

Short communication

The atopy patch test – reproducibility and comparison of different evaluation methods

Background: There is still a lack of standardization of the atopy patch test (APT) in test procedures and evaluation methods. Our aim was to examine the reproducibility of APT results and to compare visual evaluation to chromametry and laser Doppler imaging.

Methods: Fifty-two volunteers with atopic eczema/dermatitis syndrome (AEDS) were included. The APT was performed on tape-stripped and unstripped test fields on their backs using cat dander, house dust mite and grass pollen allergens from two different suppliers. Responders were re-tested 4–12 weeks later with the same allergens on their forearms.

Results: Using Allergopharma allergens, 14 (26.9%) volunteers showed one or more positive reactions. The reproducibility rate was 56.3%. The Erlangen atopy score in APT-positive and negative volunteers was 19 ± 6 vs 15 ± 6 . The test agreement in volunteers tested with both allergens, from Allergopharma and Stallergènes, was poor. Correlation of the results between the three evaluation methods was significant ($P \leq 0.001$).

Conclusions: The low reproducibility rate of APT results and the poor inter-test-agreement using allergens from different suppliers show that much work remains to make the APT a reliable tool in identifying relevant aeroallergens that lead to flare ups of AEDS. Compared to chromametry and laser Doppler imaging, visual scoring was superior in differentiation between irritative and allergic reactions.

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The role of allergic reactions in eliciting and maintaining “atopic eczema” is still controversial. In a subgroup of patients with atopic eczema/dermatitis syndrome (AEDS) (1), exacerbations of eczematous skin lesions have been described after contact with aeroallergens (e.g. house dust mite, pollen or animal dander) (2). It has been shown repeatedly that in certain patients eczematous skin lesions can be induced by epicutaneous application of aeroallergens (3–5). This procedure was named the atopy patch test (APT) (6, 7).

Positive reactions to the APT were rarely observed in patients without positive skin prick test or specific IgE antibodies to aeroallergens, although both were not absolute prerequisites (8). A history of worsening of eczematous lesions after contact to aeroallergens was described as an important predictive factor for APT positivity (9). No information is available concerning the correlation between the atopy score by Diepgen and co-workers (Erlangen atopy score) (10) as a measure of skin atopy diathesis and the APT reactivity.

Concerning the reproducibility of the APT, data are limited, but some authors observed that repeated AP testing led to satisfyingly concurrent results (11, 12).

A major problem, which is still an obstacle to

comparability of atopy patch test results from different study centres, is the lack of standardization in methodological procedure, as well as individual evaluation of the APT reactions (5). To study the usefulness of bioengineering methods for evaluation of APT results, chromametry and laser perfusion imaging were performed and compared to conventional clinical APT assessment. Recently, the use of laser Doppler perfusion imaging has been described for quantification of conventional epicutaneous patch test reactions (13).

Material and methods

Study design

The study was designed as a controlled, single-blinded study.

Study population

After approval by the local ethical committee and after obtaining informed, written consent, 52 volunteers (37 women and 15 men; mean age 25.4; range 18–43 years) with a history of AEDS and more than 10 points in the Erlangen atopy score, were included in the study. All were screened for a clear-cut positive APT reaction with aeroallergens provided by Allergopharma (Reinbek, Germany), and after 4–12 weeks responders ($n = 14$, 12 women, 2 men; median age

23.7; range 19–36 years) were retested with the same allergens. In 34 volunteers (27 women, 7 men; median age 23.5; range 18–43) additional AP tests with aeroallergens of a second supplier (Stallergènes, Rheinberg, Germany), were performed at the same time.

Allergens

Subjects ($n=52$) were tested with cat dander, house dust mite, and grass pollen allergens which were provided by Allergopharma. The concentration of the allergens was 10 000 protein nitrogen units (PNU) of lyophilisate/g petrolatum + 10% isopropylmyristate. Thirty-four of these volunteers underwent additional AP testing with allergens from a second supplier (Stallergènes, Rheinberg, Germany). The latter allergens were provided ready to use in 5 ml syringes, dissolved in petrolatum. The allergenic potency was communicated using an index of reactivity (IR) and was 200 IR/ml for all allergens.

