

Is the Atopy Patch Test with House Dust Mites Specific for Atopic Dermatitis?

Vito Ingordo^a Roberto Dalle Nogare^b Bruno Colecchia^b Corrado D'Andria^c

^aDepartment of Dermatology, Italian Navy Main Hospital 'M.O. Giulio Venticinque', ^bItalian Navy Petty Officers School, Medical Service, and ^cMedical Center 'Maugeri Foundation', Ginosa Marina, Taranto, Italy

Key Words

Atopy patch test · House dust mites · Atopic dermatitis · Atopic subjects without eczema · Nonatopic subjects · Skin hyperreactivity · Reproducibility · Specific response

Abstract

Background: The atopy patch test (APT), namely the patch test with aeroallergens, is regarded as specific for patients with atopic dermatitis (AD), but small numbers of positive APT were reported in the past also in atopic subjects without dermatitis and in healthy persons. **Objective:** The aim of this study was to evaluate the response to the APT with house dust mites (HDM) in subjects nonaffected by AD and to compare the outcomes observed in these cases with those pointed out in AD patients, evaluating also the differences between two allergen extracts manufactured at different purifications and concentrations. **Methods:** Forty-seven atopic subjects without eczema (AWE), 33 nonatopic (NA) subjects and 77 adult AD patients were patch tested with an extract of purified bodies of HDM at 20% and with another extract of whole bodies of HDM at 30%, the latter corresponding to 300 µg/g of Der p 1. The reproducibility of APT was also tested in 8 AD patients, in 37 AWE subjects and in 19 NA subjects. **Results:** Positive responses with extract at 20% were observed in 29 (37.7%) AD, in 5 (10.6%) AWE and in 4 (12.1%) NA subjects. The APT with HDM at 30% was positive in 32 (41.6%) AD, 9 (19.1%)

AWE and 4 (12.1%) NA persons. The rates of positivity and the intensity scores of responses were significantly different between AD and non-AD subjects ($p < 0.01$). The reproducibility of the APT in the three groups was satisfactory. **Conclusion:** These observations lead to conclude that the APT with HDM is positive also in non-AD subjects but it is probably more specific for AD.

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Introduction

On the ground of the observation that a subgroup of patients affected by atopic dermatitis (AD) reacted positively to the application of environmental aeroallergens to the skin [1], a patch test with these allergens has been proposed in the study of AD. This test was named 'atopy patch test' (APT) [2], and the most employed allergens were house dust mites (HDM; *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*).

Many studies on the APT have been performed [1–24], and it is regarded quite specific for patients with AD. However, small numbers of positive APT were reported in the past also in atopic subjects without dermatitis [6, 8, 18–20, 23, 24] and in healthy persons [5, 9, 11, 13, 18–20, 23, 24] and that would raise some question about the specificity and the clinical relevance of positive APT results.

The aim of this study was to evaluate the response to APT with HDM in subjects not affected by AD and to

compare the outcomes observed in these cases with those pointed out in AD patients, evaluating also the differences between two allergen extracts manufactured at different purifications and concentrations.

Patients, Materials and Methods

We have patch tested three groups of subjects with two commercial extracts of whole bodies of HDM:

(1) The first extract is manufactured by Allergopharma-Merck (Reinbek, Germany). The patch test preparation contains 20% purified mite bodies, comprised of equal amounts of *D. pteronyssinus* and *D. farinae*, suspended in white petrolatum (60%) and petrolatum oil (20%). Purified mite bodies are supplied by Allergon AB (Ängelholm, Sweden). The mite culture line is maintained by successive inoculation of new batches of culture medium. The components of the medium have either no allergenicity or minimal activity. Each batch is cultured for 3–4 months at 25 °C and 75% relative humidity. Mites are harvested, vacuum dried and placed in sealed aluminium containers at 4–8 °C. Under these conditions, the allergenic activity of the extract is stable for at least 5 years, as judged by radioallergosorbent test inhibition. No fixation process is used.

