

OUR EXPERIENCE WITH ATOPY PATCH TESTS WITH AEROALLERGENS

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Summary: Aim of our study was to evaluate the importance of atopy patch testing with aeroallergens as a diagnostic method in patients suffering from atopic dermatitis. Method: The complete dermatological and allergological examinations were performed in 29 patients; 10 men, 19 women with the average age of 27.8 years, min. 17, max. 57 years; with the median SCORAD 24.2 points, s.d. 13.3 points. Wormwood, grass, dog dander, cat dander, dermatophagoides pharinae, dermatophagoides pteronyssinus and birch pollen were examined in diagnostic procedures. Skin prick tests, specific IgE were examined; the atopy patch tests were performed with aeroallergens for skin prick tests in concentration 1× skin prick tests. Results: Specific IgE and skin prick tests to one or more tested aeroallergens were positive altogether in 27 patients; atopy patch tests were positive only in one of these patients. Conclusion: For atopy patch testing with aeroallergens the concentration of 1× skin prick tests is low to confirm the eczematous reaction in patients suffering from allergy to inhaled allergens.

Key words: Atopic dermatitis; Atopy patch tests; Specific IgE; Skin prick tests

Introduction

Atopy patch test (APT) involves epicutaneous application of type I allergens known to elicit IgE – mediated reaction, followed by evaluation of eczematous skin reaction after 48 and 72 h (1). It represents a model of cellular immunity reaction and it is presumed to reflect delayed-phase clinical reactions. According to Tonzic (2), its value is supported by the fact that atopic dermatitis is the result of complex immune interactions and involves both Coombs and Gell reactions type IV and I. APT is considered as a useful diagnostic procedure in patients with atopic dermatitis allergic to inhaled allergens (house dust mite, pollen and animal dander) and in children with food allergy younger than 2 years. The sensitivity and specificity of the test greatly depend on the tested allergens and patient age (2).

The limitations of atopy patch tests include the lack of test standardization. After standardization, the APT may provide further diagnostic information in addition to the skin prick test and serum immunoglobulin E values and may be able to evaluate the actual clinical relevance of immunoglobulin E-mediated sensitizations for eczematous lesions (3). Various concentrations of allergens for APT are described in the literature, ranging from 1× skin prick test (SPT) (10,000 AU/ml) to 1,000 × SPT (4).

Aim of our study was the evaluation if the usage of 1× skin prick tests of aeroallergens (= 100 IR/ml) is a convenient method for atopy patch testing. These tests were

performed in patients suffering from atopic dermatitis in the age 14 years and older.

Methods

Patients

29 patients over 14 years of age with atopic dermatitis (the diagnosis was made according to the Hanifin-Rajka criteria (5)) were examined at the outpatient department of Department of Dermatology and Venereology, Faculty Hospital and Medical Faculty of Charles University, Hradec Králové, Czech Republic, from September 2010 to May 2012.

Complete dermatological and allergological examinations were performed in all included patients (including the examinations for asthma bronchiale with spirometry). The occurrence of rhinoconjunctivitis was evaluated.

Scoring of atopic dermatitis

Severity of eczema was scored in agreement with SCORAD score, with assessment of topography items (affected skin area), intensity criteria (extent of erythema, oedema, crusts, excoriations, lichenification, xerosis), and subjective parameters (extent of itch and loss of sleep). Mild form to 20 points, moderate 21 to 50 points, over 50 points severe form of atopic dermatitis.

Tested allergens

Wormwood, grass pollen, dog dander, cat dander, House dust mites – *Dermatophagoides pharinae*, *Dermatophagoides pteronyssinus*, birch pollen were used in testing procedures. After discontinuation of antihistamines and topical steroids for at least 5 days and systemic steroids and UV therapy at least 2 months, the skin prick tests, the atopy patch tests were performed. Specific IgE was examined.

Skin prick test

Commercial extracts Alyostal (Stallergens, France) were used for skin prick tests (SPT).

SPTs were placed on the volar side of the forearm according to the extent of atopic dermatitis. SPTs were carried out by a standardized method using lancets with a 1 mm tip. The results were read after 15 minutes and were assessed by comparison with the wheal induced by histamine (10 mg/ml) and negative control. A wheal with a diameter greater than 3 mm in comparison with negative control was scored as positive.

Specific IgE

The serum level of the specific IgE to the tested aeroallergens has been measured with the method of CAP (system FEIA – Pharmacia Diagnostics, Uppsala, Sweden). The level of specific IgE higher than 0.35 U/ml was assessed as positive.

Atopy patch tests

Atopy patch tests were performed on non-lesional, non-abraded, untreated skin of the back during a remission.

