

## CLINICAL REPORT

# Atopy Patch Tests in Young Adult Patients with Atopic Dermatitis and Controls: Dose–response Relationship, Objective Reading, Reproducibility and Clinical Interpretation

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The clinical interpretation and reproducibility of atopy patch tests was studied in 23 selected young adult patients with atopic dermatitis and 25 healthy controls using standard inhalant allergens. Non-invasive measurements were used for objective assessment of test reactions and the participants were retested after 6 weeks. Ten of 19 (53%) evaluable patients with atopic dermatitis had at least one positive atopy patch test. However, there was no clear clinical relevance of the atopy patch test results when related to patient history and distribution of dermatitis. Reproducible and dose-dependent results were obtained with *Dermatophagoides pteronyssinus*, grass and cat with a reproducibility rate of 0.69 to 0.81 in patients and 0.60–0.96 in controls. A unique finding was a significant positive correlation between a positive atopy patch test, allergen dose and increase in transepidermal water loss and erythema, while measurement of capacitance did not distinguish between positive and negative reactions. The results of the present study do not support the routine use of atopy patch tests in the evaluation of adult patients with atopic dermatitis. **Key words:** atopic dermatitis; delayed-type hypersensitivity; non-invasive measurement.

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Patients with atopic dermatitis (AD) may experience exacerbation of dermatitis after skin contact with proteinaceous aeroallergens such as house dust mites, pollen or animal dander. These allergens often give rise to positive skin prick tests (SPTs) in patients with AD without clinical relevance for their eczema. Patch tests with proteinaceous allergens, also called atopy patch tests (APTs), may induce a local delayed-type hypersensitivity response in the skin, particularly in patients with AD. A Japanese group pioneered this field when they reported positive patch test reactions to human dander in patients with AD (1) and Mitchell et al. (2) described eczematous reactions after patch testing with well-defined aeroallergens. Since then, more than 100

articles have been published on the APT technique and performance with recently suggested improvements in vehicles and allergen concentrations (3). The aim of this investigation was to study the dose–response relationship, objective reading, clinical interpretation and reproducibility of APT in young adult patients with AD and healthy controls tested with aqueous solutions of standardized inhalant allergens up to 50 times the SPT concentration.

## MATERIAL AND METHODS

### *Subjects*

A total of 23 patients with AD (18 women and 5 men aged 19–29 years) and 25 healthy non-atopic controls (16 women and 9 men aged 18–30 years) were recruited. The patients fulfilled the Hanifin & Rajka criteria for AD (4). The SCORAD index (5) and the Rajka & Langeland scale (6) were used for scoring the severity of their eczema, and the presence of eczema on air-exposed skin was registered. The patients were asked about seasonal variation in eczema severity, and possible aggravating environmental factors to compare with patch test results. As an aid in the interpretation of a positive APT, the patients were asked whether they were aware of eczema flares and itching after lying in bed and improvement from use of an “anti-mite” bed cover. We asked for itching or rash after contact with lawn grass or cat, and presence of head and neck dermatitis and scalp dander as a possible relationship to *Pityrosporum ovale* reactivity.

The controls had no personal or family history of atopic diseases and had negative SPT to standard inhalant allergens.

The study protocol was approved by the Local Ethics Committee (# 19980029).

### *Methods*

Before entering the study, treatment with antihistamine, systemic and topical corticosteroid was discontinued for at least 7 days, and sunbathing was avoided for at least 1 month. APT was performed with aqueous solutions from ALK (Abello, Hørsholm, Denmark). The patch test concentrations included the maximal concentration obtainable from the supplier and a 4-fold dilution thereof. *Dermatophagoides pteronyssinus* (Der P), grass pollen and cat dander were tested in concentrations of 2.5 and 10 mg ml<sup>-1</sup> and *Pityrosporum ovale* in 5 mg ml<sup>-1</sup>. The maximal concentrations were from 5.9 × SPT concentration (cat) to 50 × SPT concentration (grass). Patch tests were also performed with whole milk,

and the following control substances: trypsin 0.25% aq., SLS 0.5% aq. and vehicle. The test substances in randomized order (45 µl) were applied to clinically unaffected and un-traumatized skin on the back using large Finn chambers (12 mm) with filter paper discs (Epitest OY, Helsinki, Finland) and fixed with Scanpor tape (Norgesplaster, Oslo, Norway). After 20 min the test sites were observed for immediate reactions; then occluded for 2 days and subsequently read on days 2, 3 and 7 according to standard criteria (7). The maximal APT reaction from day 2, day 3 and day 7 was registered. Reactions with a minimum of a palpable infiltrated eczema (ICDRG grade +) or greater were considered positive. A second test was performed after 6 weeks to determine reproducibility. SPTs were performed at the time of the first APT with pollen (birch, grass and mugwort), animal dander (horse, cat and dog), house dust mites, moulds, fresh milk and *Pityrosporum ovale* extracts. SPT with a mean wheal diameter of 3 mm or more was considered positive (8). Magic Light System (ALK-ABELLO) measured serum levels of total IgE and aeroallergen-specific IgE (RAST).