(2) The second extract is supplied by Euromedical srl (Calolziocorte, Italy). The preparation contains whole bodies of *D. pteronyssinus* (50%) and *D. farinae* (50%), corresponding to 15,000 mites/g, equal to 300 µg/g of Der p 1, with a titration of 26,000 IU, mixed with petrolatum (Vasetol VF Plus; 40%) and white petrolatum (VAS Pharma 21 Oil; 30%). The extract is derived by a culture of *D. pteronyssinus/D. farinae* maintained in semi-artificial diet without components of human origin at 25 °C and 75% of relative humidity. The culture is treated with interruption of food for 2 months, suddenly frozen and maintained at –15 °C in a closed box for alimentary use, carried to the laboratory of extraction in a refrigerator bag. There is absolute absence of human origin components, and the active principle is maintained at –15 °C.

In our patients, the allergen was applied in Finn chambers (Epitest Ltd., Oy, Finland) on healthy untreated upper back skin. Plain white petrolatum vehicle was used as negative control. The APT was read after 2 and 3 days, and the interpretation was performed according to the criteria suggested by Darsow et al. [3]: therefore the APT was defined positive when a clear-cut reaction was observed (i.e. erythema, infiltration, none or few papules at least occurred). Responses were also evaluated according to the following scoring scale: doubtful (+) (only erythema) = 0.5; positive + (erythema, infiltration, none or few papules) = 1.0; positive ++ (erythema, infiltration, many papules) = 2.0; positive +++ (erythema, infiltration, papules and vesicles) = 3.0.

The groups tested were the following:

(1) 47 atopic persons without eczema (AWE). It was a group of subjects, selected randomly among the cadets of the Italian Navy Petty Officers' School in Taranto, Italy, 42 males and 5 females, with a mean age of 24.7 ± 3.6 years (min.–max.: 20–35 years) and a negative history for any form of eczema. Twelve (25.5%) patients had oculorhinitis, 1 (2.1%) oculorhinitis and asthma in the past, 3 (6.4%) acute or relapsing urticaria in the past; 31 (66%) patients did not have any symptom (so-called latent atopic individuals [3] or subjects with 'atopic state' [25]). All subjects were selected by pos-

itivity to skin prick tests (SPT), performed with environmental aeroallergens (grass, mugwort, *Cupressus sempervirens*, *Chenopodium album*, *Amarantus retroflexus* and *Olea europea* pollens, moulds, *D. pteronyssinus/farinae*) and with common food allergens (corn, meat, fish, fresh fruits, dried fruits, egg, milk, soybean, yeast, legumes; Allergopharma-Merck) and by dosage of total circulating IgE (MEIA-IMX System, Abbott, Rome, Italy). The cut-off for high value of total IgE, according to age of subjects, was 120 IU/ml. The patients with urticaria were free of symptoms when the APT was performed.

(2) 33 nonatopic (NA) subjects, 27 males and 6 females, also selected randomly among the cadets, with a mean age of 25.4 ± 6.5 years (min.–max.: 20–39 years). They had a negative anamnesis for any form of eczema and other allergic diseases, negativity of SPT for the aforesaid aeroallergens/foods and normal total serum IgE.

(3) 77 patients affected by AD (75 males and 2 females), with a mean age of 23.4 ± 6.5 years (min.–max.: 18–47 years), obtained by the database of the Department of Dermatology of the Italian Navy Hospital in Taranto, Italy, in which the APT is performed routinely. The diagnosis of AD is made in all cases according to the criteria of Hanifin and Rajka [26], and all the patients undergo SPT with the aforesaid environmental aeroallergens and foods and the dosage of total circulating IgE. All the AD patients are also routinely patch tested for diagnosing a coexisting allergic contact dermatitis (ACD). In the past, the GIRDCA (Gruppo Italiano di Ricerca Dermatiti da Contatto e Ambientali) standard series was employed; since 2002, the Società Italiana di Dermatologia Allergologica, Professionale ed Ambientale series with a standard integrative series has been routinely introduced (table 1). Additional series are always added, when it is suggested by the history. The haptens (FIRMA, Florence, Italy/Chemotechnique Diagnostics, Malmö, Sweden) are applied to the healthy upper back skin in Finn chambers (Epitest Ltd.), and the interpretation of patch test results, read after 2 and 3 days, is based on the criteria of the International Contact Dermatitis Research Group, according to the GIRDCA guidelines [27], i.e. $\pm$ = weak erythema (doubtful test); + = erythema with edema, eventually papules or weak vesiculation; ++ = erythema, edema, papules and evident vesicles, possibly exceeding the site of application; +++ = erythema, edema, papules and more evident vesicles, sometimes bullae. The diagnosis of 'definite ACD' is made when the current or past relevance of positive haptens is 'possible' or 'likely', according to criteria suggested by Lachapelle [28]. Among the aforesaid 77 AD patients, 12 (15.6%) showed a coexistent 'definite' ACD. According to the classification proposed by Wüthrich [29, 30], 26 (33.8%) subjects were affected by the 'extrinsic' type of AD (i.e. they had positive SPT/high total circulating IgE), 9 (11.7%) were affected by the 'intrinsic' type (negative SPT/normal total circulating IgE), and 42 (54.5%) were affected by the 'mixed' type (i.e. they had also oculorhinitis or asthma).

