

Quantification of allergic and irritant patch test reactions using ImageJ

H. Ohshima^{1,2}, S. Kinoshita¹, M. Futagawa¹, H. Takiwaki³, K. Washizaki², A. Ishiko² and H. Kanto²

¹POLA Laboratories, POLA Chemical Industries Inc, Totsuka-ku, Yokohama, Japan,

²Department of Dermatology, School of Medicine, Toho University, Tokyo, Japan and ³Takiwaki Dermatology Clinic, Wakayama, Japan

Background/aims: It is often difficult to differentiate between allergic and irritant patch test reactions by visual inspection. The purpose of this study was to test an image analysis-based method that differentiates between the two reactions by quantifying the degree of erythema at the patch test site.

Methods: A total of 172 Japanese patients were patch-tested with sodium lauryl sulfate (SLS) and nickel sulfate, followed by digital photography and visual evaluation of the patch test areas by dermatologists at 48 and 72 h. The digital images were converted to erythema index (EI) images by image processing, and changes in ΔEI (the difference in the EI between the patch test site and the adjacent normal skin) values were analyzed.

Results: The ΔEI was significantly increased at 72 h relative to that at 48 h for positive nickel sulfate reactions ($P < 0.0001$), while no significant difference in the ΔEI was found for SLS reactions.

Conclusion: Using image analysis, allergic patch test reactions may be distinguished from irritant reactions by evaluating the change in the degree of erythema at 48 and 72 h.

Key words: erythema index – allergic – irritant – ICDRG – image processing

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DIFFERENT CHARACTERISTICS of allergic and irritant patch test reactions have been identified (1) but the two reactions are sometimes difficult to distinguish. Visual assessment of patch tests is done routinely to determine whether a positive reaction is allergic or irritant. Although visual assessment is sensitive enough to determine whether a reaction is positive or negative, subtle differences in the reaction cannot be quantified. Non-invasive instruments such as laser Doppler flowmetry (1–3), spectrophotometry (1), *in vivo* reflectance confocal microscopy (4, 5), ultrasound (6), and transepidermal water loss (TEWL) (7) have been used to evaluate test reactions and to differentiate between experimental allergic and irritant reactions. However, these instruments are expensive and are sometimes impractical and time-consuming to operate in the assessment of many test sites.

Since irritant and allergic reactions showed an increase in erythema and blood flow, the erythema index (EI) measured with contact type instruments such as Mexameter® (Courage +

Khazaka Electronic GmbH, Köln, Germany) and spectrophotometer (Cortex Technology, Hadsund, Denmark) (8, 9), and cutaneous blood flow (CBF) evaluated by non-contact laser Doppler flowmetry (10, 11) have been used to quantify the extent of inflammation at patch test sites. We reported that measurement of the (EI) using image processing is suitable for quantitative evaluation of erythema in patch tests, as neither special techniques nor expensive instruments are needed in this digital photograph-based method (12). Thus, our method may help non-experts to accurately evaluate patch test reactions (12).

In this study, we used our published method to examine whether the time course of experimental contact dermatitis caused by nickel sulfate is different from that of sodium lauryl sulfate (SLS).

Methods

Subjects

A total of 172 Japanese patients, including 34 men and 138 women (mean $\pm$ SD, 62.5 $\pm$

15.8) who visited the outpatient clinic of the Department of Dermatology, Toho University School of Medicine, underwent patch testing. The study was approved by the ethics committee of Toho University School of Medicine and by the institutional review board (Reference Number 21032). All patients gave their written informed consent to participate in this study.

Study design and visual grading

Patch testing was performed using Finn chambers[®] (Epitest Ltd Oy, Tuusula, Finland) mounted on Scanpor[®] tape (Alpharma AS, Oslo, Norway) and occluded for 48 h on the back of patients. The irritant used was SLS at concentrations of 0.5% and 1.0%; and the allergen was nickel sulfate at concentrations of 2.5% and 5% in petrolatum and water, respectively. Petrolatum and water were used as controls. Visual grading and digital photography of the patch test area were carried out approximately 0.5 and 24 h after removal of the patches. The results of patch tests were graded by dermatologists (H.K. and K.W.) who are experts in clinical studies on contact dermatitis. The patch test reactions were graded according to the criteria of the International Contact Dermatitis Research Group (ICDRG) (13). All patients were kept in a seated position during evaluation and image capture of the patch test sites.

Measurement of the erythema index of patch test sites using image analysis

All digital images were taken with PowerShot S9 (Canon, Tokyo, Japan). The EI was measured using ImageJ freeware; details of the method have been published elsewhere (12, 14–16). In brief, after splitting a brightness-adjusted original color image into RGB channel images, the (logR-logG) image was defined as the EI image (12, 14–17). As the images obtained using this conversion were too dark to see, the brightness intensity of the original EI images was uniformly multiplied by 4. A piece of Casmath (Dai Nippon Printing, Tokyo, Japan) was placed in each image as a standard for color and brightness to ensure that image brightness was kept constant (15). In each EI image, two square regions of interest (ROIs) of the same area were set: one region was inside the patch test site (the inside area covered by the Finn chamber),

and the other was normal skin adjacent to the test site (outside the area covered by the Finn chamber). The two ROIs were used to calculate the difference in EI (ΔEI) between the patch test site and normal skin (12). EI and ΔEI measurements for allergic and irritant reactions were compared to the visual assessment, which were graded as doubtful (+?) or positive (+ or ++) readings.

