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Objective Prick Test Evaluation: Non-invasive Techniques

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Prick test reactions are evaluated and quantified, comparing visual assessment with two non-invasive techniques: remittance spectroscopy and pulsed ultrasound for erythema and skin thickness measurements. Different information is provided by the two methods. Remittance spectroscopy discriminates well between negative and positive reactions (+ or ++), while failing to differentiate stronger reactions, where edema is a prominent feature. The latter reactions are better evaluated by skin thickness measurements, which, on the contrary, are less sensitive in revealing small skin thickness increases in weak reactions. ***Key words: Ultrasound; Remittance spectroscopy.***

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Prick test responses, characterized by erythema and edema, are evaluated on a visual basis, ranking the reactions from negative to positive +++. Problems may arise in discriminating between negative and positive + reactions, or when a homogeneous evaluation is required in collaborative clinical trials. Non-invasive bioengineering techniques provide objective tools to study skin function. Data based on interval scales can be monitored, evaluated, compared and analyzed statistically, utilizing parametric techniques not possible with the visual scoring.

The present study attempts to quantify both erythema and edema in prick test reactions to various antigens. Two techniques were used: remittance spectroscopy for erythema assessment and pulsed ultrasound for skin thickness measurements.

MATERIALS AND METHODS

Fifteen patients of both sexes (age range 35 ± 5) entered the study. They were reactive to allergens such as house dust mites, pollens and foods. A total of 42 positive prick tests were measured. Prick tests were performed at a concentration of 10,000 PNU/ml for pollens, 5,000 PNU/ml for dust mites. Histamine was used for control at a concentration of 10 mg/ml (Bayropharm Italy, Milan). Skin prick tests were performed on the upper back, and values were recorded 25 min after the beginning of the procedure. The same skin reactions were evaluated visually and by means of pulsed ultrasound for skin thickness and remittance spectroscopy for erythema. Visual evaluation was based on the following scoring system: − = negative reaction, + = erythema and wheal 3–5 mm diameter, ++ erythema and wheal 5–7 mm diameter, +++ erythema and wheal >7 mm diameter.

Skin thickness was measured by pulsed ultrasound (Dermascan A, Cortex Technology, Denmark) with a focused transducer of 20 MHz. Ultrasound is a form of mechanical energy produced by forcing a transducer to vibrate at a high frequency. The vibration produced is coupled into the skin and the change in the acoustic properties at the interfaces of the skin layers results in a train of pulses returning to the transducer and being displayed on an oscilloscope (1). The probe is very small and can be easily positioned in the center of the wheal. Differences between the adjacent healthy skin and the positive test site are calculated.

Erythema was evaluated with remittance spectroscopy. This optical technique is based on the reflectance of incident light radiation backscattered from the skin. Water, bilirubin, melanin, oxy- and reduced hemoglobin, carotenoids and epidermal aromatic amino acids have important effects on the diffuse reflectance spectra of human skin (2). Measurements were performed with a UV/VIS spectrophotometer with a fiberoptic probe for in vivo recordings (Perkin-Elmer, UV/VIS spectrophotometer, Lambda 5, Perkin-Elmer, Germany). The probe, connected to the device via a system of optical fibers, was applied to the skin with a specially designed frame to standardize the pressure on the skin surface. The skin area measured was 8 mm in diameter. The skin absorbance was measured at 510, 543, 560, 576 and 610 nm of wavelength and the erythema index calculated as described by Feather et al (3). The erythema index was evaluated on normal skin (basal), on negative prick test reactions and on positive reactions (+, ++/+++).

Statistical analysis of the data was performed using one-way analysis of variance and Fisher's PLSD test.

RESULTS

The distribution of reactions is shown in Figs. 1–2.