Test procedure

At the first visit, blood was taken from the 52 volunteers and serum levels of total IgE and aeroallergen-specific IgE were determined. The Erlangen atopy score was determined and a skin prick test with three relevant aeroallergens (cat dander, house dust mite, grass pollen; prick test solutions provided by Scherax-Arzneimittel; Hamburg, Germany) was performed in each subject.

At the second visit, APT were performed on unstripped and tape-stripped skin of the paravertebral midback using the same aeroallergens as for the prick test. Tape-stripping of the upper stratum corneum was performed by 20-fold application and removal of conventional adhesive tape (Tesa packaging film; Beiersdorf, Germany).

Allergopharma allergen solutions (0.05 ml/test field) were applied under occlusive conditions for 48 h by using large Finn chambers (12 mm diameter) on Scanpore tape (Hermal; Reinbek, Germany). Visual scoring, chromametry and laser Doppler perfusion imaging were performed before, 48 h and 72 h after application. Thirty-four volunteers were tested simultaneously and in the same way, using identical allergens purchased from Stallergènes.

In cases of at least one positive atopy patch test reaction to one of the Allergopharma allergens, volunteers were invited for a second APT 4–12 weeks later. On this occasion, allergens which had elicited positive reactions during the first APT were re-tested exclusively. The second APT was performed on both tape-stripped and unstripped test fields on the volunteers' forearms.

APT allergens were applied to the fields according to a randomization scheme.

Clinical assessments and bioengineering measurements

Prior to visual scoring and bioengineering measurements, subjects acclimatized for a minimum of 15 min to standardized laboratory conditions (temperature: 20–22°C, relative humidity 30–50%). Visual grading of positive APT reactions (+) to (+ + +) was similar to the criteria used in conventional contact allergy patch testing (International Contact Dermatitis Research Group recommendations) (14). Quantification of erythema was performed using a Minolta Chromameter (CR-200; Minolta, Osaka, Japan) according to published recommendations (15) (16). For the assessment of skin blood perfusion, the Moor LDI laser Doppler imager (LDPI) (Lawrenz, Sulzbach, Germany) was used. The perfusion rate is given in arbitrary perfusion units (17).

Statistical analysis

Data were analyzed by using the statistical software program SPSS 10.0 for Windows. For comparison between the Erlangen atopy

score of APT-negative vs APT-positive volunteers, the nonparametric Mann–Whitney *U*-test for unpaired data was used. The same procedure was used for comparison of the total IgE values. For correlation analysis of positive APTs and specific IgE levels, or positive APs and previous history of eczema flares, the likelihood quotient was calculated. For correlation analysis of the visual score with chromametry delta values, or LDPI perfusion units, Spearman's correlation coefficient was used. For comparison of the APT results with allergens from Allergopharma or Stallergènes, the κ -test was performed according to Douglas and Altman (18).

Results

Distribution pattern of positive reactions during the first APT

Fourteen (26.9%) of the 52 screened subjects showed one or more clear-cut positive reactions to at least one of the Allergopharma allergens. Four (7.7%) were positive to cat dander, nine (19.2%) to house dust mite and seven (13.4%) to grass pollen allergens.

History, atopy score, skin prick test, total and specific serum IgE levels

Ten volunteers (19.2%) reported a history of eczema flares after contact to cats, and nine (17.5%) were aware of exacerbations after exposure to house dust. Association between eczema flares and grass pollen exposition were reported by 11 (20.1%). The cross-table analysis showed a significant association of a prospectively obtained history of eczema exacerbations with positive APT results ($P \leq 0.01$). The sensitivity of a positive history for a positive APT result was 0.52. The specificity was 0.86 ($n=156$).