Statistical analysis

Paired *t*-test was used in the comparison of the mean values of ΔEI at 48 and 72 h, and $P < 0.05$ was considered to be significant. All statistical analyses were performed using JMP version 6.0 software (SAS Institute, Cary, NC, USA).

Results

Clinical evaluation

The results are shown in Tables 1 and 2.

Statistical analysis of the changes in ΔEI of positive test sites measured at 48 and 72 h

A large number of erythematous reactions to SLS were graded as doubtful (+?) reactions—even if diffuse erythema was present at the test site—because of the lack of substantial edema. Therefore, we included test sites that were deemed as doubtful reactions at both 48 and 72 h in the analysis. However, we excluded test sites from the analysis that were graded as negative at either 48 or 72 h.

TABLE 1. Number of doubtful (+?) and positive (+ or ++) reactions to nickel sulfate at 48 and 72 h

	72 hr	
	+?	+or++
48 hr		
+	2	3
>+?	4	18

TABLE 2. Number of doubtful (+?) and positive (+ or ++) reactions to SLS at 48 and 72 h

	72 hr	
	+?	+or++
48 hr		
+	24	10
>+?	3	1

The mean value of ΔEI of patch test sites showing either positive or doubtful reactions to nickel sulfate was significantly increased at 72 h compared to that at 48 h ($P < 0.0001$, Fig. 1). However, no significant changes were found in the case of SLS (Fig. 2).

Discussion

Irritant and allergic reactions in patch testing are usually distinguished by visual inspection (18). It may be difficult – even for experts – to visually distinguish between the two types of reactions, so palpation of the test sites and careful observation of the course of the inflammatory reaction are also performed. Attempts have been made to evaluate differences in the two reactions, including their time course, using non-invasive methods. Both laser Doppler flowmetry and spectrophotometry have been used to quantify CBF and the degree of erythema (1). Specifically, it was shown that there was significant correlation between EI and CBF as determined by laser Doppler flowmetry (1). Bjarnason et al. reported that there was an

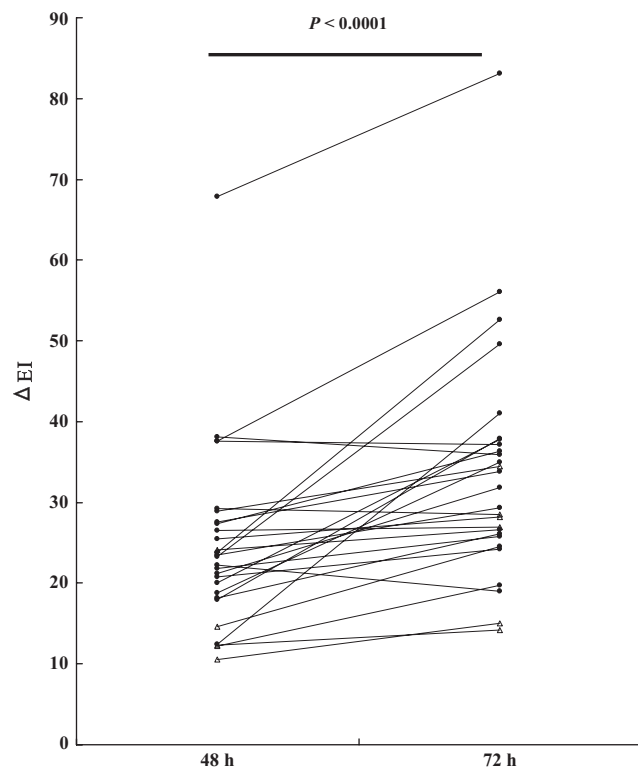


Fig. 1. Changes in ΔEI of patch test sites showing doubtful or positive reactions to nickel sulfate at 48 and 72 h. Δ : +?, $\bullet$: + or ++ P , probability.

