Acta Dermatovenerol Croat 2005;13(1):3-16 ORIGINAL SCIENTIFIC ARTICLE

Atopy Patch Test Reaction to Airborne Allergens in the Diagnosis of Atopic Dermatitis

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SUMMARY The aim of the study was to evaluate the possible use of atopy patch test in the diagnosis of atopic dermatitis and to characterize an optimal standardized system for atopy patch test in terms of allergen concentrations and time of allergen exposure. The study included 36 patients with atopic dermatitis and IgE-mediated airborne allergy. Patients presented positive results of skin prick tests and serum antigen specific IgE against house dust mite allergens and/or selected grass pollen allergens. Control groups consisted either of patients with allergic rhinitis (control group 1) or healthy volunteers with no signs or symptoms of atopy (control group 2). Allergologic diagnostic workup consisted of skin prick test, serum antigen specific IgE and total IgE evaluation, atopy patch test with selected airborne allergens of different concentrations (0.1xSPT, 1xSPT and 10xSPT), time of allergen exposure (8, 24 and 28 h), and readings of the results (8, 24, 48 and 72 h). Positive results of atopy patch test with airborne allergens were obtained in 47.2% of atopic dermatitis patients and none of control subjects. Contact reaction itself and the intensity of reaction were demonstrated to correlate with allergen concentration and time of allergen exposure on atopy patch test. The dose and time response analysis showed the optimal concentration of allergens for atopy patch test to be 10xSPT - 500000 SBE/ml, and optimal evaluation time 24 and 48 h of allergen application. There was no correlation between atopy patch test results and mean serum concentrations of total or antigen specific IgE. Atopy patch test results did not correlate with localization of skin lesions, severity and extensiveness of skin inflammation. A significantly higher contact reactivity to airborne allergens was recorded in the group of atopic dermatitis patients with polyvalent allergy in comparison with atopic dermatitis patients allergic to only one aeroallergen. It is concluded that atopy patch test is the only provocation test currently available with clinical relevance for contact IgE-mediated sensitization in atopic dermatitis patients. Using petrolatum as a vehicle, allergen concentration of 500000 SBE/ml and evaluation time of 24 and 48 h of allergen application may lead to improved atopy patch test results.

KEY WORDS: airborne allergens; atopy patch test; atopic dermatitis; standardization

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INTRODUCTION

Skin inflammation in patients suffering from atopic dermatitis (AD) may be aggravated after exposure to environmental allergens and may improve with allergen avoidance (1-3). Airborne allergens like house dust mites (HDM), plant pollen allergens, animal epithelia or moulds may be responsible for severe exacerbations of skin lesions in AD patients and there are basically two possible routes of such an influence. One route is by inhalation and so far only a few allergen inhalation studies in patients with AD were performed (4,5). Another route which seems to be more important is by skin contact with environmental allergens. An increasing number of reports indicate that in selected patients with AD, eczematous skin lesions can be induced after epicutaneous patch testing with airborne allergens (e.g., HDM) (6-10). For this procedure, epicutaneous patch test with airborne allergens known to elicit IgE-mediated reactions and evolution of eczematous skin lesions, the term atopy patch test (APT) has been proposed (6,11,12). It seems that airborne allergens after penetrating the skin may bind to FcεR1-bound IgE on Langerhans cells, which subsequently can present the antigens to T cells leading to activation of Th2 subpopulation in an acute stage and Th1 subpopulation in case of chronic stage of the reaction (13-15).

Studies on patch testing with airborne allergens were first carried out by Mitchel *et al.* (16) in 1982. Further investigations and scientific projects varied widely in the methods used. Skin abrasion (8,16,17), tape stripping (10,18) and sodium lauryl sulfate (SLS) application (19) were frequently used in order to increase allergen penetration. There were also studies on APT applied on nonabraded, nonpretreated skin (6,12,20,21) reporting different frequency of positive APT reaction. Different results were also related to various allergen concentrations in the preparations used for APT.

It seems that the variables in studies on APT that might have affected the outcome of testing were allergen concentrations, tape stripping, reading time and application site. Therefore, the first objective of our study was to re-evaluate the effects of the main variables, i.e. allergen concentrations and time of exposure.

The occurrence of positive APT in atopic patients without AD has been reported by some authors (22,23), therefore the second objective of the study was to evaluate the frequency of positive APT reaction in AD patients and to determine whether APT reaction was only specific for this population. The third objective was to analyze the results of APT in relation to the clinical course of AD, and finally to assess the correlation of APT results with other allergologic evaluations of IgE-mediated reaction.

MATERIAL AND METHODS

Patients and control subjects

Thirty-six patients with AD (22 female and 14 male) aged 6-45 (mean age 19) years, diagnosed and treated at Allergic Diseases Diagnostic Center and Department of Dermatology, University of Medical Sciences in Poznań, Poland, were selected for the study. Patients were recruited according to the following inclusion criteria: monovalent or polyvalent type of IgE-mediated airborne allergy diagnosed on the basis of clinical course of AD (exacerbations of skin inflammation after exposure to airborne allergens); results of SPT and evaluations of antigen specific IgE (asIgE) directed against common aeroallergens; clinical remission of AD symptoms at the time of APT; at least 12 months free from systemic glucocorticotherapy, phototherapy and allergen specific immunotherapy; and at least 2 weeks free from antihistamine administration.

