be easily performed, can be used for a quick quantification of a positive reaction.

Regarding our image analysis procedure, the most important aspect is the choice of the band of interest. Using this software package, a great number of echo-amplitude intervals, varying in width and amplitude positioning, can be selected and used for highlighting different parts of the image. Every image can therefore be considered for its different aspects and described and quantified in different ways.

The characteristic echographic aspect of a positive patch test reaction is due to the presence of a lower density and a greater homogeneity of the tissue corresponding to a decrease in reflectivity. This hypo-reflectivity of the tissue is observed in inflammatory conditions of the skin, including not only allergic contact dermatitis, but also atopic dermatitis and psoriasis.

Consequently, we chose an echo-amplitude interval marking hyporeflecting parts of the image corresponding to edema and inflammatory infiltration. Delimiting the whole skin block by the ROI function enables the automatic determination of the extension of the area reflecting within the chosen echo-amplitude interval, whose value is expressed in mm^2 or in number of pixels. According to measurements of increase in number of pixels forming areas reflecting in the 0–30 interval (average differences), the quantification of the degree of positivity can be defined as follows: 1 + reactions = 679; 2 + reactions = 1242; 3 + reactions = 1673; 4 + reactions = 2195.

Moreover, in strong patch test reactions, a slight decrease of reflectivity in the epidermis and a marked decrease in the lower dermis can be observed. Therefore, an echo-amplitude interval, corresponding to the highest amplitude levels, was chosen. These decreases could thus be quantified and an inverse correlation between pixel increase in the 0–30 band and

values of pixels reflecting in the 201–255 range could be observed.

Compared to the echographic assessment with image analysis, performed by the standard Dermascan C software, this new method enables a more reproducible evaluation. In fact, by image analysis the delimitation of the whole skin block on the screen is easier and more precise than the hand outlining of the margins of the hypo-echoic area required by the traditional method.

However, to obtain reproducible results we would stress the importance of performing all measurements on images which have been recorded by the same swept gain curve and at the same magnification. In fact, by changing the recording procedure, data show great variations and are no longer comparable.

Ultimately, unlike other non invasive methods, B scanning enables the assessment of reactions of all degrees of intensity, regardless of environmental conditions or the emotional state of the subject undergoing examination.

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B Scanning Evaluation of Irritant Reactions with Binary Transformation and Image analysis

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In order to characterize and quantify irritant skin reactions, 12 women aged 18 to 45 underwent 5 patch tests with sodium lauryl sulfate at the following concentrations: 0.5, 1, 2, 3 and 5%. The tests, applied on volar forearm skin, were removed after a 24-h application. Evaporimetry and B scanning were carried out at the beginning and at 24, 48 and 72 h after patch test application. Echographic recordings were performed by Dermascan C (Cortex Technology, Hadsund, Denmark). After the acquisition, the echographic images were processed by a dedicated software (Dermavision 2D, Cortex Technology, Hadsund, Denmark) enabling the selection of amplitudes of interest and the transformation into a binary colour system. By attributing one colour to a selected amplitude band, part of an image can be highlighted and assessed by a value corresponding to the number of pixels (picture elements). For the evaluation of the images, 2 bands were chosen. The first, ranging from 201 to 255 is able to highlight hyperreflecting parts of the pictures (epidermis, lower part of the dermis); the second, ranging from 0 to 30, marks the hypo-echogenic part of the tissue, which is the site of inflammation.

The evaluation by means of the 201–255 amplitude band showed a marked decrease of the echogenicity of the epidermis which was more pronounced at 24-h determinations and for higher concentrations of sodium lauryl sulphate (SLS), whereas the increase in pixel numbers, shown by the 0–30 band, was slight and apparent only for high SLS concentrations.

Echographic data and transepidermal water loss (TEWL) values showed a good correlation. Thus, we can conclude that this evaluation method proved useful in assessing superficial skin damage induced by irritants. ***Key words: SLS patch test; Echographic evaluation; Image analysis; Evaporimetry.***

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Irritant contact dermatitis and subclinical skin irritation are common but complex clinical problems. Unlike allergic contact dermatitis, which is always accompanied by subjective and objective signs, skin damage induced by an irritant substance can exist in the absence of clear clinical alterations thus making the skin more vulnerable to further irritation (1). For this reason, instrumental assessment of subclinical irritation is particularly important.