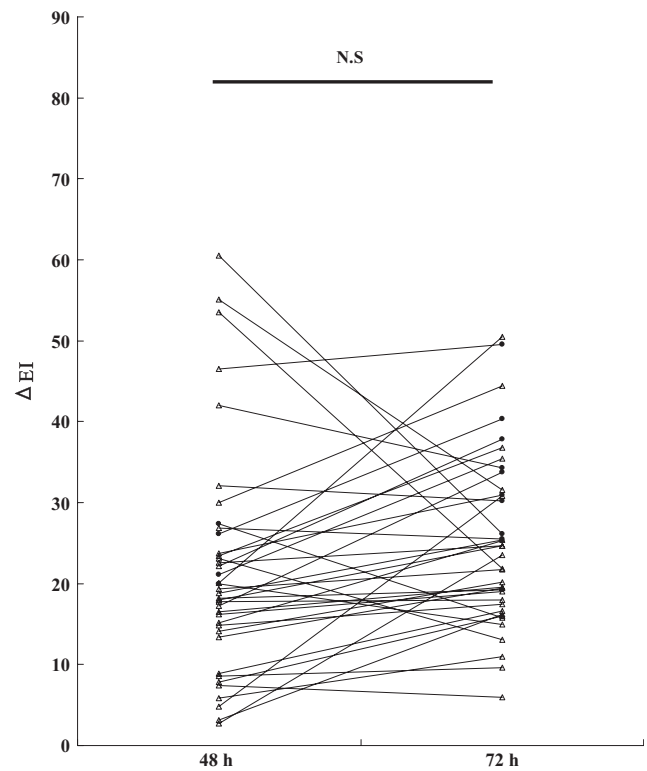


Fig. 2. Changes in ΔEI of patch test sites showing doubtful or positive reactions to SLS at 48 and 72 h. Δ : +?, $\bullet$: + or ++ N.S., not significant.

agreement between CBF measured by laser Doppler perfusion imaging (LDPI) and visual gradings in positive nickel sulfate patch tests (10). Goon et al. reported that LDPI correlated with visual scores in contact allergic reactions (2). However, utilization of laser Doppler instruments in clinical practice may be limited as these instruments are expensive and time-consuming to operate in the assessment of many test sites. EI imaging developed by our group (16) is an ImageJ-based method that requires only a digital camera and a computer, and is suitable for quantifying the degree of erythema and CBF of various skin lesions. We used this ImageJ-based method to quantify the difference between allergic contact dermatitis due to nickel sulfate and irritant contact dermatitis due to SLS.

According to ICDRG grading, the presence of papular or edematous reactions distinguishes positive reactions from doubtful reactions. Our method quantifies erythema but does not assess the presence of papular or edematous reactions. However, we previously reported that ΔEI values increase significantly in a stepwise fashion as the clinical score increases from doubtful (+?)

to positive (++) , suggesting that ΔEI values reflect the degree of inflammation at patch test sites (12). Therefore, we focused on the value of ΔEI without assessing the presence of papular or edematous reactions. This study showed that the mean value of ΔEI was significantly increased at 72 h compared with that at 48 h for allergic reactions to nickel sulfate (Fig. 1), but this was not seen in irritant reactions to SLS (Fig. 2).

Using our method, evaluation of ΔEI at both 48 and 72 h may allow clinicians to distinguish between positive irritant or allergic patch test reactions. However, this finding was based on statistical analysis, and individual patch test reactions did not always agree with this finding. Therefore, measurement of ΔEI may be used to statistically determine whether a substance induces an allergic and irritant dermatitis reaction in a large number of patch tests, rather than to assess the nature of the contact dermatitis at a single patch test site. In this study, only one irritant (SLS) and one allergen (nickel sulfate) were evaluated. As Agner and Serup reported that three different irritants (SLS, hydrochloric acid and croton oil) cause different patterns of skin damage (19), it would be necessary to examine the time course of the ΔEI changes of different patch test substances, especially if the substance is a suspected irritant.

Measurement of physiological parameters other than erythema or dermal blood flow may also be useful in differentiating irritant and allergic reactions. Serup et al. reported that TEWL measurement at 24 h after occlusion helped to distinguish between the two reactions (7). Specifically, the TEWL values of irritant reactions were significantly increased compared

with allergic reactions after 24 h of occlusion; however, measurement of TEWL values after 48 h of occlusion was unhelpful in distinguishing between allergic and irritant reactions (7). In a separate study using high-frequency ultrasound, Serup et al. reported that the edema was greater in allergic than irritant reactions (6). Benjamin et al. (5) and Astner et al. (4) reported that evaluation of epidermal changes using reflectance confocal microscopy may be useful in distinguishing irritant and allergic reactions. The abovementioned findings may relate to histopathological observations that irritant reactions show greater disruption of the stratum corneum, parakeratosis and increased epidermal thickness compared with allergic contact dermatitis. On the other hand, allergic contact dermatitis shows greater vesicle formation and intercellular edema. Therefore, we suggest that several techniques examining different skin parameters be used to confirm if the patch test reaction is allergic or irritant in nature.

In conclusion, the degree of erythema at patch test sites at both 48 and 72 h may enable differentiation between allergic and irritant contact dermatitis, such as those caused by nickel sulfate and SLS, respectively. Image analysis of patch tests may be especially useful when evaluation of patch test reactions is performed remotely through electronic transmission of digital photographs of patch test sites or by teledermatology (20).

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Address:

Hiroshi Ohshima
 POLA Laboratories, POLA Chemical
 Industries Inc
 560 Kashio-cho
 Totsuka-ku, Yokohama 244-0812, Japan
 Tel: +81 45 826 7134
 Fax: +81 45 826 7239
 e-mail: ooshima@pola.co.jp