

Reproducibility of atopy patch tests with *Dermatophagoides*: a study on 85 patients with atopic dermatitis

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Atopy patch tests (APTs) are considered a useful addition to standard testing procedures for the identification of allergens triggering the appearance of eczema in patients with atopic dermatitis (AD). The aim of the study was to evaluate the reproducibility of APTs with house dust mites by simultaneously patch testing AD patients. 85 subjects affected by AD, 36 males and 48 females, aged from 1 to 52 years, were patch tested with *Dermatophagoides* mix (*D. pteron*/*D. farinae*) 20% pet. In our patients, the percentage of agreement between the frequency of positive and negative responses to *Dermatophagoides* mix was 96.7%. Reproducibility was highly satisfactory, as evaluated by *k*-value (0.953). As regards the agreement in the intensity of APT responses to *Dermatophagoides* mix between the right and left sides, we found that reproducibility was also highly satisfactory (*k* = 0.851). In conclusion, for *Dermatophagoides* APTs, the agreement rate was similar to that of standard patch tests, and therefore APTs may be considered sufficiently reproducible to be employed as a diagnostic testing procedure.

Key words: atopic dermatitis; atopy patch tests; *Dermatophagoides*; reproducibility. © Blackwell Munksgaard, 2004.

Accepted for publication 27 January 2004

Atopic dermatitis (AD) is an inflammatory skin disorder of relapsing course, which is associated with non-specific skin hyperreactivity, hypersensitivity to environmental allergens and immunoglobulin E (IgE) hyperproduction. Clinical data, pointing at a relationship between exposure to house dust mite (HDM) allergens and exacerbation of the dermatitis, together with the presence of mite-specific IgE in many AD patients, and the finding of IgE receptors on Langerhans' cells (1, 2), indicate that the role of mite allergens can be carried out through their transdermal penetration, binding to Langerhans' cells, and activation of lymphocytes triggering a delayed-type hypersensitivity reaction.

Skin lesions characterized by erythema, papules and vesicles, showing microscopic and immunopathological similarities to eczematous lesions in AD, can be artificially elicited by applying aeroallergens on the skin of AD patients. Approximately 20 years ago, Mitchell et al. started patch testing AD patients with aeroallergens to experimentally reproduce the normal route of exposure of atopic skin to environmental allergens (3). This procedure, later called the

atopy patch test (APT) by Ring et al. (4), has been employed since 1982 by many other authors using HDMs, pollen and animal dander, obtaining different positivity rates according to patch test materials and modalities (5–16).

Thus, APTs are considered a useful addition to standard testing procedure for the identification of aeroallergens eliciting the appearance of eczema in AD patients. However, in spite of the trend increasingly to consider APTs as a completion of the diagnostic procedure for AD, their reproducibility has so far been poorly investigated (6, 13, 16–20).

Our aim was to check the reproducibility of APT reactions to HDMs by simultaneously patch testing AD subjects.

Materials and Methods

At the Department of Dermatology in Modena, 85 subjects affected by AD, 36 males and 49 females, aged from 1 to 52 years (mean age $\pm$ SD = 13.1 $\pm$ 12.1 years), were simultaneously patch tested with *Dermatophagoides* mix (*D. pteron*/*D. farinae*) 20% pet. (Allergopharma,

Milton Keynes, UK). Approximately 20 mg of each preparation was applied to healthy skin on the upper right and the upper left of the back with large Finn Chambers[®] on Scanpor tape (Epitest, Tuusula, Finland). Reading was taken at 72 h, 30–120 min after removal of the test chambers. Test results were evaluated according to the following scale: erythema and oedema = 1; erythema, oedema and few papules = 2; erythema, oedema and papules covering most of the patch test area = 3; and erythema, oedema and papules spreading outside the patch test area or vesicles = 4. Irritant or doubtful reactions included redness with no infiltration.

The reproducibility of the APT was evaluated by assessing the percentage of agreement and by statistical κ calculation (Cohen test): $k < 0.20$ = unsatisfactory; 0.21–0.40 = barely satisfactory; 0.41–0.60 = fairly satisfactory; 0.61–0.80 = satisfactory; and 0.81–1 = highly satisfactory.

Results

In our study population, 45 AD patients showed positive responses to APTs on both sides, whereas 1 patient reacted only on the right side and 1 patient only on the left side. Overall, the percentage of agreement in the frequency of positive and negative responses between the right and left sides was 97.6%. Reproducibility as expressed by k -value was highly satisfactory ($k = 0.953$).

Figure 1 shows the percentage of positive responses in 4 different age groups. It ranged from 44.4% in children under 6 years of age to 64.3% in subjects aged from 6 to 12 years. No significant differences in skin reactivity to HDMs were observed according to age. The percentage of agreement between the right and the left sides proved high in all age groups, ranging from 93.3 to 100%.

Evaluating the agreement in the intensity of the response to APTs with *Dermatophagoides* between the right and left sides, we found that

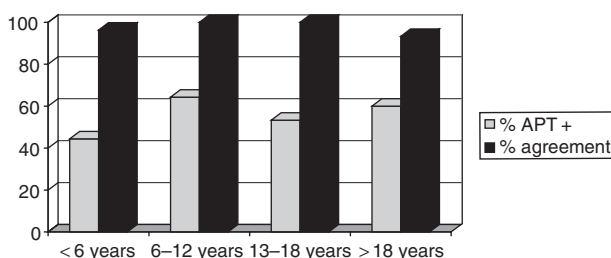


Fig. 1. Percentage of positive atopy patch test (APT) responses to *Dermatophagoides* and agreement between right and left sides according to age.

reproducibility was highly satisfactory ($k = 0.851$) (Table 1).