Control groups consisted of 25 adults: control group 1 of 10 patients (6 female and 4 male) with allergic rhinitis (seasonal or perennial) aged 8-45 (mean age 21); and control group 2 of 15 healthy volunteers (9 female and 6 male) aged 7-49 (mean age 20) years, with no signs and symptoms of atopy.

Physical examination in patients with AD

AD was diagnosed according to Hanifin and Rajka criteria and in all cases complete physical examination was performed. Case history included the following elements: onset of the disease (patient age); coexistence of any other atopic diseases; family history of atopic diseases; exacerbations of skin inflammation after exposure to airborne allergens; seasonal course of exacerbations; treatment applied during the previous year; and coexistence of any systemic pathologies or administration of any therapies that might influence study results. Clinical evaluation of patients with AD was based on W-AZS index (Severity and extensiveness of skin inflammation in AD score) proposed by Silny *et al*. (24).

W-AZS I

I EVALUATION OF PRURITUS AND LOSS OF SLEEP IN PATIENTS WITH ATOPIC DERMATITIS

A. PRURITUS EVALUATION:

POINTS

No pruritus .....□

Pruritus is present:

Extensiveness:

1. Single or multiple localization of pruritus 2
2. Extensive pruritus involving the whole body surface 6

Frequency:

1. Short episodes of pruritus – less than 30 minutes 2
2. Longlasting pruritus episodes 4
3. C□ 8

Severity:

1. Scratching is not necessary 2
2. Scratching is necessary 4
3. Anxiety and irritation caused by pruritus 8

B. LOSS OF SLEEP EVALUATION

1. No□ 0
2. Problems in falling asleep 3
3. Night awakening caused by pruritus 6
4. Slee□

TOTAL I = A + B

II EVALUATION OF EXTENSIVENESS AND SEVERITY OF SKIN INFLAMMATION IN PATIENTS WITH ATOPIC DERMATITIS

Extensiveness of skin	lesions A	Severity of skin inflammation: erythema oedema	vesicles erosions	crusts scaling	lichenisation pigmentation B	AxB / 10
1. Face and neck	( )x1=	( )x3 +	( )x3 +	( )x2 +	( ) = ...	.....
2. Scalp and nucha	( )x1=	( )x3 +	( )x3 +	( )x2 +	( ) = ...	.....
3. Trunk (anterior surface)	( )x4=	( )x3 +	( )x3 +	( )x2 +	( ) = ...	.....
4. Trunk (posterior surface)	( )x4=	( )x3 +	( )x3 +	( )x2 +	( ) = ...	.....
5. Right arm	( )x1=	( )x3 +	( )x3 +	( )x2 +	( ) = ...	.....
6. Right forearm and hand	( )x1=	( )x3 +	( )x3 +	( )x2 +	( ) = ...	.....
7. Left arm	( )x1=	( )x3 +	( )x3 +	( )x2 +	( ) = ...	.....
8. Left forearm and hand	( )x1=	( )x3 +	( )x3 +	( )x2 +	( ) = ...	.....
9. Right thigh	( )x2=	( )x3 +	( )x3 +	( )x2 +	( ) = ...	.....
10. Right shank and foot	( )x2=	( )x3 +	( )x3 +	( )x2 +	( ) = ...	.....
11. Left thigh	( )x2=	( )x3 +	( )x3 +	( )x2 +	( ) = ...	.....
12. Left shank and foot	( )x2=	( )x3 +	( )x3 +	( )x2 +	( ) = ...	.....
					↑TOTAL	.....

- Score extensiveness of skin lesions from 0 to 3:
  - 0 = absent
  - 1 = 1%-10% of skin surface involved
  - 2 = 11%-30% of skin surface involved
  - 3 = 31%-100% of skin surface involved
- Score severity of skin inflammation from 0 to 3:
  - 0 = absent
  - 1 = mild
  - 2 = moderate
  - 3 = severe

TOTAL W-AZS : I +II

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Skin prick test

SPT was performed with 20 airborne allergens (Nexter Allergopharma, Reinbek, Germany) such as: *Dermatophagoides pteronyssinus* 50000 SBE/ml; *Dermatophagoides farinae* 50000 SBE/ml; cultivated rye 50000 SBE/ml; timothy grass 50000 SBE/ml; rye grass 50000 SBE/ml; meadow fescue 50000 SBE/ml. Results of SPT were evaluated after 20 minutes according to the following scale:

0 reaction to allergen equal to negative control;
+ mean diameter of allergen-induced wheal bigger than negative control but smaller than histamine-induced wheal;
++ mean diameter of allergen-induced wheal bigger than half or nearly equal to the mean diameter of histamine-induced wheal;
+++ mean diameter of allergen-induced wheal equal or slightly bigger than the mean diameter of histamine-induced wheal;
++++ mean diameter of allergen-induced wheal at least twice as big as the mean diameter of histamine-induced wheal, and all pseudopodial reactions.

Results evaluated as ++, +++ and ++++ were recorded as positive, and those evaluated as 0 and + as negative.