Skin thickness: Mean skin thickness was 2.3 mm in +, 3.0 mm in ++, 3.6 mm in +++, 2.4 mm in negative reactions and 1.9 mm in basal skin. Mean increase in skin thickness in + reactions was 0.11 mm, compared to 0.50 mm in ++ reactions and 1.28 mm in +++ reactions. Analysis of variance is significant (<0.01). Comparison between subgroups showed significant differences (<0.05) in skin thickness between basal skin, negative, + reactions and +++ reactions. The mean increase in skin thickness compared to baseline resulted in a more sensitive parameter with significant differences between negative, +, ++, versus +++ reactions. The mean increase in skin

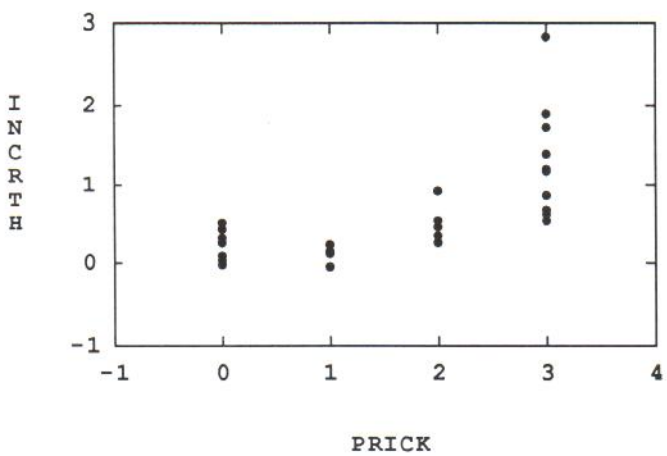

Fig. 1. Increase in skin thickness (mm) in relation to visual scoring of prick test reactions (0 = negative, 1 = +, = ++, 3 = +++).

Table I. *Evaluation of prick test reactions with pulsed ultrasound. Skin thickness and increase in skin thickness in relation to baseline compared to visual score (values in mm). *° Significant differences between groups (multiple comparison, <0.05, see text)*

Vis.score	Skin Thickness		Increase in Thickness	
	Mean	±SD	Mean	±SD
+	2.30*	0.64	0.11°	0.11
++	3.01	0.48	0.5°	0.25
+++*°	3.66	0.69	1.28	0.70
Negative	2.47*	0.69	0.26°	0.26
Basal	1.93*	1.17	0°	0

thickness was not significant between + and ++ reactions (Table I, Fig. 1).

Remittance spectroscopy: Basal skin gave mean erythema index scores of 36.6 compared to 47.7 in negative reactions and 71.3 of + reactions. ++ and +++ reactions gave 56.8 and 60.2 respectively (Fig. 2). Analysis of variance revealed a significant value (<0.01) among the groups. Fisher test showed significant values in the comparison between basal, negative, ++ and + reactions, (<0.05) (Table II). Basal and negative reactions were not significantly different. Discrimination between strong reactions (++ vs +++) failed.

DISCUSSION

Skin reactions, such as patch tests, have been investigated by objective and non-invasive techniques (4–9). Serup et al. (7), using a specially designed high-frequency pulsed ultrasound technique, reported differentiation of positive patch test reactions on the basis of skin thickness: doubtful reactions <0.2 mm., + reactions 0.3–0.7 mm., ++ reactions >0.8 mm.; ++ and +++ reactions could be differentiated by the absolute increase in skin thickness, but less significantly. Mendelow et al. (6), using remittance spectroscopy, reported a good differentiation among different patch test responses. The technique was sensitive and allowed, among reactions classified with the same score, of the differentiation of allergen formulation. Using laser Doppler velocimetry, a different optical technique to measure blood flow, Staberg et al. (4) found a fivefold increase of blood flow in sites scored "doubtful" and a tenfold increase in "positive" reactions. Laser Doppler velocimetry has been demonstrated to be more helpful in evaluating doubtful reaction rather than quantifying strong reactions (4, 10, 11). Serup in 1984 (12) monitored for the first time with a 15MHz probe the formation and expansion of cutaneous edema, reporting the usefulness of skin thickness measurements via ultrasound in the assessment of small and medium-sized prick tests. Later, Serup & Staberg (13) quantified prick test reactions using laser Doppler flowmetry, reporting that edema formation strongly influences the flow (and erythema) in the center of the wheal.