A better understanding of the pathophysiological mechanisms underlying irritant skin damage has been achieved by experimental irritant patch testing. Functional investigation of skin blood flow, skin colour and skin thickness changes, as measurements of the inflammatory process, and of trans-epidermal water loss, reflecting alterations of the barrier function of the stratum corneum, have been performed by many researchers (2–9).

B scanning assessment of allergic patch test reactions has proved a reliable method for their quantification, and is well correlated to clinical scoring (10). We have described an evaluation method employing a new software package to be used on images recorded by a 20-MHz B scanner, enabling the determination of the surface of image areas reflecting within homogeneous echo-amplitudes (11). Using this method, we demonstrated that the number of pixels mirrored within a pre-selected range shows an increase that is proportional to the intensity of a positive allergic reaction.

Our study aims at evaluating irritant reactions using this new method, comparing the results of the echographic assessment with clinical scoring and with values for transepidermal water loss (TEWL), which is considered a reliable method for the evaluation of subclinical water barrier function damage.

MATERIAL AND METHODS

Patients and patch tests

Twelve women, aged 18 to 45 years, underwent patch tests with 0.5, 1, 2, 3 and 5% sodium lauryl sulphate (SLS) (SIGMA Chemical Co., purity according to monograph 95%) with a 24-h application time. A filter paper disk was put into a large Finn Chamber (Epitest, Ltd., Finland) and wet with 30 µl of each solution. After basal recordings, patch tests were applied on the flexural aspect of the left forearm, fixed to the skin by Scanpor tape (Norgesplaster, Finland). Two patch tests were placed, in pairs, 4 cm below the antecubital fossa, two more patches 4 cm lower, the fifth 4 cm below these, at mid distance between the pairs. The positioning of the chambers was systematically varied, but blinded to the examiner. On the distal part of the forearm, a patch test with sterile water and an empty chamber without the paper disk were applied. After patch test removal and between assessments, patch test sites were covered by a light gauze dressing.

Clinical evaluation

Clinical scoring was carried out, before instrumental evaluations, by using a five-point intensity scale, as follows: normal skin = 0; scaling or very weak erythema = 1; weak erythema and slight infiltration = 2; marked erythema, infiltration and vesicles = 3; marked erythema, infiltration, vesicles, abrasion and possibly exudation = 4.

Instrumental assessments

Instrumental assessments were performed after a 30-min acclimatization period, at the beginning of the experiment, after 24 h, 30 min after patch test removal, and after 48 and 72 h. All measurements were carried out on relaxed reclining subjects in a climatic chamber with temperature set at 21–22°C and humidity at 45–50%.

Evaporimetry

TEWL was determined by an evaporimeter (EP1, Servomed, Stockholm, Sweden). This instrument uses the method of water pressure gradient calculation described in detail by Nilsson (12). During all measurements, the probe was hand held using an insulating glove, thus avoiding heating of the probe. After stabilisation of the TEWL value, 30–45 s after application of the probe to the skin, damping filter buttons were pressed in order to smooth fluctuations in TEWL. TEWL value displayed during the next 30-s period was considered the

Table I. *Skin thickness values in mm (± SD) at SLS patch test sites*

Concentration	Baseline	24 h	48 h	72 h
0.5%	1.02 (0.07)	1.12 (0.10)	1.12 (0.11)	1.1 (0.11)
1%	1.01 (0.08)	1.1 (0.10)	1.13 (0.07)	1.19 (0.36)
2%	1.07 (0.09)	1.24 (0.22)	1.29 (0.20)	1.32 (0.42)
3%	1.09 (0.10)	1.22 (0.19)	1.27 (0.17)	1.32 (0.39)
5%	1.1 (0.08)	1.42 (0.28)	1.41 (0.20)	1.38 (0.49)

measured value (13). TEWL measurements were only performed at SLS treated skin sites.

Echographic evaluations

The echographic recordings were carried out using a 20-MHz B scanner (Dermascan C, Cortex Technology, Hadsund, Denmark). This instrument has already been described in detail elsewhere (10). For all recordings a standard swept gain curve at a constant level of 22 dB was used. The evaluations were performed by employing the zoom function in axial direction at the first magnification (at factor 2) which enables exploring of the tissue up to a depth of 6.71 mm. The automatic calculation of skin thickness was based on velocity of sound in skin of 1580 m/s. Assessment of thickness of epidermis and dermis was performed in B mode, outlining the image of the whole skin block on the screen and determining the extension by the region of interest (ROI) function. From this area a mean thickness, based on the values of 224 A scan lines (obtained by dividing the value of the area by 224) can be calculated (14).