### Bioengineering methods

Non-invasive measurements of test sites were performed in accordance with published guidelines (9–11). The skin barrier function was assessed by transepidermal water loss (TEWL) with an Evaporimeter EPI<sup>®</sup> (ServoMed, Stockholm, Sweden). The electrical capacitance was measured with a Corneometer CM 825<sup>®</sup> (Courage/Khazaka, Germany), and erythema with a Minolta ChromaMeter CR-300 (Osaka, Japan). Skin surface temperature was measured with a digital thermometer, Ellab type DM852, probe diameter 18 mm. The measurements were performed in relation to the first test procedure before application of the APT and at the day 3 reading. The TEWL and skin surface temperature were measured once and the electrical capacitance and erythema three times and expressed as mean.

### Statistics

The results are given as proportions or medians. The Fisher exact test was used to evaluate the correlation between air-exposed eczema and a positive APT. The difference in bioengineering readings between day 0 and day 3 from each test site was recorded and presented as delta values. The non-parametric Kruskal-Wallis test and Mann-Whitney U-test were used for statistical analysis ( $p < 0.05$ ).

## RESULTS

The patients with AD had moderate to severe eczema with a median of 38 points in the SCORAD index (maximum score 103) and 6 in the Rajka & Langeland scale (maximum score 9). A history of allergic rhinitis or asthma was found in 56%.

All participated in the two test procedures. Because four patients developed widespread dermatitis of the test area at both test and retest procedures, the reactions were considered not evaluable and the patients were excluded from further analysis. Four of the remaining 19 patients with AD (21%) developed widespread dermatitis on the back at one of the test

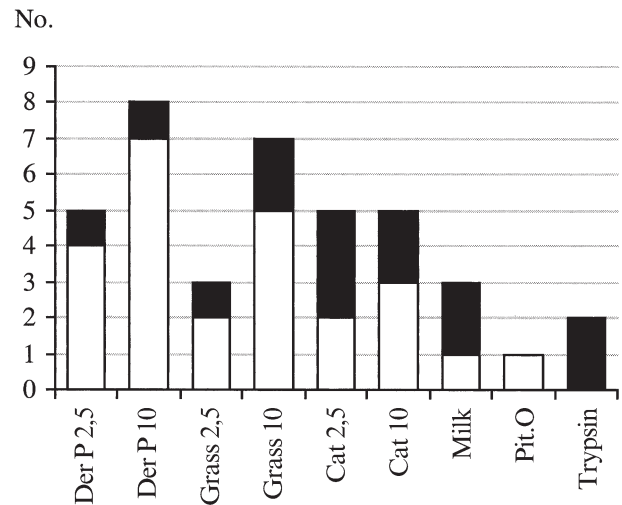


Fig. 1. Positive (□) and doubtful positive (■) atopy patch tests in 19 patients (HDM=house dust mite, Der P=Dermatophagoides pteronyssinus, Pit.O=Pityrosporum ovale).

procedures. One of these had widespread dermatitis at the time of the first test and was therefore excluded from the skin barrier function tests.

Ten of 19 (53%) patients with AD had at least one positive APT: 7/19 (37%) to Der P, 5/19 (26%) to grass and 3/19 (16%) to cat (Fig. 1). All 7 patients positive to Der P reacted to the highest concentration ( $10 \text{ mg ml}^{-1}$ ), and 4/7 also to the lowest concentration ( $2.5 \text{ mg ml}^{-1}$ ). All 5 positive grass pollen patients reacted to the highest concentration ( $10 \text{ mg ml}^{-1}$ ), and 2/5 reacted to the lower ( $2.5 \text{ mg ml}^{-1}$ ). All 3 positive cat dander patients reacted to the highest concentration ( $10 \text{ mg ml}^{-1}$ ) and 2/3 reacted to the lowest ( $2.5 \text{ mg ml}^{-1}$ ). Three patients reacted to more than one allergen. Three of the patients showed an urticarial reaction to Der P, two to cat and three to grass. Two patients experienced flare up of dermatitis during the test procedure. We saw 2 doubtful (+?) and 1 positive reaction to milk, 1 positive reaction to *Pityrosporum ovale*, and 2 doubtful reactions to trypsin. SLS gave irritant reactions in all patients, and 23/25 controls had a clearly irritant reaction (soap effect) and 2 showed a weak reaction to SLS. The SLS reactions were easily distinguished from responses to the allergen both visually and by measurement of TEWL (Table I). None reacted to the empty chamber (occlusion) or vehicle. In the control group, two had a positive APT to Der P but none reacted to grass or cat.