At the time of testing, most patients had a clinically active disease, but no lesion either on the place of APT or patch testing for ACD was present. None of the subjects of the 3 groups was treated with systemic steroids, antihistamines or other immunosuppressant drugs before patch testing.

For evaluating the reproducibility of the test, the APT was also repeated in 8 AD patients, in 37 AWE subjects and in 19 NA subjects, after a time varying from 3 months (in control groups) to 4 years (in some AD patients). The test was performed in the same conditions and was read by the same observer (V.I.).

The favorable opinion of the local independent ethical committee had previously been obtained, and the members of the control groups gave their informed consent.

The differences in results of the APT between the groups were evaluated by Fisher's exact test. For evaluating possible variations in intensity of response among the three groups, the differences in the scores were evaluated by the one-way ANOVA test. Using the unpaired Student's *t* test, differences in the mean two by two scores of positivity between groups were also searched for. Reproducibility of the APT in re-tested subjects was evaluated by κ statistic (Cohen test) and by the paired Student's *t* test.

Results

APT Positivity

The outcome of APT with the two extracts in AD, AWE and NA groups is reported in table 2. The differences in APT positivities between the AD group and the AWE, NA and total non-AD (AWE + NA) groups were statistically significant ($p < 0.01$). On the contrary, no significant difference was shown between AWE and NA subjects. The rate of positivity with the extract at 30% was

Table 1. Patch test series: GIRDCA standard series

1	White petrolatum ¹	17	Disperse yellow 3 1% ¹
2	Thiuram mix 1% ¹	18	Fragrance mix 8% ¹
3	Potassium dichromate 0.5% ¹	19	Paraben mix 15% ¹
4	Balsam of Peru 25% ¹	20	Disperse blue 124 1% ¹
5	Phenyl-isopropyl-4- phenylenediamine 0.1% ¹	21	Benzocaine 5% ¹
6	Kathon CG 0.01% ¹	22	Cobalt chloride 1% ¹
7	4-Phenylenediamine base 1% ¹	23	Quinoline mix 6% ²
8	Wool alcohols 30% ¹	24	Quaternium 15 1% ²
9	Colophony 20% ¹	25	4,4'-Diaminodiphenylmethane 0.5% ²
10	Neomycin sulfate 20% ¹	26	Imidazolidinyl urea 1% ²
11	Ethylenediamine dihydrochloride 1% ¹	27	Mercury ammonium chloride 1% ²
12	Formaldehyde 1% ¹	28	Disperse red 1 1% ²
13	Mercaptobenzothiazole 2% ¹	29	Thimerosal 0.1% ²
14	4-tert-Butylphenolformaldehyde resin 1% ¹	30	Carba mix 3% ²
15	Epoxy resin 1% ¹	31	Mercapto mix 2% ²
16	Nickel sulfate 5% ¹		
<i>Other haptens</i>			
32	Corticosteroid mix 3.1% ¹	38	Dimethylaminopropylamine 1% ²
33	Compositae mix 5% ¹	39	Toluene sulfonamide formaldehyde resin 10% ²
34	Euxyl K 400 1.5% ¹	40	Propolis 10% ²
35	Gold sodium thiosulfate 0.5% ¹	41	Allyldisulfur 1% ²
36	Lactone sesquiterpene 0.1% ²	42	Disperse dye mix 1% ²
37	Promethazine hydrochloride 1% ²	43	Tixocortol-21-pivalate 1% ²

¹ Società Italiana di Dermatologia Allergologica Professionale ed Ambientale standard series.