Image analysis

After the acquisition, the echographic images were processed by a dedicated programme (Dermavision 2D, Cortex Technology, Hadsund, Denmark), set up on an IBM compatible system (11). This programme has been described elsewhere (15). For the evaluation of the images two bands were chosen. The first, ranging from 0 to 30, marks hyporeflecting areas corresponding to oedema and inflammatory infiltration; the second, ranging from 201 to 255, is able to highlight hyper-reflecting parts of the picture. For every image, pixel values of areas reflecting in these ranges were determined. The chosen regions of interest were, for the 0–30 band, the entire skin block appearing on the screen; for the 201–255 interval, the hyper-reflecting band, corresponding to the epidermis and, separately, the lower part of the dermis.

Statistics

For each SLS dilution and for control tests, TEWL, thickness and pixel values referring to baseline skin and to 24-, 48- and 72-h assessments were compared using the ANOVA test for repeated values and the Student-Newman-Keuls (SNK) test. The Spearman rank correlation coefficient was calculated to study the relationship between the visual grading system and the echographic evaluations. Regression analysis was used to detect linearity between TEWL data and the number of pixels. Finally, the Student's *t*-test for paired values was used, to compare baseline values of pixels to values obtained from SLS treated skin sites, which had been scored with 0.

RESULTS

Clinical evaluation

Out of 180 evaluations of patch test reactions (12 patients × 5 patch tests × 3 times of assessment), 112 resulted in a positive clinical score. Forty-nine scored 1, 34 scored 2, 17 scored 3 and 12 scored 4. No positive reactions were detected at control test sites. Reactions were at their maximum intensity at 24 h: for each test area, the total score was at its highest at 24–h assessment and decreased progressively at 48 and 72 h. The reaction,

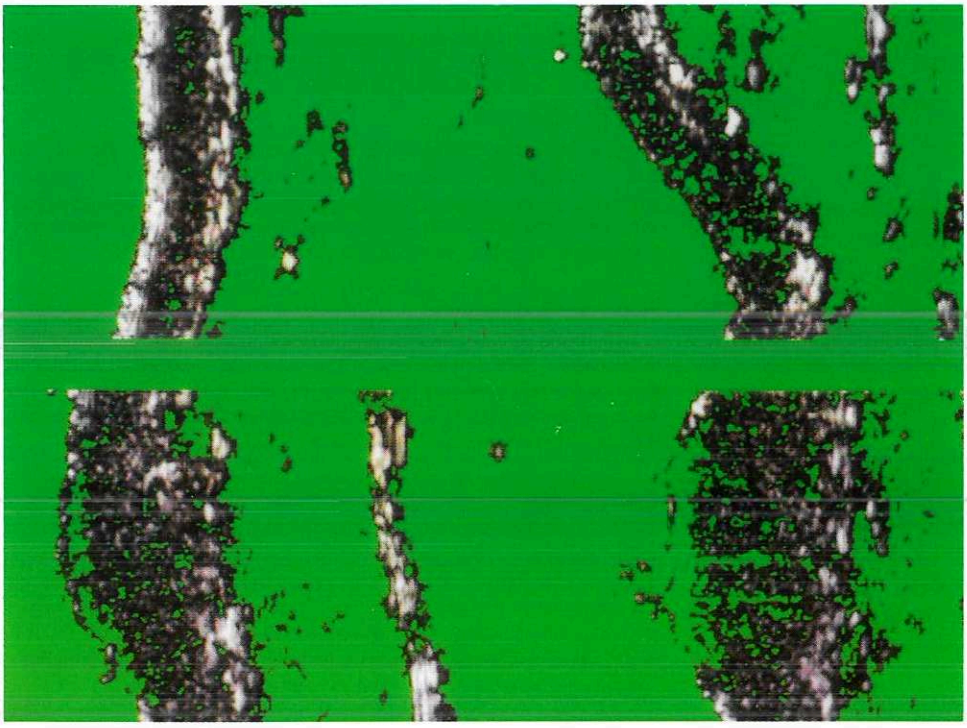


Fig. 1. Images of normal skin of the volar forearm (left upper corner) and of patch test reactions of different degrees of positivity, after selection of the 0–30 amplitude band and binary transformation. The area reflecting in the selected interval appears green in the dermis and increases according to positivity degree.