The relationship between APT results and type I allergic reactions (positive SPT and/or specific IgE > class 3) is given in Table II.

There was a dose-related increase in TEWL and erythema at test sites in AD patients with a positive APT compared to those with a negative APT and controls (Table I), while measurements of capacitance could not distinguish between positive and negative

Table I. Non-invasive evaluation of atopy patch tests for Der P 2.5 and 10 mg ml<sup>-1</sup>, positive (SLS) and negative (vehicle) control in patients with a positive and negative result and in controls.  $\Delta$ TEWL/ $\Delta$ Erythema is the intra-individual difference in TEWL/erythema on test site, when subtracting the pretest value from the test reaction value on day 3.

		$\Delta$ TEWL median (25–75 percentiles)	$\Delta$ Erythema median (25–75 percentiles)
Der P 2.5 mg	Patients with positive APT (n=4)	3.5 (2–7) <sup>a</sup>	5.79 (3.46–8.48) <sup>c</sup>
	Patients with negative APT (n=14)	0.0 (–1–1)	–0.20 (–0.87–0.73)
	Controls (n=25)	1.0 (0–1)	0.41 (–0.33–1.7)
Der P 10 mg	Patients with positive APT (n=4)	17.5 (9–19) <sup>b</sup>	8.03 (6.08–8.59) <sup>d</sup>
	Patients with negative APT (n=14)	1.0 (–1–2)	–0.05 (–0.39–2.38)
	Controls (n=25)	1.0 (0–1)	0.58 (–0.49–1.5)
SLS	Patients (n=18)	24.0 (8–36)	2.68 (1.68–5.7)
	Controls (n=25)	15.0 (12–22)	4.18 (2.24–5.42)
Vehicle	Patients (n=18)	–1.0 (–1–1)	0.10 (–0.92–0.69)
	Controls (n=25)	1.0 (0–2)	–0.08 (–0.35–1.09)

P-values for a difference between the 3 groups (Kruskal-Wallis): <sup>a</sup> $p < 0.04$ , <sup>b</sup> $p < 0.01$ , <sup>c</sup> $p < 0.005$ , <sup>d</sup> $p < 0.007$ .

Median basic TEWL in the 3 groups was 7 (range 3–16), and median basic erythema in controls was 9.7 (range 4.5–15.0), 7.1 in atopic dermatitis patients with positive APT (range 5.9–8.5) and 7.9 (range 3.3–10.8) in those with negative APT.

TEWL=transepidermal water loss; Der P=Dermatophagoides pteronyssinus; APT=atopy patch test; SLS=sodium lauryl sulphate.

tests (data not shown). Following a significant outcome in the Kruskal-Wallis test, a Mann-Whitney test showed that the differences were attributed to the patients with a positive APT compared to the other two groups. The reproducibility rates for each allergen and concentration varied between 0.69 to 0.81 in patients and 0.60 to 0.96 in controls and were determined as the number of reproducible results divided by the total number of tests with that allergen concentration (number pos (test and retest)+number neg (test and retest)+number doubtful (test and retest)/total).

There was no convincing relationship between a positive APT, patient history and distribution of dermatitis ( $p = 0.17$ ).

Table II. Cumulative atopy patch test (APT) results day 2, day 3 or day 7 at test or retest with standardized ALK<sup>®</sup> extracts.

	Number of patients with	
	Positive APT	Negative APT
<i>Dermatophagoides pteronyssinus 10 mg/ml</i>		
Positive SPT	6	1
Negative SPT	1	11
Positive RAST	5	0
Negative RAST	2	12
<i>Cat dander 10 mg/ml</i>		
Positive SPT	3	7
Negative SPT	0	9
Positive RAST	3	3
Negative RAST	0	13
<i>Grass pollen 10 mg/ml</i>		
Positive SPT	5	4
Negative SPT	0	10
Positive RAST	5	3
Negative RAST	0	11

SPT=skin prick test.