There was a significant difference ($P \leq 0.05$) between the atopy score of those volunteers who were APT-positive (mean score $\pm$ standard deviation = 19 ± 6) compared to the APT-negative volunteers (15 ± 6).

Of the 52 subjects 24 (46%) had a positive skin prick test to cat dander allergen, 23 (44%) to house dust mite allergen and 36 (69%) to grass pollen allergen. Only one of the APT-positive volunteers had a negative skin prick test to the APT reaction eliciting allergens (house dust mite and grass pollen allergen).

APT-positive volunteers ($n=14$) had a higher total IgE level (median 1210 IU/ml, mean 1349 IU/ml) compared to APT-negative volunteers (median 251 IU/ml, mean 644 IU/ml) ($P \leq 0.01$). Specific IgE to cat dander, house dust mite and grass pollen was assessed using the RAST (radioallergosorbent test) and values were recorded as CAP (capacity) classes ranging from 1–6. The sensitivity of CAP class above 1 for a positive APT was 0.86 and the specificity was 0.52.

Correlation of APT results between unstripped and tape-stripped test sites

All APTs were performed on both unstripped and tape-stripped test sites. On unstripped and tape-stripped areas, 10 and 17 reactions, respectively, were assessed as

Table 1. Cross-tabulation of APT results obtained from 34 volunteers tested against cat dander, house dust mite, and grass pollen (102 reactions) both with test solutions from Allergopharma as well as from Stallergènes

	Stallergènes allergens	
	Positive	Negative
Allergopharma allergens		
Positive	6	7
Negative	7	82

positive (Allergopharma allergens). Concurrent positive results between unstripped and tape-stripped test sites were obtained in only five out of 22 cases.

All test fields were assessed at 48 h and 72 h. On unstripped test fields, four crescendo reactions and four decrescendo reactions were assessed and two reactions remained unchanged. On tape-stripped test fields, one crescendo reaction and six decrescendo reactions were observed, whereas 10 reactions did not change.

In 10 volunteers, a slight erythema (visual score 1) was observed on the tape-stripped control areas without allergens. Two reactions reached a visual score of 2 and were thus classified as clear-cut positive.

Comparison between the APT results obtained by allergens from two different suppliers

Out of 34 volunteers that were tested with both Allergopharma and Stallergènes allergens simultaneously, a total of nine (26.5%) showed at least one positive reaction to the Allergopharma products, whereas 10 (29.4%) were positive when using Stallergènes allergens.

The APT results for both Allergopharma allergens and Stallergènes allergens were compared for each volunteer. A total of 13 volunteers out of 34 showed at least one positive APT reaction either to Allergopharma allergens and/or to Stallergènes allergens. Comparison of results for each specific allergen shows a poor test agreement (Table 1). Statistical analysis was performed by calculation of the κ -value (0.18) (18).

Reproducibility of results with Allergopharma allergens

In 13 volunteers with at least one positive APT obtained by using Allergopharma allergens (16 positive APT reactions altogether), the APT was repeated after 4–12 weeks by applying an APT on their forearms. Again, APT was performed on both unstripped and tape-stripped areas. Altogether, nine of 16 APTs were positive again, which corresponds to a reproducibility rate of 56.3%. On unstripped fields, only seven positive reactions were reproducible (43.8%) in repeated APTs. Interestingly, this time no positive reactions were seen on unstripped sites, although they were negative on tapes-tripped sites.

Quantification of APT reactions: chromametry and LDPI-measurements vs visual score

The boxplot shows a linear correlation between chromametric a^* values and the visual scores (Fig. 1). Correlation was confirmed statistically ($P < 0.0001$). Analysis of the LDPI data revealed correlation ($P < 0.0001$) between relative perfusion units and the visual score (boxplots shown in Fig. 2).