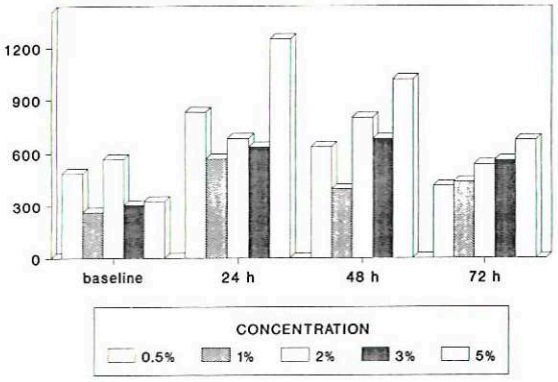


Fig. 2. Number of pixels forming areas reflecting in the 0–30 amplitude interval at SLS patch test sites, performed with 0.5% to 5% SLS in distilled water, assessed at different times. Number of pixels is at its maximum at 24-h determinations.

as assessed clinically, was proportional to the concentration of the irritant solution.

Echographic evaluation

Skin thickness, as assessed in B mode, showed an increase in time which was more evident with high SLS concentrations (Table I). The increase was at its maximum at 24 h for 0.5 and 5% concentrations, and at 72 h for 1, 2 and 3% SLS patch tests. Comparing consecutive times of assessment, differences in skin thickness values were significant only for 0.5 and 5% SLS concentrations. At a level of significance of 0.05 it was possible to distinguish between baseline values and 24-, 48- and 72-h values.

Processing by the 0–30 band showed an increase in the extension of the area reflecting in this range for all dilutions (Fig. 1). The highest increase was observed for the 5% solution. For all concentrations, the increase was more evident at 24-h determinations and values dropped again after that time (Fig. 2). Statistical differences between pixel values, assessed at different times for the same SLS dilution, were observed for 3 and 5% patch tests. SNK evaluation showed significant differences between baseline values and those assessed at 24, 48 and 72 h for 3% patches, and differences between baseline and 24- and 48-h values and between 48- and 72-h ones for 5% patch tests. The correlation coefficient r between number of pixels and visual scoring was 0.432.

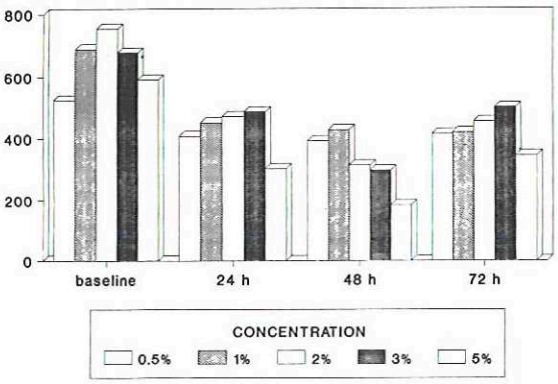


Fig. 3. Number of pixels forming areas reflecting in the lower dermis in the 201–255 amplitude interval, at sites of SLS patch tests, performed with 0.5% to 5% SLS in distilled water, assessed at different times. Pixel values decrease especially at 5% SLS patch tests.

Dermis evaluation by the 201–255 band showed a slight decrease at all SLS treated skin sites and at all times, which was more evident at 48-h assessment (Fig. 3). This decrease reached statistical significance for 2 to 5% patch tests. For 5% patch tests, at a level of significance of 0.05, it was possible to distinguish between baseline values and 24-, 48- and 72-h values.

Considerable information could be obtained by processing with the 201–255 band to highlight areas reflecting in the upper part of the skin (Fig. 4). Pixel values showed a marked decrease for all concentrations especially at 24-h determinations. This decrease was more pronounced for patches performed with 3 and 5% solutions (Fig. 5). Variations in pixel numbers were statistically significant for 1 to 5% patches. Differences between groups were detectable for 1 to 5% solutions between baseline values and 24- and 48-h values and between 24- and 72-h values; moreover, for the 2% concentration, differences between baseline and 72-h and between 48- and 72-h values were evident. Finally, 3% patch test determinations differed between 48-h and 24- and 72-h measurements.

No significant variations were observed for values of echographic measurements at control test sites.

The correlation coefficient r between the echographic evaluation by the 201–255 band and the visual grading system was -0.277. In view of this poor correlation, values of pixels reflecting in the 201–255 range of SLS treated skin sites scoring 0 were compared to baseline values for the same skin site by the Student's t-test for paired values. This test showed a highly significant difference between the 2 groups ($p < 0.001$).

Evaporimetry

TEWL at SLS treated sites had significantly increased at 24 h compared to basal skin. Readings fell gradually, but the increases were still significant at 48 and 72 h. Increases were more evident for the highest SLS concentrations (Fig. 6).