## DISCUSSION

Contrary to respiratory atopic diseases, the role of standard inhalant allergens in the pathophysiology of AD remains controversial. However, some patients experience exacerbation after contact with aeroallergens, especially house dust mites. We aimed to test the APT procedure under standardized test conditions, using well-characterized allergens in young adults with a high propensity to mount an inflammatory response. With the allergens tested we were not able to see a significant relationship between a positive APT, the patient history and dermatitis pattern, and therefore conclude that the interpretation of a positive APT is doubtful and of little help in the clinical evaluation of the patient. However, the size of the study must be taken into account in the evaluation of this negative result. There is no gold standard to prove the relevance of a positive APT to aeroallergens in patients with AD. The inference in ascribing relevance is problematic, as several factors may be responsible for the patient's answer, i.e. itching in bed could just as well be due to sweating or other autonomic factors and not related to house dust mite exposure. A relationship between a positive APT and eczema on air-exposed skin has been reported (12), but this was not confirmed in our study. Darsow et al. (13) performed the first controlled, double-blind designed study to describe significant associations of APT results and parameters of clinical relevance. They calculated sensitivity and specificity of an APT procedure based on patient history as a relevance parameter and found that APT had higher specificity compared with SPT and RAST. This is in agreement with our study, where there was a high concordance between a positive APT and a positive SPT in patients with AD (Table II).

Disregarding the doubtful reactions (+?) with only macular erythema, the aeroallergens in the control

group gave only one positive APT to Der P 2.5 and 10 mg/ml. Other investigators have shown that healthy controls and patients with only respiratory atopy do not react to APT (3, 14). The reactions to milk, trypsin and SLS were similar in patients and controls. The frequency of positive APT in patients with AD in the literature varied from 15% to 100% owing to different test techniques and selection of patients (15). In our study, 10/19 (53%) patients with AD had a positive APT. Technical aspects of APT with regard to vehicle, dose response, mode of application, reading times, scoring and clinical covariates have been studied by several groups (3, 12, 13, 16). Further standardization of the test procedure and evaluation of the APT reactions are needed in agreement with a recent German study (17), especially with regard to the choice of allergen extracts, as currently available dust mite allergens show an inordinately high number of false-positive reactions (18, 19). We selected allergens in solution because this ensures more precise dosing and distribution of allergens compared to petrolatum preparations. The test substances were applied on clinically uninvolved, untreated back skin; tape stripping, skin abrasion or addition of detergents to the test substances were omitted because these procedures may result in false-positive tests. Furthermore, the visual readings were supported by non-invasive measurements. A dose-related increase in TEWL and erythema were seen at test sites in patients with a positive APT to Der P, while measurement of stratum corneum hydration did not distinguish between positive and negative APT. TEWL measurements may be used in the evaluation of ordinary patch tests (20) and have also been used twice in APT studies (21, 22). The epidermal barrier function in patients with AD was altered only in positive APT reactions to aeroallergens and not in the positive classical patch test reactions to contact allergens (21). Chromametry and laser Doppler imaging has also been used to evaluate APT reactions and showed a significant positive correlation with the visual scores (17). The advantage of non-invasive measurements is the objectiveness and suitability for dose-response analysis, but none of these techniques can replace the eyes and fingers of the experienced clinician (23).

Almost all positive APT reactions were seen on day 2, and nothing was gained from later readings. Some reactions became more infiltrated on day 3 (Fig. 2), and reactivity decreased on day 7.

Although the APT method has been in use for about 20 years, few studies have used highly concentrated and purified antigens, which are expensive and difficult to acquire. Only a few studies have measured the allergen dose in units permitting comparison with environmental allergen levels, and dose-response studies are sparse (24). The reported concentration of