Total and antigen specific IgE evaluations

Serum levels of total and asIgE were measured by use of CAP-FEIA system (Pharmacia, Uppsala, Sweden). Serum samples were collected on the first day of APT from each patient with AD and allergic rhinitis. Evaluation criteria for asIgE serum concentrations were as follows :

class 0: conc.<0.35 kU/l;
class 1: conc. 0.35-0.7 kU/l (low conc.);
class 2: conc. 0.7-3.5 kU/l (medium conc.);
class 3: conc. 3.5-17.5 kU/l (high conc.);
class 4: conc. 17.5-50 kU/l (very high conc.);
class 5: conc. 50-100 kU/l (very high conc.);
class 6: conc. >100 kU/l (very high conc.);

Results evaluated as class 3, 4 ,5 and 6 were recorded as positive.

Atopy patch tests

APTs were performed with the following airborne allergens: *Dermatophagoides pteronyssinus, Dermatophagoides farinae*, cultivated rye, timothy grass, rye grass and meadow fescue. Lyophilized airborne allergens and solvent composed of 0.9% NaCl with the addition of 0.4% phenol were supplied by Allergopharma, Reinbek, Germany. These allergens were used in concentrations of 1xSPT concentration (50000 SBE/ml), 10xSPT concentration (500000 SBE/ml) and 0.1xSPT concentration (5000 SBE/ml). The vehicle for APT allergens was composed of *Eucerini anhydrati* and *vaselini albi* āā and control reaction was evaluated with the vehicle and solvent.
Allergens were applied on clinically uninvolved, untreated back skin in Finn Chambers on Scanpor (diameter 12 mm; Epitest Ltd., Oy, Finland). Results were evaluated at 8, 24, 48 and 72 hours of allergen application.
APT results were evaluated according to the following scale:

0 no reaction;
+ erythema, no edematous papules;
++ erythema, inflammatory infiltration, singular edematous papules;
+++ erythema, multiple edematous papules, singular vesicles;
++++ intensive inflammatory infiltration, multiple vesicles.

In the group of 36 patients with AD and in 10 patients with allergic rhinitis the following investigations were performed: SPT (Allergopharma, Reinbek, Germany); serum total IgE (FEIA CAP System, Pharmacia, Uppsala, Sweden); serum asIgE evaluation (FEIA CAP System, Pharmacia, Uppsala, Sweden); and APT. The group of 15 healthy volunteers underwent SPT (Allergopharma, Reinbek, Germany) and APT.

Statistical analysis

Statistical analysis was performed by use of Mann-Whitney nonparametric test, Spearman nonparametric correlation coefficient, Fisher or Fisher-Freeman-Halton test and u-Gauss test. Calculations were performed with STATISTICA v 5.5 and StatXact-3.

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RESULTS

Results of clinical evaluation in AD patients

In patients with AD and IgE-mediated airborne allergy, exacerbations of skin inflammation after exposure to airborne allergens such as HDM or plant pollen allergens can be expected. In such cases, localization of skin lesions at least at the starting point of the reaction should include so-called "airborne" regions like face, neck, upper trunk, forearms, etc. In our study, 25 (69%) AD patients reported periodical exacerbations after exposure to airborne allergens. In this population house dust was considered to be the causative factor in 13 (36%) patients, seasonal exacerbations after exposure to grass pollen allergens were recorded in 8 (22%) patients, whereas four (11%) patients described exacerbations of clinical status after exposure to both HDM and pollen allergens. In the group of 26 patients with well documented IgE-mediated allergy to *D. pteronyssinus* and *D. farinae*, 17 (65%) patients noticed exacerbations of the inflammatory process after exposure to the allergens; the same phenomenon was observed in 12 (67%) subjects from the group of 18 AD patients allergic to grass pollen allergens.

On clinical evaluation of AD patients, we analyzed localization of skin lesions according to airborne regions. Twenty-seven (75%) patients presented typical airborne localization of skin inflammatory lesions ("protected" regions, 25%).

The clinical score of AD patients ranged from 3 to 100 points (W-AZS maximal value 178 points, median 21.2 points). Patients were then divided into two subgroups according to W-AZS score: group 1, mild severity (up to 40 points) and group 2, moderate severity (over 40 points). Mild severity of AD process (median 16 points) was recorded in 27 (75%) and moderate severity of AD (median 61.3 points) in the remaining nine (25%) patients.

Results of skin prick tests

In the population of all AD patients (n=36), positive SPT results with HDM allergens and negative with grass pollen allergens were recorded in 18 patients, whereas ten patients showed positive SPT results for grass pollen allergens and negative SPT with HDM allergens. In eight AD patients SPT results were positive for both HDM and grass pollen allergens.

In control group 1 (allergic rhinitis), three patients showed positive SPT with HDM allergens (in two patients SPT result was positive for *D. pteronyssinus* and *D. farinae*; in one patient SPT was positive for *D. pteronyssinus* allergen only). For grass pollen allergens SPT results were positive in five AD patients. In total, positive SPT results for *D. pteronyssinus* allergens were recorded in 26 AD patients and five patients with allergic rhinitis. Positive SPT results for *D. farinae* were obtained in 26 AD patients and four allergic rhinitis patients. SPT with grass pollen allergens (rye grass, timothy grass and cultivated rye) produced positive results in 18 AD patients and six patients with allergic rhinitis. In control group 2 (healthy volunteers), SPT results were negative with all tested allergens. The characteristics of skin reactivity (point scale) to selected airborne allergens in AD patients are presented in Table 1. Most patients showed well defined positive skin reactivity on SPT testing (+++ or ++++).

