

Objective Non-invasive Assessment of Irritant Patch-test Reactions with Laser Doppler Perfusion Imaging (LDPI)

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Background : Traditional visual reading of patch-test reactions is a rather subjective method, lacking the sensitivity and reproducibility needed in experimental studies. Recently the laser Doppler perfusion imaging (LDPI) has been used to measure objectively the increase in superficial blood flow which results in the appearance of erythema.

Objective : We designed this study to examine the relationship between the LDPI measurement and visual reading after patch test to several different irritants.

Methods : In this study, reading of erythema in experimentally-induced irritant contact dermatitis was performed visually and by laser Doppler perfusion imaging (LDPI). In addition, we investigated whether the LDPI measurement was appropriate in the routine patch test clinic.

Results : A close correlation was shown between the 2 methods ($r = 0.9046$, $p < 0.001$) and the LDPI producing mean adjusted perfusion values (APVs) was able to discriminate between the different visual grades.

Conclusion : LDPI is a valuable instrument to objectively assess intensity of irritant patch-test reaction, and is indeed one of the few methods which overcomes the inter-individual variations in visual reading, but this instrument is not appropriate to use routinely in patch test clinic because of unacceptably long measurement time.

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Key Words : Erythema, Irritant contact dermatitis, Laser doppler perfusion imaging

Patch testing has been widely used by dermatologists to identify the cause of eczema aggravated by contact sensitizers or irritants. In routine clinical practice, patch-test reactions are commonly evaluated by visual rating scale based on the degree of erythema, edema and the presence or absence of vesicles. Although this grading system is useful as an indicator of the clinical significance of any given re-

action, it is a rather subjective method, lacking the sensitivity and reproducibility needed in experimental studies^{1,2}. For these reasons, quantitative readings of patch-test reactions would be preferable.

Previously, a number of instrumental methods, such as skin fold thickness measurement³, high-frequency pulsed ultrasound⁴, transcutaneous oxygen tension (tc-PO₂)⁵, skin temperature⁶, erythema index⁷, etc., have been used to quantify patch-test reactions in an attempt to introduce objectivity and reproducibility. These techniques produce data on a continuous scale which is appropriate for dose-response analysis. However, most of the above quantitative methods do not discriminate between the visual grades of patch-test reactions³⁻⁷.

As vasodilatation with increased blood flow is

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Table 1. The grading system accepted by the north american contact dermatitis research group

Visual grade	Assigned value	Morphology
0	0	negative reaction
+/-	1	doubtful reaction, faint erythema only
1+	2	weak (nonvascular) positive reaction, erythema, infiltration, possibly papules
2+	3	strong (vesicular) positive reaction, erythema, infiltration, papules, vesicles
3+	4	extreme positive reaction, bullous reaction

Table 2. The mean APVs and standard deviations (S.D.) of LDPI readings in relation to visual grades

Visual grade	No. of reactions	Adjusted perfusion values (Mean value \pm S.D.)	Student <i>t</i> -test
0	67	0.06 \pm 0.02	between 0 and +/-, $t=1.0$: $p<0.001$
+/-	19	0.30 \pm 0.07	between +/- and 1+, $t=2.1$: $p<0.001$
1+	10	0.62 \pm 0.16	between 1+ and 2+,
2+	4	1.14 \pm 0.32	too few reactions within grade 2+
3+	0	-	

Table 3. The mean perfusion values and standard deviations (S.D.) of control patches and visual grade 0

	Mean perfusion value \pm S.D.	Student <i>t</i> -test
Controls	1.07 \pm 0.41	between controls and grade 0,
Visual grade 0	1.18 \pm 0.35	$t=5.0$: $p > 0.1$