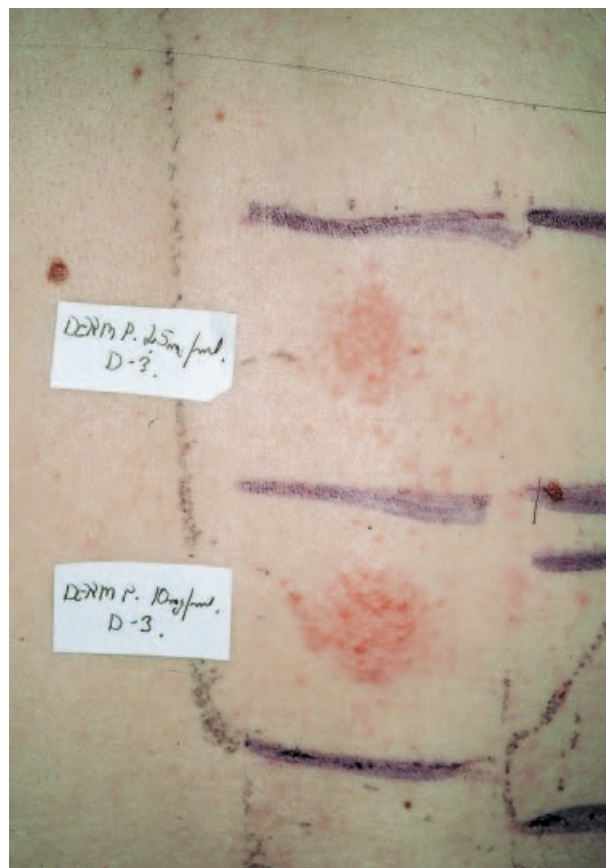


Fig. 2. Positive atopy patch test to Der P 2.5 mg ml<sup>-1</sup> and 10 mg ml<sup>-1</sup> day 3.

Der P1 in floor dust is about 280 µg/cm<sup>2</sup> (25). We tested with 354 µg Der P/cm<sup>2</sup> (10 mg ml<sup>-1</sup>), that is in the relevant range. It is not known if antigens for type IV reactions are the same as for type I (26). Therefore, we tested with the full extracts of house dust mite and not the isolated major allergens (e.g. Der P1). *Pityrosporum ovale* was only available in the usual prick test concentration, which is probably too low to give positive reactions. In fact, we saw one positive reaction in a patient with head and neck dermatitis. Milk has previously been used for APT on infants; therefore, we included whole milk as an extra control substance, as none of our patients had milk allergy. However, one patient had a positive APT to milk without clinical relevance, as oral intake of milk did not provoke exacerbation of AD. The clinical significance of a positive APT to food allergens can be evaluated by double-blind, placebo-controlled food challenge. In fact, the APT has recently been introduced into the diagnostic armamentarium for food allergy (27), and was found to have a high sensitivity and specificity with milk, egg, wheat and soybean (28, 29).

Enzymatic activity in a mite preparation can cause cutaneous inflammation without any specific immune

reactions caused by proteolytic activity (30). Tests with a trypsin solution gave only doubtful reactions in the concentration used.

The reproducibility of APT is rarely studied. Only one previous study included systematic retesting with APT in all participants (31). Seventeen young persons with AD, 12 non-eczematous atopic male volunteers and 11 healthy volunteers, were tested twice with crushed house dust mites with an interval of 15 days to 18 months between the tests. Reproducibility was satisfactory with a  $\kappa$ -value between 0.63 and 1. Heinemann et al. (17) found a reproducibility rate of only 56.3%, when retesting 14 (out of 52) with a positive APT to a specific allergen. However, they used allergens in petrolatum with poor inter-test agreement. We obtained reproducible and dose-dependent results with aqueous extracts of Der P, cat dander and grass pollen with a reproducibility rate of 0.69 to 0.81 in patients and 0.60 to 0.96 in control persons. The results of duplicate patch tests in an ordinary standard patch test procedure are equivocal, with concordances from 56% to 93% (32, 33).

A positive APT alone should not be taken as an indication of allergen exclusion measures in patients with AD, as no simple correlation exists between the severity of AD and house dust mite exposure. It is generally accepted that AD may aggravate following skin exposure to aeroallergens like house dust mite, animal dander and pollen. Most of the patients have elevated allergen-specific IgE and improvement after house dust mite elimination is described (34). However, improvement following use of bed covers occurred in all patients with AD irrespective of the reactivity pattern towards Der P (35). Possibly, it is not the house dust mite allergen concentration but more the individual genetic background, status of the skin and degree of sensitivity that are decisive for allergic manifestations. It is important to show that a positive APT has clinical relevance: that exposure makes eczema worse and elimination makes the disease less active — but no intervention studies have yet been completed after patients have been tested with APT. At the moment, no gold standard exists for the provocation of eczematous skin lesions in AD. Future studies with standardized allergens, reading, elimination and provocation procedures, and long-term follow-up must be performed before this diagnostic tool can be recommended for use in the clinical routine.

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