Discussion

Our results confirm that epicutaneously applied aeroallergens may elicit eczematous lesions in individuals with AEDS (6). Compared to the results of a recent multicenter study (4), the rate of responders to cat dander, house dust mite or grass pollen allergen in our study was significantly lower. The explanation for this difference is not evident, since we used identical allergens in the same concentrations and vehicles. The relations between the rates of responders to the different allergens were similar. Confirming results reported in the literature (4, 8), we found a significant correlation both between skin prick test and a positive APT reaction, as well as between specific IgE antibody level and positive APT reaction. A positive history of eczema flares after allergen contact turned out to be highly specific (specificity 0.86) for a positive APT reaction to the same allergen. However, information about the relevance of APT results from challenge tests is more reliable since it is not influenced by individual differences in the perception of allergic symptoms. In spite of the significant difference of a mean atopy score of 19 in APT-positive volunteers vs a mean score of 15 in APT-negative volunteers, no threshold value could be calculated, which was of interest for APT reactivity. The hypothesis that a volunteer with a higher atopy score is more likely to react positively to epicutaneous application of aeroallergens than a volunteer with a lower score was confirmed with statistical significance ($P < 0.05$). This may be explained by compromised epidermal barrier function in more severe patients. The APT was performed simultaneously both on unstripped and tape-stripped sites to study the impact of tape-stripping on the number of positive results. This effect was reported in the literature (19) and was confirmed by our study. Tape-stripping leads to enhanced penetration of allergens by removing the upper layers of the stratum corneum. One disadvantage of this method is augmentation of the number of false-positive nonspecific irritant reactions (20). However, we did not expect to observe positive reactions on unstripped fields, while obtaining negative results on corresponding tape-stripped fields. This was the case in five of a total of 22 positive reactions. These findings strongly suggest that we had a considerably higher number of false-positive APT results, due to irritant reactions, implying

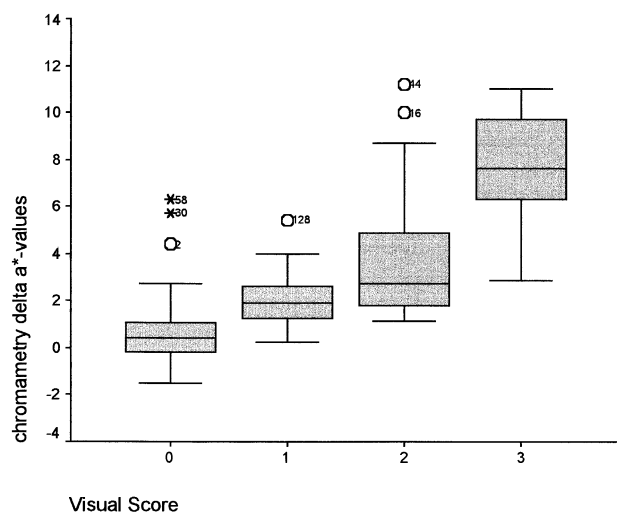


Figure 1. The boxplots show the chromametry delta values from 84 APT test fields (three different allergens on both tape-stripped and unstripped test fields) in the 14 volunteers who had at least one positive reaction on Allergopharma allergens. The chromametry delta values were compared with their corresponding visual scores. Correlation was significant ($P < 0.001$).

poor reliability of the APT. An indicator that some reactions might have been of irritant nature is the high proportion of decrescendo reactions from 48 h to 72 h on both unstripped and tape-stripped test fields. Darsow et al. reported less irritant reactions when using smaller concentrations of allergens (7000 (PNU)/g for house dust mite, 7000 PNU/g for cat dander and 5000 PNU/g for grass pollen allergens) (4). Interestingly, when the APTs were repeated on forearms, the results on tape-stripped and unstripped fields showed the expected pattern (two more positive results on tape-stripped fields, no positive results on unstripped fields, when tape-stripped fields were negative). The reproducibility rate for the APT was 56.25%, which is less than described by other authors (11, 19). In part, our lower reproducibility rate might be explained by the fact that the first and second APTs were performed on different body regions and at different times (back then forearm). Magnusson and colleagues reported stronger reactions on the upper back compared to forearms in conventional patch testing (21). However, Langeveld-Wildschut et al. patch-tested on test fields on the back and the antecubital fossa in 10 patients with AEDS, but failed to find different responses between the two test sites (19).