Table 1. Evaluation of skin reaction to selected airborne allergens in skin prick test (SPT) in the group of atopic dermatitis patients

	SPT reaction to selected airborne allergens (results presented by point scale)		
	++	+++	++++
D. pteronyssinus (n=28)	1*	13	14
D. farinae (n=28)	3	13	12
Rye grass (n=18)	1	8	9
Timothy grass (n=18)	1	7	10
Meadow fescue (n=18)	1	7	10
Cultivated rye (n=18)	1	5	12

*patient with mild reaction to *D. farinae* allergens

Results of serum IgE evaluation

Results of total and asIgE evaluations in serum of AD patients and control group 1 are presented in Table 2. The mean concentration of total IgE was significantly higher in AD patients (998.5±813.7 kU/l) than in control group 1 (allergic rhinitis; 397.7±590.4 kU/l; $p<0.05$). Serum asIgE directed against selected airborne allergens were detectable in all AD patients. The mean concentration of serum asIgE for grass pollen allergens was comparable in AD patients and allergic rhinitis patients. The mean concentration of asIgE directed against HDM allergens was higher in AD patients, however, without statistical significance.

Table 2. Mean serum concentrations of total and antigen specific IgE (kU/l) ± SD in atopic dermatitis patients and control group 1

		(n)	Atopic dermatitis patients	(n)	Control group 1*	Level of significance
Mean concentration of aslgE ±SD	Mean concentration clgE ± SD	36	998.5 ± 813.7[a]	10	397 ± 590.4[a]	[a/a] - p <0.05
	D. pteronyssinus	26	49.4 ± 39.5[b]	5	31.4 ± 18.9[b]	[b/b] - NS
	D. farinae	26	53.5 ± 41.7[c]	5	38.4 ± 8.7[c]	[c/c] - NS
	Rye grass	18	48.4 ± 39.4[d]	7	50.8 ± 36.5[d]	[d/d] - NS
	Meadow fescue	18	48.0 ± 40.2[e]	7	50.7 ± 37.5[e]	[e/e] - NS
	Timothy grass	18	48.5 ± 38.1[f]	7	52.0 ± 37.5[f]	[f/f] - NS
	Cultivated rye	18	46.1 ± 40.5[g]	7	48.6 ± 34.8[g]	[g/g] - NS

Table 3. Number and percentage of atopic dermatitis patients with positive APT results according to different allergen concentrations and exposure time points

	8 h	24 h	48 h	Total number of patients with positive results to different allergen concentrations
Concentration 0.1xSPT n=36 (100%)	0 (0%)	4 (11.1%)	8 (22.2%)	9
Concentration 1xSPT n=36 (100%)	1 (2.8%)	8 (22.2%)	11 (30.6%)	16
Concentration 10xSPT n=36 (100%)	2 (5.6%)	15 (41.7%)	16 (44.4%)	17
Total number of patients with positive results in different exposure time subgroups	3	15	16	

Results of atopy patch tests

Patients with positive APT reaction were older than patients with negative APT results (mean age 21.9±11.5 *vs* 16.6±7.69 years). This difference was not statistically significant. APT reaction was positive in 17 (47.2%) AD patients and negative in all subjects from both control groups ($p<0.05$). In AD patients APT results correlated with the concentration of allergens used on testing and time of skin exposure to airborne allergens (Table 3). Nine patients showed reaction to a concentration of 0.1xSPT, 16 patients to a concentration of 1xSPT, and 17 patients to a concentration of 10xSPT. The following pattern of correlation with the time of allergen exposure was observed: with the lowest concentration of allergens, no reaction occurred after 8 h, four patients reacted after 24 h and eight patients after 48 h of exposure; with a concentration equal to SPT allergen concentration, the respective figures were one, eight and 11 patients; and with a concentration of 10xSPT reaction occurred at the respective time points in two, 15 and 16 patients. According to time of exposure, positive reaction was recorded in three patients at 8 h, in 15 patients at 24 h, and in 16 patients at 48 h of exposure to airborne allergens. At 8 h of exposure, there was no reaction to a concentration of 0.1xSPT; there was 1 reaction to 1xSPT concentration and 2 cases

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of positive reaction to 10xSPT concentration. At 24 h or exposure, four patients reacted to a concentration of 0.1xSPT, eight patients to a concentration of 1xSPT, and 15 patients to the highest concentration of 10xSPT. The longest time of exposure to airborne allergens (48 h) resulted in 8 positive reactions to the lowest concentration, 11 positive reactions to the concentration of 1xSPT and 16 positive reactions to the highest allergen concentration of 10xSPT.

All AD patients underwent APT with multiple airborne allergens (2-6 allergens). Patients showed various reactions to different allergens of equal concentrations after the same periods of exposure. Patients were considered as reacting positively in case of at least one reaction to the airborne allergen tested. This method of calculation may be somehow misleading, therefore we decided to calculate all positive reactions to the allergens not in consideration for particular patients. Results were then summed up, are discussed below and presented in Tables 4 and 5.