² Integrative standard series since 2002.

Table 2. Response to APT in the tested groups

	AD		AWE		NA		Controls (AWE + NA)	
	n	%	n	%	n	%	n	%
APT 20% +	29	37.7	5	10.6	4	12.1	9	11.2
APT 30% +	32	41.6	9	19.1	4	12.1	13	16.2

Fisher's exact test: AD versus AWE, $p < 0.01$; AD versus NA, $p < 0.01$; AD versus controls, $p < 0.01$; AWE versus NA, $p =$ not significant.

higher in AD and AWE individuals than the rate obtained with the extract at 20%. Analysis of variance of the reading scores obtained from the APT-positive subjects showed a statistically significant difference among the three groups (table 3). Moreover, comparing the mean scores in the groups two by two, significant differences with both the extracts were pointed out between the AD and AWE group and between the AD and NA group, whereas no difference was noticed between AWE and NA subjects (table 3).

Outcome of APT and Clinical Covariates in the AD Group

The AD patients were also subdivided into two subgroups according to their eczema localization, in agreement with Darsow et al. [31]. A subgroup encompassed the subjects with an *air-exposed* pattern, i.e. the sites involved were exclusively or predominantly the hands, wrists, fore-

arms, head and neck. The other group had skin lesions predominantly or exclusively in the *covered areas*, i.e. the trunk, arms, thighs, cubital and popliteal folds. Using the extract at 20%, the APT was positive in 23 out of 54 subjects (42.6%) with the air-exposed pattern and in 6 out of 23 patients (26.1%) with clinical lesions localized on covered sites. Using the extract at 30%, 21 (38.9%) subjects with the air-exposed pattern and 11 (47.8%) subjects with the non-air-exposed pattern had an APT-positive test. No significant difference in APT positivity using both extracts was pointed out between the two subgroups ($p = \text{n.s.}$). Unfortunately, we were not able to record the history of AD patients with regard to worsening/improvement of the dermatitis after dust mite exposition/avoidance.

Concordance of SPT and APT with HDM

Mite-specific IgE by prick tests were pointed out in 53.2% of AD subjects and in 61.3% of AWE subjects. Using the extract at 20%, in 13.8 and 41.6%, respectively, of AWE and AD subjects with HDM-positive SPT, APTs were positive too, whereas 20 and 51.6% of AWE subjects and AD patients reacting to APTs with HDM, respectively, showed positive HDM SPT responses. On the contrary, employing the extract at 30%, AWE and AD subjects reacting to SPT with HDM had, respectively, 20.7 and 46.3% of APT responses, whereas in 66.7 and 59.4% of AWE and AD individuals reacting to APT a positive SPT with HDM was observed.

APT Reproducibility

Reproducibility of APT with both extracts is summarized in table 4. κ calculus showed a reproducibility varying from *fairly satisfactory* to *highly satisfactory*. The extract at 20% showed among the three groups higher variations in κ values than the extract at 30%, with which

Table 3. Differences among the mean reading scores in the APT-positive groups

	AD subjects	AWE subjects	NA subjects
APT 20%	1.51 $\pm$ 0.59	0.75 $\pm$ 0.26	0.81 $\pm$ 0.53
APT 30%	1.64 $\pm$ 0.66	1.00 $\pm$ 0.58	0.77 $\pm$ 0.50

APT 20%: ANOVA (one-way test, comparing the three groups), $p < 0.01$. Student's t test (unpaired, comparing the groups two by two), AD versus AWE, $p < 0.01$; AD versus NA, $p < 0.01$; AWE versus NA, $p = \text{not significant}$.

APT 30%: ANOVA, $p < 0.01$. Student's t test, AD versus AWE, $p < 0.01$; AD versus NA, $p < 0.01$; AWE versus NA, $p = \text{not significant}$.