To our knowledge, in this study the association between APT results obtained by testing the same allergens provided by different suppliers has been investigated for the first time. For comparing results between different studies/investigators, that might use different allergen preparations, good inter-test agreement for particular allergens (e.g. cat dander, house dust mite or grass pollen allergen) would be desirable.

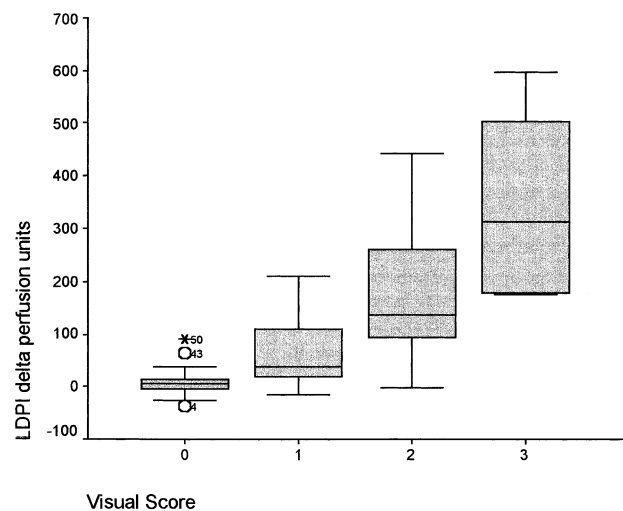


Figure 2 The boxplots show the laser Doppler flowmetry delta perfusion values from 84 APT test fields (three different allergens on both tape-stripped and unstripped test fields) in the 14 volunteers who had at least one positive reaction on Allergopharma allergens. The Doppler flowmetry delta-perfusion values were compared with their corresponding visual scores. Correlation was significant ($P < 0.001$).

Nevertheless, statistical analysis of our data showed a rather poor agreement in results. This might be because of different allergen isolation procedures, resulting in a variability of the allergenic components in the allergen solutions (22, 23). Furthermore, allergen concentration lacks unique standardization: Depending on the company, allergen concentration is often given in protein nitrogen units, PNU (Allergopharma), or in major allergen units. Stallergènes used an index of reactivity to provide information about the allergenic potency of their extracts. Since the index of reactivity is an internal standard that refers to a reference allergen extract, comparison with allergen products provided by other suppliers is not possible. Considering these facts and the observed poor reproducibility of test results, even when the same product was used, a low degree of inter-test result agreement is not surprising.

Visual scoring systems have not yet been replaced in evaluation of epicutaneous test reactions. The most promising noninvasive bioengineering techniques for obtaining objective and reliable assessments of allergic reactions are probably chromametry and, particularly, laser Doppler perfusion imaging (24). In our study, both visual scores and a^* values obtained by chromametry, as well as visual scores and LDPI values, showed a significant positive correlation ($P \leq 0.001$). It must be considered that Spearman's correlation coefficient does not give information about the diagnostic efficiency of the measuring procedures. For this purpose it would be necessary to divide the perfusion units or the chromametry values into categories, similar to those of the visual score. However, this seemed unreasonable, as some of the reactions which were assessed to be

(undoubtedly) positive or negative by the visual score, would have been judged contrarily by the technical measurements. Now there seems to be no practical benefit in the assessment of allergic reactions by chromametry or laser Doppler perfusion imaging. Further work should be done to improve standardiza-

tion of allergen solutions with respect to their allergenic components and concentration units. Finally, the same internationally accepted visual scoring system should be used by all investigators, to allow better comparability of APT reaction assessment in different laboratories.

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