A significant linear correlation was demonstrated between TEWL values and number of hyper-reflecting pixels (201–255) in the epidermis. The linear regressions were as follows: $y = -0.06 \times +29.19$ ($r = -0.832$).

DISCUSSION

It is well known that surfactant induced changes are present after applications of the irritant even though the skin appears normal from a clinical and a subjective view point. Moreover, it has been demonstrated that the inflammatory response associated with skin barrier function damage induced by SLS is correlated with the concentration of SLS (8). In fact, even in the absence of clinical signs of skin irritation, increases in TEWL were detected before increases in skin blood flow, sustaining the view that barrier function damage precedes the inflammatory process (7, 16). Thus, TEWL and other correlated dynamic parameters, such as skin surface water loss, have proved to be reliable methods for the assessment of skin irritation.

For this reason, we chose the TEWL measurement as a

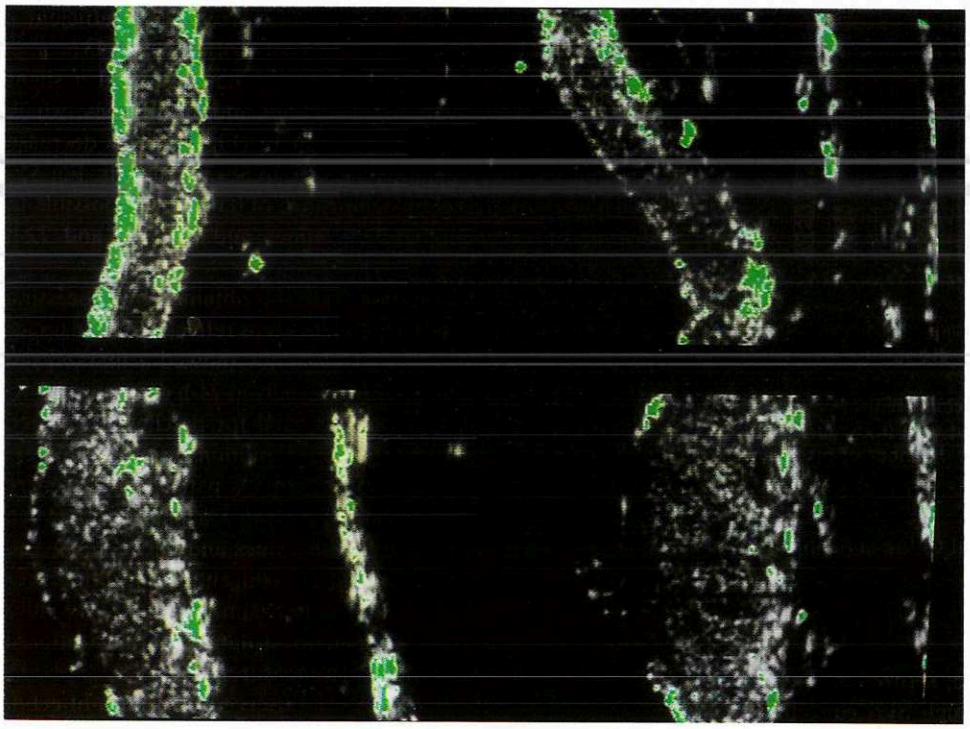


Fig. 4. Images of normal skin of the volar forearm (left upper corner) and of patch test reactions of different degrees of positivity, after selection of the 201–255 band and binary transformation. Area reflecting in the selected interval appears green. Epidermis echo and echogenicity of the lower dermis are clearly decreased at positive patch tist sites compared to normal skin.

method which is both comparable to clinical scoring and to echographic data referring to subclinical irritation.

Our results confirm a positive dose response correlation between irritant doses of SLS and skin damage as assessed clinically. TEWL results are in accordance with previous data referring to irritant patch tests performed with a 24-h application time (7, 16, 18).

The increase in skin thickness, already evidenced by Serup and co-workers using a 15 MHz A scan equipment (19), was confirmed by our B scan assessments which showed that this rise, which is tied to the inflammatory process, reaches its highest level later than TEWL value evaluating skin barrier damage.

Our image analysis procedure, based on the selection of amplitude intervals of interest and on the determination of the values of the pixels forming areas reflecting within the chosen amplitude bands, enables a numerical description of physiological and pathological conditions of the skin. The choice of the bands for evaluating irritant skin reactions was based on a previous experience concerning assessment of allergic patch test responses (11).