Table 4. Reproducibility of APT in the tested groups

Groups	APT 20%				κ^1	APT 30%				κ^1
	1st reading		2nd reading			1st reading		2nd reading		
	n	%	n	%		n	%	n	%	
AD (8 subjects)	6	75	7	87.5	0.60	5	62.5	6	75	0.71
AWE (37 subjects)	9	24.3	6	16.2	0.75	10	27	7	18.9	0.77
NA (19 subjects)	5	26.3	4	21	0.85	6	31.5	4	21	0.73

¹ κ (Cohen's coefficient): <0.20 = unsatisfactory; $0.21-0.40$ = barely satisfactory; $0.41-0.60$ = fairly satisfactory; $0.61-0.80$ = satisfactory; $0.81-1$ = highly satisfactory.

κ values were unvarying. The evaluation of the mean reading scores, performed by the paired Student's *t* test, did not reveal in all the groups any statistical difference between the first and the second APT, using both the extract at 20% and the extract at 30% (data not shown).

Discussion

In studies performed on APT the percentages of positive subjects varied [7, 10, 14, 16], and this was ascribed to the absence of standardization in employed techniques and to the different purification and concentration of allergens [3, 9, 16–18, 32]. At present, the technique most frequently employed is the application of extracts on healthy untreated upper back skin [3]. The concentration and the purification of allergens are still discussed [3, 9, 15–18, 32]. The main antigens of *D. pteronyssinus* and *D. farinae* have been isolated by feces or bodies (Der p 1 and Der f 1/Der p 2 and Der f 2), but most patch test studies were performed using whole mite culture or culture extracts, because of the high cost of purified allergens. Consequently the protein content is not limited to the allergenic proteins, and the concentration of the allergens is indefinable. The extract at 30% employed in the present study, which contained 15,000 mites/g, was titrated with regard to its content in Der p 1. This makes the results reproducible and the dilutions valid. We tested the subjects with the less purified extract at 20% as well, to point out if some significant difference in APT responses was present, using two allergens with different purification and concentration.

Studies on the tested skin have explained the immunopathologic mechanism involved in the APT, which includes the T cells' intervention via the IgE-bearing Langerhans cells. That was suggested by the observation of a Th2 cytokine pattern into the T cells infiltrate in the skin during the precocious phases of APT reaction [33, 34] and was confirmed when in the APT a switch from an initial Th2 response to a late Th1 response in situ was shown [35, 36]. Furthermore Langeveld-Wildschut et al. [37] demonstrated that a positive APT reaction requires the presence of epidermal IgE+CD1a+ cells in clinically uninvolved skin. It was also pointed out that the APT positivity is associated with proliferation and activation of allergen-specific circulating T cells, confirming that an immunologically mediated mechanism leads to positive reactions [38].

Therefore, the APT can be considered a diagnostic procedure enabling the identification of subjects who re-

act with eczema after exposure to mites [4, 5, 15]. However, the outcome of the APT in subjects unaffected by AD has been poorly investigated with contrasting results. In 85 healthy volunteers no reactions to APT with house dust mites and grass pollens were observed by Langeveld-Wildschut et al. [17]. No positive APT responses to aeroallergens in patients with respiratory atopy or controls were detected by Darsow et al. [3]. On the contrary, rates of positivities ranging from 5 to 40% in AWE [5, 6, 8, 11, 18, 24] and healthy subjects [6, 8, 9, 11–13, 18–20, 22–24] were reported in the past by other authors. Recently, Seidenari et al. [32] have observed positive responses to mite patch tests also in subjects with respiratory atopy and nonatopic subjects without AD, but with a frequency and intensity significantly lower compared to AD patients. In the present work, we have detected a rate of positivity in our patients with AD roughly comparable to rates observed in AD patients by other authors and by us in the past, when the same allergen extracts were employed [11, 13, 19, 20, 23, 24, 32], and we have confirmed in agreement with Seidenari et al. [32] that the APT is positive also in AWE and NA subjects, with a frequency significantly lower than in AD patients. Moreover, the evaluation of reading scores confirmed a significant difference of intensity in APT responses in AD patients. However, unlike other works [20, 24, 32], no difference was pointed out by us between AWE and NA subjects. Furthermore the rate of contemporaneously positive SPT and APT with HDM was roughly higher in AD subjects than in controls. These observations lead us to conclude that the APT with HDM is more specific for AD, although it is also positive in a proportion of non-AD subjects.