an essential part of the inflammatory response, cutaneous blood flow measurements might provide a useful technique for the quantification of patch-test reactions⁸. This is made possible with laser-Doppler technology based on the Doppler phenomenon⁹. The laser Doppler perfusion imager® PIM 1.0 (LDPI, Lisca Development AB, Linköping, Sweden), an instrument for laser Doppler perfusion scanning, used in this study supports this technique (Fig. 1A & 1B). It is a data acquisition and analysis system that generates images of tissue perfusion. The scanner, located in the laser head (Fig. 1B), guides a low-power helium-neon laser beam from one skin measurement site to another. At each site, light including a part of back-scattered laser light, is detected by a photo-detector in the laser head. Further processing by a computer system generates arbitrary values (0.00–10.00 volts) for each measurement site in proportion to perfusion. After each reading, a color-coded picture made of scanned measurement sites is displayed on a monitor where each color indicates a different perfusion interval. The principle used by this scanning tech-

nology is the same as that used for the laser Doppler flowmetry (LDF)¹⁰. The main advantage of the LDPI over LDF for measurement of patch-test reactions is its scanning property¹¹. It makes possible quick assessment of superficial blood perfusion and eliminates the unavoidable skin touch of the conventional flowmeter that may interfere with reading results. The technique has been used to measure blood perfusion in patch-test reactions^{11–13} and in other applications^{14,15}.

In this study, we compared the results of the LDPI measurements with those of visual readings, after patch test to several different irritants. In addition, we investigated the suitability of the LDPI measurement in the routine patch test clinic.

MATERIALS AND METHODS

Subjects

20 healthy, non-atopic volunteers, with no past or present history of skin disease, participated in the study during the month of March. Their ages ranged from 23–38 years with a mean age of 28

Fig. 1. (A) The laser Doppler perfusion imager®PIM 1.0; (B) The laser head.

Fig. 2. The IQ Chamber®.

years.

Test substances

5 different irritants offered from the 'P. Corp.' were tested. As a control, 1 empty patch was also included.

Patch testing

Patch tests were performed using IQ Chamber® (Fig. 2, Chemotechnique Diagnostics, Malmö, Sweden). The material of the IQ Chamber® is inert additive free polyethylene plastic and the opening of

Fig. 3. The black sheet with a squared opening of 9×9 mm (c, scanning area; d, patch test area).

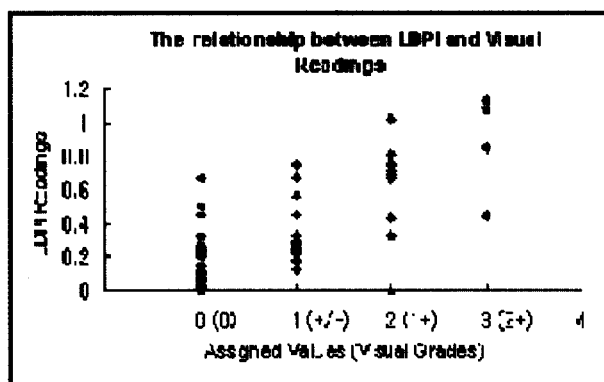


Fig. 4. The correlation between visual grades and LDPI readings.

the chamber is square to make it easier to measure the erythema with the LDPI. The volume of the chamber is $65 \mu\text{l}$ and the inside area of the chamber is 9×9 mm (81 mm^2).

6 patches (5 irritants and 1 control) were applied to the volar area of the forearm. After 48 h, the patches were removed. Measurements were taken about 30 min after removal. Patch-test reactions were first read visually and then assessed with the LDPI.

Visual readings

Visual readings were made by 2 dermatologists independently of each other, prior to LDPI measurements, according to the grading system accepted by the North American Contact Dermatitis Research Group¹⁶ (Table 1).

LDPI measurements***Environment & patient position :***

LDPI readings were performed with the volunteers, in the sitting position, after a rest of not less than 5 min. They were asked to breathe easily and not to talk or move, as these could affect the readings. The only light allowed during readings was that from the computer monitor which was set to a minimum. Room temperature was kept between 22 ~28 °C.