Further analysis of APT results according to allergen concentration used and time of exposure

APT results according to allergen concentrations at different time points are presented in Table 4. In total, we recorded 27 reactions to the lowest concentration, 65 reactions to the medium concentration, and 107 positive reactions to the highest concentration of tested allergens. Statistical analysis of the results revealed a significant increase of positive results with the increase in airborne allergen concentrations. Then, the results were analyzed according to the three different periods of exposure (Table 5). Prolongation of the period of exposure resulted in an increase in the rate of positive reactions to airborne allergens. In total, we recorded 7 reactions at 8 h, 81 reactions at 24 h and 111 positive reactions at 48 h of exposure. Statistical analysis of these results yielded a significant increase in the rate of positive results with exposure prolongation. Correlation between APT reaction and time of exposure for different allergen concentrations is illustrated in Figure 1.

Table 4. Number and percentage of positive APT reactions in atopic dermatitis patients and results of comparative statistical analysis according to allergen concentrations tested

	Time of exposure			Positive reactions (number of cases)	Statistical significance
	8 h	24 h	48 h		
Concentration 0.1xSPT	0 (0%)	8 (29.6%)	19 (70.4%)	27[a] (100%)	a/b p=0.00001
Concentration 1xSPT	1 (1.5%)	25 (38.5%)	39 (60.0%)	65[b] (100%)	a/c p=0.00001
Concentration 10xSPT	6 (5.6%)	48 (44.9%)	53 (49.5%)	107[c] (100%)	b/c p=0.00037

Table 5. Number of positive APT reactions in atopic dermatitis patients and results of statistical comparative analysis according to time of exposure to airborne allergens

	Concentration of allergens			Positive reactions (number of cases)	Statistical significance
	0.1xSPT	1xSPT	10xSPT		
8 h	0	1	6	7[a]	a/b - p=0.00001
24 h	8	25	48	81[b]	a/c - p=0.00001
48 h	19	39	53	111[c]	b/c - p=0.0012

Table 4 and Figure 2 present percentage of positive reactions at each time of exposure evaluated relative to the total number of positive reactions for particular allergen concentrations. The increase of allergen concentration to 1xSPT led to an increase in the rate of positive reactions after 24 h of exposure, however, the highest percentage of positive reactions were recorded after 48 h (38.5% and 60%, respectively). At allergen concentration of 10xSPT, a similar role for the exposure time (24 and 48 h) was observed, yielding 44.9% and 49.5% of positive reactions, respectively. Statistical comparative analysis revealed a significant difference in the percentage of positive results between 24-h and 48-h time points at allergen concentrations of 0.1xSPT ($p<0.001$) and 1xSPT ($p<0.05$). There was no significant difference in the percentage of positive APT reactions between 24-h and 48-h time points at allergen concentration of 10xSPT. We were unable to perform statistical analysis for the exposure time of 8 h because of the low number of positive reactions observed.

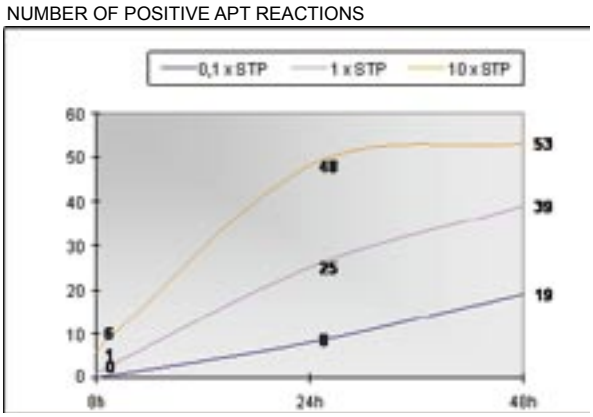

Figure 1. Relation between positive APT reaction and time of exposure at different allergen concentrations.

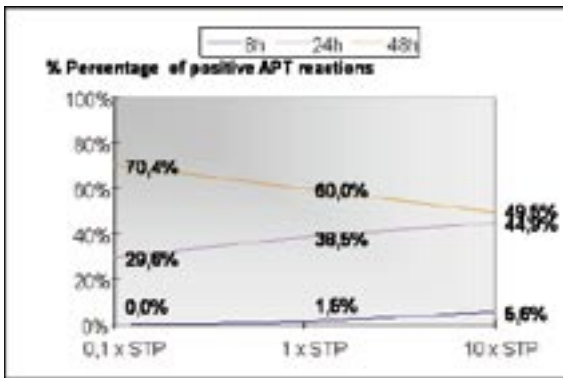

Figure 2. Positive APT reactions at different time of exposure to different airborne allergen concentrations.

Onset of positive APT reaction

In the group of AD patients with positive APT results we observed 4 different patterns of reaction according to time of exposure (Table 6). In three patients, positive reaction was recorded at all three time points, whereas 11 patients showed reaction at 24 and 48 h of exposure. Only one patient developed reaction at 24 h of exposure, whereafter the result was negative again. In two patients, positive reaction was only observed at 48 h of allergen exposure. The onset of positive APT reaction was recorded after 8 h of exposure in three, at 24 h in 12, and at 48 h in two patients.

Table 6. Onset of positive APT reaction in atopic dermatitis patients at allergen concentration of 10xSPT

		Onset of positive APT reaction after allergen application		
No.	Number of patients with positive reaction	8 h	24 h	48 h
1	3	+	+	+
2	11	−	+	+
3	1	−	+	−
4	2	−	−	+
Total	17	3	15	16

We also evaluated mean serum concentrations of asIgE in patients with positive APT reaction at different time points (Table 7). The mean concentrations of all airborne allergens tested were higher with the onset at 8 and 24 h in comparison to the onset at 48 h of exposure.