Measurements of skin thickness with pulsed ultrasound discriminate between basal, negative, + and +++ reactions but

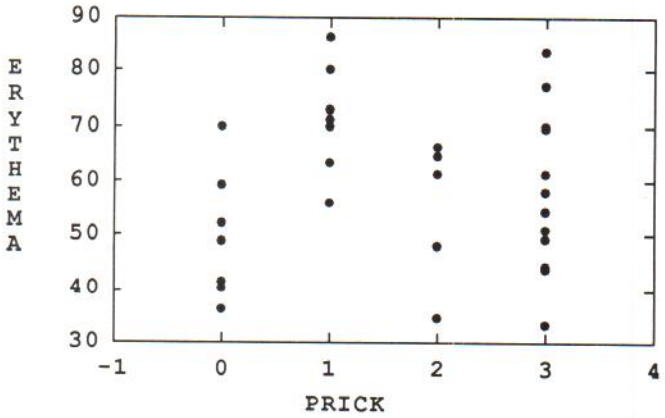

Fig. 2. Erythema index in relation to visual scoring in prick test reactions (0 = negative, 1 = +, 2 = ++, 3 = +++).

cannot detect skin thickness changes between ++ and +++ reactions. Apparently, when both the wheal and the edema are greater, skin thickness reveals significant changes. With smaller skin thickness increases, data are biased by the reproducibility of the technique (less accurate on the back, where the skin is thicker) (7). Indeed, the analysis of the increase in skin thickness compared to the basal measurement (Table I, Fig. 1) reveals significant changes between ++ and +++ reactions (Fisher PLSD, <0.05). Our findings are partially in contrast with those reported by Serup (12) that showed the usefulness of ultrasound in quantifying small and medium wheals. This is consistent with the different site investigated: the volar forearm has a thinner and a more uniform subcutaneous fat distribution, which explains the decreased variability of the recordings.

Remittance spectroscopy has been used in dermatology to assess topical corticoid-induced blanching (3, 14) and to monitor PUVA therapy for psoriasis (15). This technique is sensitive and allows of reliable estimation of the hemoglobin content of the skin and thus of skin erythema.

Remittance spectroscopy provides important information on the non-invasive assessment of prick test reactions. Discrimination between negative and positive (+,++/+++) reactions is significant. The technique is of questionable value in assessing stronger reactions (++/+++); two possible mechanisms may be involved: 1) stronger reactions (+++) have noticeable edema with reduction of blood flow and hemoglobin content with skin whitening; 2) erythema is maximum in

Table II. *Evaluation of prick test reactions with remittance spectroscopy. Erythema index scores compared to visual assessment. Analysis of variance between groups is significant (<0.01). *° Significant differences between groups (multiple comparison, <0.05, see text).*

Visual score	Mean	±SD
+*	71.3	9.9
++°	56.8	12.8
+++	60.2	13.3
Negative	47.7*	12.4
Basal	36.6*°	11.4

++ reactions and does not increase further at +++ level. This technique is reliable in differentiating negative from + and ++ reactions. Our data confirms what has previously been reported by Serup & Staber using a different technique (13). Nevertheless, the big (8 mm) aperture of the probe can negatively influence the results, and a smaller probe is recommended. Indeed the aperture, in small reactions, covers both the wheal and the flare and the measurement is biased by the size of the wheal. Negative skin sites show a slightly higher, but not significant, erythema index compared to basal skin, due to a dermographic reaction related to the procedure.

These two techniques are helpful tools in monitoring prick test reactions. Their combined use should allow the investigator to monitor two important parameters characterizing prick test reactions. The main drawbacks of remittance spectroscopy are the difficult positioning of the probe during measurements and the time required for one measurement (~ 3 min). Moreover, measurements are highly reproducible and sensitive to small erythematous changes. Ultrasound is faster but detects macroscopic changes, usually more easily detected with the naked eye which, after all, remains a useful tool for the clinician. The bioengineering techniques may be of value for specialized investigations, benefitting from the objective data and parametric statistics.

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