LDPI reading parameters :

Perfusion was read and analyzed with an LDPI PIM 1.0 using LDI 2.5 software (Lisca Development AB, Linköping, Sweden). Readings were performed with the LDPI using a distance of 15 cm between the laser head aperture and the reading area, a background threshold of 6.1V(volts), low resolution, and an angle as close to 90° as possible between the LDPI laser beam and the reading area. The perfusion scale ranged from 0.00~10.00 V and the amplification factor was 1. The laser head was adjusted parallel to the reading area. The size format was set to read 10×10 measurement sites for each reaction. This size format was able to scan 12×12mm area. To simulate 9×9mm sized inside area of the chamber, a black sheet with a square opening of 9×9mm was used (Fig. 3).

Assessment of perfusion :

The mean perfusion value of each patch test area could be calculated and that of control patch was subtracted from each irritant patch. Perfusion obtained this way is termed "adjusted perfusion value (APV)", indicated in V (volts).

Statistics

To investigate the relationship between the visual grading system and LDPI method, the Spearman rank correlation coefficient was calculated. In addition, the Student t-test was also performed to study whether the APVs were able to discriminate between the different visual grades.

Assessment of the ease of use of the LDPI

The views of 2 independent investigators concerning practical aspects of the use of the LDPI were recorded. A comparison was made between the time taken for visual readings at 6 patch test sites with that required for LDPI measurements.

RESULTS***Visual and LDPI readings***

The irritants applied produced a variety of patch-test reactions. The visual grades ranged from 0 ~ 2+ and APV profiles ranged from 0.00~1.57 V (Table 2). No detectable erythema was found in the control patches by the naked eye.

The relationship between LDPI and visual reading

APVs in relation to visual grades are shown in Fig. 4. The Spearman rank correlation coefficient r was 0.9046 ($p < 0.001$), indicating that there was good correlation between the two methods. Also included in Table 2 are the results of the Student t -tests which show the significant differences between the APVs in each visual grade group.

The mean perfusion values of control patches and visual grade 0

There was no difference in the mean perfusion values between control patches and visual grade 0. It means that patch test with no visible reaction had blood flow values similar to those of control patches.

Ease of use of the LDPI

The general opinion of the investigators was that the LDPI was not easy to handle. The minimum time required to measure the patch-test reactions at 6 patch test sites was 15 min, compared with an average of 1 min for visual assessment.

DISCUSSION

There have been many trials to quantify the visual reading score of the patch-test reaction, especially in the weak reactions. As previously mentioned, many instrumental methods have been used for that purpose, but most of them were proved inappropriate.

To detect adjusted perfusion value (APV) using LDPI, the mean perfusion value of a control patch should be subtracted from those of the irritant patches on the same strip applied equally long and read at the same session. A control patch can be expected to give a good reference value, but is affected by test- and non-test-related factors, such as irritation, spatial heterogeneity of skin blood perfu-

sion¹⁷ and pathological skin conditions¹⁸. Such factors can affect assessment of perfusion, but this occurred rarely¹¹. This subtraction of the mean perfusion value of a control patch is a very important process to evaluate the true increment of blood flow beyond the basal level induced by patch test.

In our experiments, patch test sites with no visible reaction gave similar perfusion values as those of control patches. These findings suggest that a reliance on visual assessment is unlikely to lead to 'false negative' results. In addition, there was a 5-fold increase in mean APV between visual grade 0 and +/-, with an approximately doubling of mean APV between +/- and 1+, and between 1+ and 2+. That means there were very good correlations between LDPI measurements and visual readings, and the LDPI producing mean APVs were able to discriminate between the different visual grades. These good correlations and discrimination of LDPI measurements with visual readings are interesting results compared with the other instrumental methods³⁻⁷.

There is no one instrumental method to read the patch-test reaction instead of visual reading till now. Every instrumental method has its own merits and drawbacks at the same time. In our experiments, we found that LDPI is a valuable instrument to objectively assess intensity of irritant patch-test reactions, that there is a good correlation between LDPI measurements and visual readings, and that it is indeed one of the few methods which overcomes the inter-individual variations in visual reading. But it took an unacceptably long time to read patch-test reaction in comparison with visual reading. Although we did not compare the results of LDPI with other instrumental methods, we believe that LDPI is one of the best instrument to objectively measure the intensity of irritant patch-test reactions, but that LDPI is not appropriate to use routinely in the patch test clinic because of its long measurement time.

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