Characteristics of APT reaction according to allergen concentrations and time of exposure

Results of these evaluations are presented in Figures 3 and 4. Results for all investigated allergen concentrations and exposure periods were mostly designated as ++ (erythema, inflammatory infiltration and few papules). The increase in allergen concentration and prolongation of exposure period resulted in a significantly greater severity of topical reaction ($p=0.0001$). Results described as + (erythema) were considered nonspecific for eczematous contact reaction and doubtful, thus they were not recorded as positive.

Table 7. Mean concentration of asIgE (kU/l)±SD directed against selected airborne allergens in patients with atopic dermatitis and positive APT reaction according to exposure time

		Mean concentration of asIgE (kU/l)±SD			
Exposure time to onset of positive APT reaction	Number of patients with positive APT reaction	*D. pteronyssinus*		*D. farinae*	
8 h	1 + 1*	49.9 ± 45.05		53.1 ± 41.89	
24 h	5 + 2**	53.1 ± 40.63		58.2 ± 45.11	
48 h	1	19.4		12.8	
		Rye grass	Timothy grass	Meadow fescue	Cultivated rye
8 h	1 + 1*	55.0 ± 21.43	50.9 ± 8.84	55.5 ± 19.23	51.3 ± 22.13
24 h	5 + 2**	57.7 ± 44.67	59.8 ± 47.20	60.3 ± 43.97	60.4 ± 46.74
48 h	1	11.6	12.5	11.0	9.85

*;**these patients reacted to HDM and grass pollen allergens

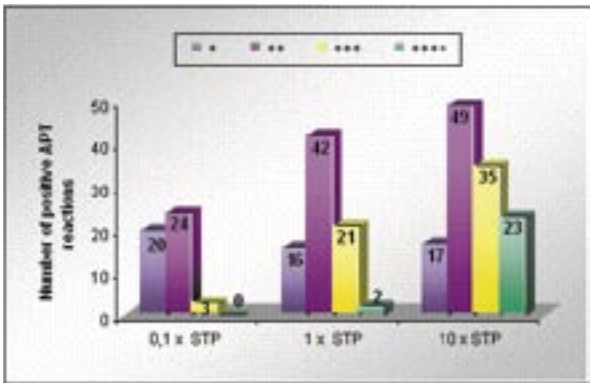

Figure 3. Relation between positive APT reaction of different characteristics and allergen concentration used on testing.

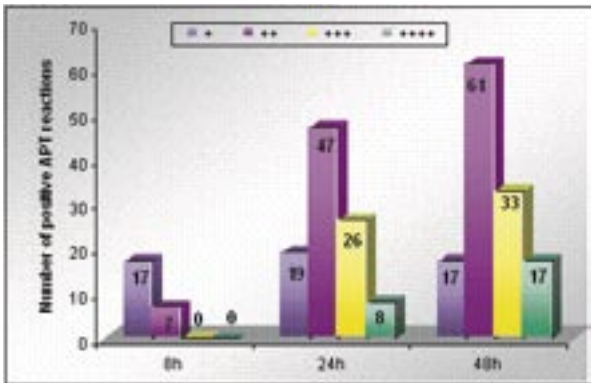

Figure 4. Relation between positive APT results of different characteristics and different periods of exposure.

Evaluation of APT results according to the evolution of positive reaction upon termination of allergen exposure (6 airborne allergens tested at allergen concentration of 10xSPT)

Evolution of APT reaction upon termination of allergen exposure is presented in Table 8. After 8-h allergen exposure 6 positive reactions were recorded. They were still positive at 24 h, however, only 3 positive reactions were observed at 48 h. At 72-h time point, all results were negative. Of 48 positive results obtained at 24 h of exposure, all remained positive at the 48-h time point and 26 were still positive at 72-h time point. Forty-two of 53 positive reactions recorded after 48 h of allergen exposure remained positive at 72 h. In total, out of 107 positive reactions, 68 (63.5%) persisted at the time point of 72 h. Intensification of APT reaction in different time-related evaluations is presented in Table 9. In total, out of 107 positive APT reactions on the first reading, 15 (14%) intensified on further readings.

Positive APT reaction according to clinical characteristics of AD patients

Within the group of patients reporting exacerbations of skin inflammation after exposure to airborne allergens (n=25), APT reaction was positive in 13 and negative in 12 cases. No exacerbations after exposure were recorded by 11 patients, four patients showed positive and seven patients negative APT results. Airborne localization of skin lesions was observed in 27 patients, 14 of them with positive and 13 with negative APT reaction. Localization of skin lesions within “protected” areas was recorded in nine patients. In this population, APT results were positive in three and negative in six

Table 8. Evaluation of positive APT reactions upon termination of allergen exposure

	Initial evaluation of APT results			Further evaluations of APT results		
	8 h	24 h	48 h	24 h	48 h	72 h
Number of positive reactions	6	–	–	6	3	0
	–	48	–	–	48	26
	53	–	–	–	–	42
	Total number of positive reactions on initial evaluation			Total number of positive reactions on further evaluations		
				24h	48h	72h
	107			6	51	68

patients. We were not able to show any significant difference in skin reactivity in APT according to localization of the skin inflammatory process.