A technique similar to conventional patch tests has been used by performing of atopy patch testing – CURATEST F strip (Lohmann & Rauscher International GmbH & Co. KG D-56579, Rengsdorf, Germany) with 12mm cup size. For atopy patch testing the concentrations of allergens 1× SPT was used – commercial extracts Alyostal (Stallergens, France), 1 ml of this allergen was administered to 12 mm cup size of patch testing. The reactions were evaluated in 48 and 72 hours after the first application of allergens.

Grading of positive APTs reactions was similar to the criteria used in conventional contact allergy patch testing with the modifications of the European task Force on Atopic Dermatitis (EFTAD) Consensus Meetings; i.e. + erythema, infiltration, ++ erythema, infiltration, papules (up to 3), +++ erythema, papules from 4 to many, ++++ erythema, many or spreading papules and vesicles. Test application and reading was performed by an investigator with no knowledge of the patient's history. Only reactions from + (erythema, infiltration) onwards were designated positive (6).

Results

Patients

Altogether 29 persons suffering from atopic dermatitis were included in the study: 10 men, 19 women with the average age of 27.8 years, min. 17, max. 57 years; with the median SCORAD 24.17 points, s.d. 13.3 points.

Personal history

Rhinoconjunctivitis was recorded in 21 patients.

Spirometry examination

Asthma bronchiale was recorded in 12 patients.

Specific IgE

Specific IgE to tested aeroallergens were recorded in 16 patients (to birch in 2 patients, to grass in 7 patients, to wormwood in 4 patients, to cat or dog dander in 6 patients, and to *dermatophagoides farinae* or *pteronyssinus* in 9 patients) (table 1).

Skin prick tests

Positive results in skin prick tests to tested aeroallergens were recorded in 25 patients (to birch in 8 patients, to grass in 17 patients, to wormwood in 2 patients, to cat or dog dander in 5 patients, and to *dermatophagoides farinae* or *pteronyssinus* in 11 patients) (table 1).

Atopy patch tests

Atopy patch test were recorded as positive in 1 patient – to wormwood, grass and *dermatophagoides farinae* (table 1, patient No 23), evaluated as ++. The positive results in skin prick tests were recorded to *dermatophagoides farinae*, grass and cat dander, in sIgE to grass and *dermatophagoides farinae* (table 1).

Discussion

The first experimental study on patch test with aeroallergens was published in 1937 by Rostenberg and Sulzberger, and in 1982 by Mitchell (7, 8) and various APT techniques have been described in the literature. In order to enhance the penetration of the allergen into the skin, skin abrasion, tape – stripping and sodium lauryl sulfate application were used (4). Today, APT is performed on nonlesional, untreated skin in remission (4). The European Task Force on Atopic Dermatitis (EFTAD) has developed a standardized APT technique. It consists of purified allergen preparation in petrolatum, applied in 12 mm diameter Finn chambers mounted on Scanpor tape to non-irritated,

Tab. 1: Results of examination in 29 patients

Patient, sex, age	Specific IgE	Skin prick test	Atopy patch test	SCORAD (points)	Total IgE IU/ml	AB	RC
1, W, 20	D, G, CD	–	–	15	1224	–	+
2, M, 20	W, CD	D, W	–	30	608	+	+
3, W, 27	B, W	D, CD, G	–	20	748	–	+
4, M, 28	G	D		26	1071	+	+
5, W, 15	CD	G	–	32	200	–	+
6, W, 39	D, CD, W	G,	–	32	2475	+	+
7, W, 23	D,	G	–	31	74	–	–
8, W, 24	G	G, CD	–	27	86	–	–
9, M, 38	–	D, B, CD	–	42	2331	+	+
10, M, 38	G,	B, G	–	20	89	–	+
11, W, 26	D, G, CD	G, D, B	–	35	335	+	+
12, W, 26	D, G	–	–	31	632	+	+
13, M, 20	–	–	–	32	4473	+	+
14, M, 21	–	D, CD, G	–	18	181	+	+
15, W, 21	–	G, B	–	22	1864	–	+
16, M, 26	–	G	–	19	547	–	–
17, W, 30	–	G, B	–	28	25	–	–
18, W, 57	–	G, D	–	15	100	+	+
19, W, 19	W, CD, D	G, B	–	12	53	–	–
20, W, 41	–	–	–	12	120	–	–
21, W, 38	–	CD	–	19	23	+	+
22, M, 19	–	D, G	–	25	4022	–	+
23, M, 17	D, G	D, G, CD	W, G, D ++	28	5000	+	+
24, M, 19	–	D, W	–	29	768	–	+
25, W, 25	–	G	–	10	9	–	+
26, W, 30	–	B	–	11	527	–	–
27, W, 39	D	G, D	–	18	163	–	+
28, W, 43	G	G	–	32	822	–	–
29, W, 20	D, B	G, B	–	30	1055	+	+

W: wormwood; G: grass; CD: dog, cat dander; D: Dermatophagoides pharinae, Dermatophagoides pteronyssinus; B: birch; AB: asthma bronchiale + yes, – no; RC: rhinoconjunctivitis + yes, – no

non-abraded, or tape-stripped skin on the upper back (8). The test is read after 48 hours and 72 hours and the reading key is the appearance of erythema, and the number and distribution pattern of the papules. Usage of aeroallergens concentration over 5,000 PNU (protein nitrogen units/g in petrolatum allows for testing on clinically uninvolved skin without potentially irritating tape-stripping (9).