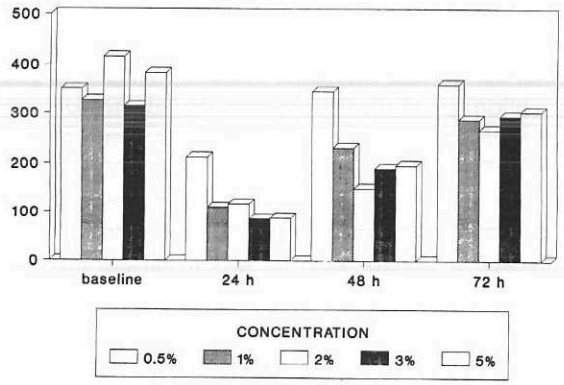


Fig. 5. Number of pixel forming areas reflecting in the upper part of the skin in the 201–255 interval at sites of 0.5% to 5% SLS patch tests, assessed at different times. Pixel values decrease at 24-h determinations.

The echographic aspect of an irritant reaction is characterized by the presence of a lower density and a greater homogeneity of the dermis corresponding to a decrease in reflectivity as in allergic reactions. Unlike the latter, irritant reactions show a clear decrease in the epidermis echo which is hyporeflecting and interrupted or has totally disappeared.

Thus, an evaluation interval covering the lowest amplitudes can quantify the appearance of dermal hyporeflectivity, whereas a band comprising the highest amplitudes is helpful in determining the damage of the entrace echo. Data calculated by the 0–30 band evaluation show a fair correlation to clinical scoring, whereas reflectivity of the epidermis, as assessed by the 201–255 band, is inversely proportional to TEWL values. The significance of the superficial hypo-echogenicity is difficult to explain. It is interesting to observe that it is proportional to functional damage, as demonstrated by TEWL, and could be considered as a visual equivalent of impaired water barrier function of the skin.

Moreover, this method seems very sensitive and is therefore useful in evaluating subclinical irritation. In fact, SLS treated skin areas which had been clinically evaluated as negative test showed echographic modifications which could be quantified by image analysis and were significant in comparison to normal skin values.

Compared to other non invasive techniques in assessing irritation, the echographic evaluation has the advantage of a quick execution. In fact, no pre-conditioning of the subjects is necessary, whereas subjects have to rest for at least 15 min

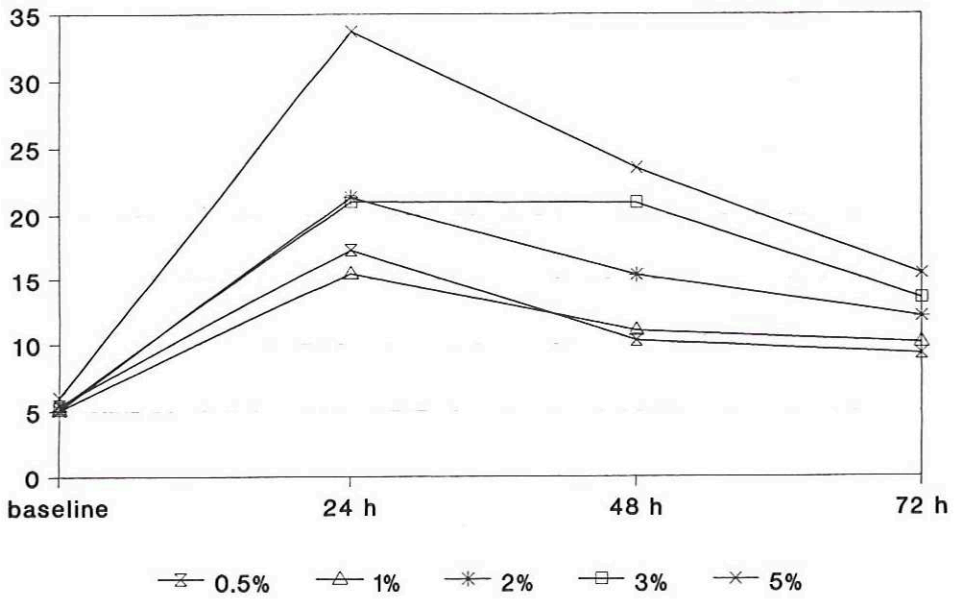


Fig. 6. TEWL average values assessed at different times at 0.5% to 5% SLS patch test sites. The increase in TEWL reaches its highest level at 24-h assessment.

before measurements of TEWL, skin colour and skin blood flow.

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