Correlation of APT results with extensiveness and severity of skin inflammation in AD patients (W-AZS score)

Results of APT reaction in patients with mild and medium severity of AD are presented in Table 10. There was no statistically significant difference in the frequency of positive APT results between these two subgroups of AD patients.

Table 9. Evolution of positive APT reactions on different time-related evaluations

	Initial evaluation at			Intensification of APT reaction		
	8 h	24 h	48 h	24 h	48 h	72 h
Number of positive reactions	6	–	–	1	0	–
	–	48	–	–	11	0
	–	–	53	–	–	3
		Total			Total	
		107			15	

Results of APT in AD patients with monovalent and polyvalent airborne allergy

Results of this analysis are presented in Table 11. Subgroup 1 included 18 patients allergic to HDM; subgroup 2 included ten patients allergic to grass pollen allergens; and subgroup 3 included eight patients allergic to both HDM and grass pollen allergens. This analysis was performed in relation to the reactions recorded at 24 and 48 h of allergen exposure and concentration of 10xSPT. Comparative statistical analysis yielded a significantly higher percentage of positive APT reactions in the group of AD patients with polyvalent allergy.

Table 10. Correlation of APT results with extensiveness and severity of skin inflammation in 36 atopic dermatitis patients (W-AZS index score)

		APT results			
		Positive		Negative	
		Number of patients	Median W-AZS	Number of patients	Median W-AZS
Clinical score W-AZS	Mild severity of skin inflammation W-AZS <40 points	11	19	16	16
	n=27 (100%)	(40.7%)		(59.3%	
	Medium severity of skin inflammation W-AZS >40 points	6	63.4	3	44.9
	n=9 (100%)	(67%)		(33%)	

No statistically significant between-group difference (Fisher test)

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Table 11. Number and percentage of positive and negative APT reactions in atopic dermatitis patients with monovalent and polyvalent airborne allergy

		APT reactions	
		Positive (n = 101)	Negative (n = 147)
Patients with monovalent allergy	Reactions in subgroup 1 (n=72)	14	58
	Reactions in subgroup 2 (n=80)	30	50
	Total groups 1 and 2 (n=152)	44[a]	108
Patients with polyvalent allergy	Reactions in subgroup 3 (n=96)	57 [b]	39

Patients with positive APT reaction: a/b $p<0.01$

DISCUSSION

Results of our study indicated that airborne allergens such as HDM or grass pollen allergens are able to elicit eczematous skin lesions in AD patients when applied epicutaneously in a dose and time dependent way. In this study, different variables that may influence the outcome of APT were evaluated. These variables were allergen concentration, time of exposure to airborne allergens, and reading time. Positive APT results were recorded in 47.2% of AD patients. Literature data indicate that positive APT reaction with airborne allergens may occur in 15%-90% of AD patients, depending on the methodology used on testing (10,16,25). In order to obtain the most natural skin conditions for APT in our study, we did not perform stripping although many authors perform initial stripping before the application of allergens (10,16,18,26). It seems to be important to note that in these studies stripping was performed for water solutions of allergens used for APT. Water solutions lack the occlusive conditions provided by ointments and therefore initial stripping may somehow improve skin reactivity. We decided to use vaseline and eucerine containing base for allergens in order to obtain good skin penetration and permeability. Such conditions resemble the natural status of atopic skin, which is frequently being moistened and greased. Our results clearly indicate that this base is a good carrier for airborne allergens in APT reaction.

In the literature, various concentrations of allergens ranging from 1xSPT (10,23,27) to 1000xSPT (28) and native powdered form (29) have been used. Van Voorst Vader *et al.* (10) concluded their studies with the optimal allergen concentration of 500xSPT and exposure time of 48 h. According to Langeveld-Wildschut *et al.* (30), allergen concentration should be equal to 10000 AU/ml (1xSPT) and increasing allergen concentration to 1 000 000 AU/ml (10xSPT) did not significantly influence the number of patients with positive APT results. The authors advise to perform evaluation of APT after 24 and 48 h of allergen exposure, so that no eczematous reactions are missed. Darsow *et al.* (31) recorded a significant increase of positive APT reactions with allergen concentration of 10000 PNU/g in comparison to a concentration of 1000 PNU/g when testing the same population of AD patients (exposure time, 48 h). Further studies by the same authors revealed the optimal concentration of HDM allergens to be 7000 PNU/g and 5000 PNU/g for grass pollen allergens (32).

Our results revealed close correlation of APT results with the concentration of allergens used on testing. For the exposure periods of 8 and 24 h, the increase in allergen concentration (0.1xSPT; 1xSPT; and 10xSPT) nearly doubled the number of patients with positive results; at 48-h exposure time, the increase in allergen concentration also resulted in a higher number of patients with positive APT results. We recorded 199 positive results in total: 27 reactions at the lowest concentration, 65 reactions at a concentration of 1xSPT, and 107 reactions at the highest concentration of 10xSPT. The increase in allergen concentration also resulted in significant intensification of topical reaction. Therefore, we assumed that the concentration of 0.1xSPT was too low for adequate APT reaction, and further analysis was focused on the other two allergen concentrations. The highest concentration of allergens (10xSPT) resulted in a significantly higher rate of positive APT results irrespective of the time of exposure in comparison to the concentration of 1xSPT ($p<0.01$ for the overall num-

ber of positive reactions). Considering the gradual increase of positive APT reactions in parallel with the increase of allergen concentration, and the highest intensity of reaction at the concentration of 10xSPT, we chose this particular allergen concentration as being most adequate for APT. Prolongation of exposure to airborne allergens used at this concentration did not result in any significant variation of APT result. The concentration of 10xSPT was found to be a significant point defining the moment when skin contact reactivity was getting less dependent on the time of exposure to airborne allergens. This observation should be further investigated in a larger population of AD patients.