Various concentrations of allergens are described in the literature, ranging from $1 \times$ SPT (10,000 AU/ml) to $1,000 \times$ SPT (4). Van Voorst Vader et al. conclude that the optimal allergen concentration should be $500 \times$ SPT with exposure time of 48 h (10). Langeveld-Wildschut et al. conclude that concentration should be equal to $1 \times$ SPT and according to their results increasing the allergen concentration to up to $10 \times$ SPT did not significantly influence the number of positive results (11). In their study, APTs were performed in 84 patients with atopic dermatitis, 30 control patients with atopic disease, and 85 healthy volunteers, with house dust mite and grass pollen allergens in concentrations of 100, 1,000, 10,000, and 100,000 allergenic units/ml.

The authors from Poland also studied the impact of allergen concentration and found that $0.1 \times$ SPT was too low, while $10 \times$ SPT concentration had significantly more positive reactions than $1 \times$ SPT (12).

When biopsy is performed from allergen-induced eczematous APT site, allergen specific T cells are cloned (13)). The TH2 cytokine pattern is initially present and after 48 h TH1 pattern is predominant (13). An early influx of inflammatory dendritic epidermal cells into lesional skin has been demonstrated (14). When allergen is captured by IgE molecules, it binds to IgE receptor on Langerhans cells. Antigen presentation results in specific T cell reaction which is responsible for eczematous reaction observed clinically (14). T cells are responsible for the reaction occurring in lesional skin in atopic dermatitis and also in the skin in APT, and macroscopic and microscopic similarities indicate that APT is valid model for inflammation found in atopic dermatitis (15).

According to our results in specific IgE and in skin prick test, the allergy to aeroallergens is common in patients suffering from atopic dermatitis; 12 patients of our study suffer from asthma bronchiale and 21 patients from rhinoconjunctivitis. The positive result in atopy patch test was recorded only in one patient. This patient suffers from moderate form of atopic dermatitis, from allergy to aeroallergens according to the result in sIgE and SPT, suffers from asthma bronchiale and rhinoconjunctivitis; his level of total IgE is 5,000 IU/ml, this level is the highest in comparison with the results of other patients.

Literature data indicate that positive APT reactions can occur in 15–90% of atopic eczema patients, depending on the methodology used in testing (12, 16). Healthy individuals as well as patients with respiratory atopy without a history of eczema have negative APT or react to house dust with lower frequency and intensity compared with atopic patients (12).

Ronchetti et al. found positive APT with food in 4–11% and with aeroallergens in 4–30% of an unselected children population, depending on allergen tested (17), and these results are in conflict to other study results (4, 18).

For our atopy patch testing the concentrations of allergens $1 \times$ SPT was used from commercial extracts Alyostal (Stallergenes, France). This company makes use of IR units in skin prick tests. The definition is that it is a measure of allergenic efficacy; it is extract of allergens with the content of 100 IR, which provokes in skin prick test with the use of lancet Stallerpoint a wheal about the size 7 mm in 30 patients with sensibilisation to this allergen. The other company producing skin prick tests ALK Abelló has units of allergens in HEP (histamin equivalent prick) and Sevapharma makes use of PNU/g (Protein nitrogen units/g). That is why it is difficult to identify the solutions in skin prick tests with high concentration of allergens, because each company represented on the market (Sevapharma, Stallergenes, ALK Abelló) has chosen its own allergen standardization with its own unit of measure. The direct conversion from one unit to another is not possible. What is missing is the 'gold standard' upon which the various 'currencies' orient themselves and which allows clear comparability. We can conclude, that the concentration of $1 \times$ skin prick tests of Stallergenes is not right for atopy patch testing.

The European APT model used with standardization of allergen concentration and vehicle may provide an important diagnostic tool to select patients for avoidance and for procedures of allergen-specific immunotherapy, but the clinical relevance of positive APT reactions awaits standardized provocation and avoidance testing (3).

Conclusion

The concentration of aeroallergens of $1 \times$ skin prick tests (extracts Alyostal, Stallergenes, France) is too low to confirm the eczematous reaction in patients suffering from allergy to inhaled allergens in atopy patch testing.

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