Discussion

Most authors consider aeroallergen APTs as a diagnostic tool for identifying subjects presenting with eczematous skin lesions upon exposure to allergens generally inducing IgE-mediated reactions (3–16), which may provide further diagnostic information in addition to the patients' history and prick test results. In practice, the parallel use of prick and patch tests has been demonstrated to improve the rate of positive reactions, probably because several immunologic mechanisms are involved in allergic responses in AD. Initially, some groups observed that aeroallergen patch test results were dependent on the presence of specific IgE to aeroallergens. IgE on the surface of Langerhans' cells in the skin of patients with AD was regarded as the major event leading to eczematous lesions after exposure to aeroallergens (1, 2). However, in many patients with negative prick test responses and negative specific IgE serum levels, clear-cut positive patch test reactions are observable (7, 9, 10). In a study performed by us in 1992, the percentage of positive patch test responses was 51% in mite IgE-positive patients and 44% in the negative (7).

From our findings on 313 AD patients undergoing skin tests with *Dermatophagoides*, APTs were positive in 39% of cases, whereas prick tests were in 31% (10). In particular, 15% of AD patients reacted to both skin tests, 24% to APTs alone, 17% only to skin prick test (SPT), whereas 44% proved negative. The percentage of agreement between APTs and SPTs was 58%, showing that allergen-specific IgE is not mandatory for a positive patch test reaction.

Studies on APTs with mites, beginning with that by Mitchell et al. in 1982 (3), have varied widely in the allergenic materials and methods used, unsurprisingly yielding different percentages (15–100%) of positive responses related to variables such as skin integrity, type and concentration of allergenic material and vehicle (9, 13). Our data confirm that delayed-type reactions to *Dermatophagoides* allergens in AD subjects are frequent, because positive responses were observed in 55% of the patients, suggesting that dust mites may often be involved in the development of skin lesions in APT-positive patients. The positivity rate in our study population ranged from 44 to 64% according to age, being higher in children aged 6–12 years.

Allergen-specific T-cell clones deriving from skin specimens taken from aeroallergen patch test sites have been characterized (21), and a

Table 1. Results of atopy patch tests with *Dermatophagoides* according to the intensity of the response

		Left				
		Negative responses	Score 1	Score 2	Score 3	Score 4
Right	Negative responses	38	1	0	0	0
	Score 1	0	6	0	2	0
	Score 2	0	0	7	0	0
	Score 3	0	2	0	13	0
	Score 4	1	0	0	3	12

Scores indicate the different grades of positive responses.

typical time-dependent cytokine pattern expression *in situ* identified (22), thus demonstrating the specific nature of the APT response. However, atopic subjects without dermatitis and non-atopics have also been reported to show positive patch test responses (6, 10, 15, 17), even if less frequently and intensely compared to AD patients. Thus, the eczematous reaction upon exposure to aeroallergens does not apply only to patients affected by AD. We could imagine a spectrum of reactivities characterizing different skin types modulated by percutaneous penetration capability, sensitivity threshold and efficiency of specific and unspecific effector mechanisms promoting and enhancing the inflammatory response (15). Moreover, the irritant effects linked to the proteolytic activity of mite allergens should not be underrated, because this seems to account for a consistent number of positive responses (23). Because of the finding of specific IgE by prick testing and radio allergen sorbent test (RAST), positive APT responses in subjects not affected by AD may indicate an atopic diathesis and identify patients predisposed to develop dermatitis on allergen exposure.

However, before introducing APTs in the routine diagnostic work-up of AD patients, the issue of reproducibility of APT results should be considered. So far, it has been studied by re-testing a small number of subjects after 15 days–18 months (6, 13, 16, 17, 19, 20). When investigating the concordance of APTs with whole HDM bodies in 40 subjects at a second testing performed at a time interval of 15 days–18 months, reproducibility was highly satisfactory in AD patients and in healthy volunteers (17). It is well known, however, that skin reactivity to allergens may show time-dependent variations, because new sensitizations may be acquired or lost with time. Moreover, in sensitized subjects, responses to patch tests may vary according to factors modulating the local or the general immunologic state, inducing transitory skin hypo- or hyper-reactivity (24), and this may influence the results

and give rise to poor reproducibility when testing at different time points. Simultaneous skin contact on different areas of healthy skin should give the same response if the test material is suitable for absorption by the skin and comes into contact with the immune system. On the contrary, if the material induces an irritant reaction or is not properly absorbed by the skin, such responses may show pronounced variations. Moreover, when applying patch tests in children, adhesiveness to the back is not uniform because of the convexity of the scapular area and concavity of the paravertebral regions. This may also hamper allergen penetration and skin reactivity. The percentage of agreement for standard patch test substances varied between 97 and 100% in a study performed on 155 patients with suspected allergic contact dermatitis, simultaneously tested on the right and left sides of their back with the same patch test series (25). Our preliminary data on 30 AD patients contemporaneously patch tested with *Dermatophagoides* mix, and *Alternaria alternata* demonstrated that APT reproducibility was highly satisfactory ($k = 1$) (18). The agreement in the frequency of positive and negative responses between the right and left sides was 100% for both aeroallergens.

In this study performed on a larger number of patients, the agreement between ATPs with *Dermatophagoides* proved to be high (97.6%) similar to that of standard patch tests. Moreover, the degree of positivity of responses to patch tests performed on the right and left sides of the back showed a high agreement, indicating that the intensity of the immunologic reaction also shows high reproducibility.

In conclusion, from our findings, APTs with *Dermatophagoides* may be considered sufficiently reproducible to be employed as a diagnostic testing procedure. Now, further studies on the reproducibility of APTs need to be performed in larger number of patients, and with different aeroallergens and food allergens, to confirm our data.

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