Then, various periods of exposure to the selected airborne allergens were analyzed according to number of patients and total number of reactions. At all three concentrations of allergens, the highest number of patients showed reaction after 48 h of exposure. However, with the optimal concentration of allergens (10xSPT) positive reactions were recorded at 24 h of exposure in 15 and at 48 h of exposure in 16 patients. In one patient allergic to HDM, we observed positive reactions (10xSPT) at 24 h of exposure (*D. pteronyssinus* and *D. farinae* allergens) and no reaction at 48 h. In two patients allergic to grass pollen allergens, positive reactions were only recorded at 48 h of exposure. Similar observations have been reported elsewhere (10,30). Considering the total number of positive APT reactions, 55.8% were recorded at 48 h, 40.7% at 24 h and 3.5% at 8 h of allergen exposure ($p<0.01$). The dynamics of the increase of positive reactions was clearly related to the concentration of allergens used on testing. Prolongation of exposure time from 8 to 24 h resulted in a rapid increase of positive reactions to all allergen concentrations tested. Further prolongation of the time of exposure (from 24 to 48 h) resulted in the increase of positive reactions at lower allergen concentration. Thus, as mentioned above, the time of exposure appeared to be less important at higher concentrations used on testing, and prolongation of exposure time caused an increase in the intensity of contact reaction. The latter phenomenon was not as spectacular as in case of allergen concentration increase, yet it was statistically significant.

Accordingly, our study indicated that for HDM allergens as well as for grass pollen allergens the following conditions result in an optimal outcome of APT: allergen concentration of at least 10xSPT (500000 SBE/ml), and exposure time and evaluation of the reaction at 24 and 48 hours.

The second objective of our study was to evaluate the frequency of positive APT reaction in the population of AD patients, and to determine whether APT reaction was specific only for this population of patients. The frequency of positive results was already discussed above and we did not record any positive APT reaction in either control group (patients with allergic rhinitis and healthy volunteers). These results were highly expected in subjects with no signs and symptoms of atopy, however, in patients with allergic respiratory diseases there are some data indicating APT skin reactivity (14,19,20,22,23,25). However, positive skin reaction is generally described in the literature as erythematous or urticarial, and most probably related to the immediate type of contact reaction. Therefore, it is not the same process that we are looking at.

The third objective was to analyze APT results in relation to the clinical course of AD, and to correlate APT results with total and asIgE serum concentrations. Twenty-five AD patients allergic to HDM and/or grass pollen allergens (69%) experienced exacerbations of skin inflammation after exposure to airborne allergens. In all 25 patients, SPT results and serum asIgE evaluations indicated airborne IgE-mediated allergy to allergens described by the patients as “provoking” exacerbations of skin inflammation. APT results proved contact reactivity to the mentioned allergens in 13 (52%) of these patients. On the other hand, four of 11 (36%) patients who did not observe any impact of exposure to airborne allergens on the course of AD showed positive APT results. There was no significant difference between these two subpopulations of AD patients. Therefore, it seems that in order to evaluate skin contact reactivity to airborne allergens all patients should be tested regardless of the information on the exposure impact on the course of AD. Most probably nonspecific factors and constant exposure to multiple factors may cause difficulties in proper patients’ evaluation of the provoking exposures. Literature data on this topic are also conflicting (10,31-35). We found no significant correlation between “airborne” localization and APT results either, although we were expecting it. Again, this indicates the role of APT in proper evaluation of skin contact reactivity to airborne environmental atopens. Neither the information obtained from the patient’s case history nor the clinical picture of AD seems to be sufficient in the diagnosis of airborne contact skin reactivity. Patients with more severe AD measured as W-AZS score showed a higher rate of positive APT reaction (66.7%) in comparison to patients with

mild disease (40%). However, in order to evaluate this phenomenon properly the population of AD patients should be enlarged. However, other authors did not record any relation between positive APT and severity of AD (36,37). Finally, we investigated the possible correlation of positive APT reaction with serum total and asIgE in AD patients. Patients with positive APT had slightly higher concentrations of serum total and asIgE in comparison to patients with negative APT results, however, the difference was not significant. Literature data are conflicting (10,18,32-33,38). Some authors were able to detect significantly higher serum concentrations of total and asIgE in patients with positive APT results (10,30), whereas others found no such correlation (22,32,39). We only found a significantly higher rate of positive APT results in AD patients with polyvalent airborne allergy in comparison to those with monovalent allergy.

In conclusion, APT may provide further diagnostic information in addition to SPT and serum IgE evaluations. With a standardized APT, the actual clinical relevance of IgE-mediated sensitizations for eczematous lesions might be evaluated. Further investigations correlating APT positive reaction with immunohistochemical skin analysis and evaluations of the possible influence of selected therapeutic approaches, e.g., allergen specific immunotherapy, on APT results should be performed.

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