Whole-body extracts of mites contain not only proteic antigens but also lipidic antigens and exhibit a proteolytic enzyme activity [18, 39–42], causing potentially irritative reactions too. False-positive reactions in healthy subjects, when a high concentration of allergen is used, have been consequently suggested [18]. However, a recent study has shown that the *D. pteronyssinus* allergens, although provided by enzymatic activity, are not able to exert direct proinflammatory effects on both AD and normal human keratinocytes [43], and Bygum et al. [22] showed no skin reaction to trypsin in their APT-positive patients. The hypothesis that the positivity of APT could be related to the aspecific hyperreactivity of atopic skin can be denied also by the observation that no difference in skin irritation with sodium lauryl sulfate between APT-positive and APT-negative AD patients has been detected [37] and by the good reproducibility of the test in the same AD subjects [16, 17, 20–22].

Moreover, the reproducibility of the APT was shown by us in the past, using the extract at 20%, in a small number of AWE and NA subjects too [20], and Bygum et al. [22], using another extract, observed a good coefficient of reproducibility in AD patients and controls. In the present work, these observations were confirmed, APT showing κ values varying from fairly satisfactory (0.60) to highly satisfactory (0.85) in all the examined groups. This is in agreement with the available data, according to which the results of duplicate patch tests in an ordinary standard patch test procedure have concordances from 60 to 93% [44, 45]. The variability of κ values in this work could also depend on the loss or acquisition of immunologic responses in some subjects tested after a long time. Nevertheless, with the extract at 30% κ values were unvarying, whereas the extract at 20% showed among the 3 groups some variations in coefficient values. This can lead to conclude that the allergen concentration at 30% produces more specific responses. The good reproducibility of the APT in all the groups was also confirmed by the evaluation of the mean reading scores, performed by the paired Student's *t* test, which in all the groups did not reveal any statistical difference between the first and the second APT, using both the extract at 20% and the extract at 30%.

Some authors consider the APT as a diagnostic tool, related to the hypothesis that it represents a 'provocation test' for a subgroup of AD patients, as the bronchial or nasal provocation tests in asthma and rhinitis [4]. This concept was supported by some previous reports which showed a significant association of positive APT with HDM and the air-exposed pattern of eczema [4, 31], but this observation was not confirmed by other works [5, 7, 15, 17, 24]. Afterwards, the provocation test hypothesis was confirmed by some studies that demonstrated both the exacerbation of symptoms after exposition to aeroallergens [15] and the correlation between clinical worsening of APT-positive patients and exposure of them to HDM in their homes [5, 15]. Recently, the exposure to high concentrations of HDM allergen in early infancy has been related to the development of AD as well [46]. The improvement of AD after removal of environmental aeroallergens has also been described [47–49]. On the contrary, some other reports did not notice any improvement of overall disease activity in adult AD patients after HDM avoidance [50] and showed an unexpected significant inverse correlation between the quantity of mite antigen in mattress dust and APT reactivity [51]. Our observation, which shows no statistical association between APT outcome and the air-exposed or non air-exposed pat-

tern of eczema, is in agreement with recent works. Unfortunately, we were not able to record the exposure of AD patients to the dust and we did not evaluate the APT positivity according to their clinical history.

In this work, we also attempt to compare the results of APT obtained using two HDM extracts with different concentrations and purifications of the allergens. By our results, we pointed out that the more purified and standardized extract at 30% yielded a higher rate of positivity and a higher mean reading score in AD patients. The coefficient of reproducibility in the three groups, using this extract, was also more unvarying. This can lead us to conclude that the APT with the extract at 30% could be more specific for AD, although we were not able to relate the results to the history of the patients.

The statistical evaluation of results of this work suggests nevertheless that an immunologic specific mechanism is involved in the APT reaction in atopic subjects without eczema and nonatopic subjects as well. Seidenari et al. [32] imagine a spectrum of reactivity in different skin types, modulated by percutaneous penetration capability, sensitivity threshold and efficiency of effector mechanisms of the inflammatory response. They hypothesize also that a positive response of APT in healthy subjects may be equivalent to the finding of specific IgE by SPT or RAST, indicating an atopic state [32]. If we accept this hypothesis, other surveys on atopic individuals without eczema and nonatopic persons, with immunohistochemical studies too, are needed to show if the immunologic mechanism locally involved in APT-positive AD patients plays a role also in these subjects.

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