Pediatr Allergy Immunol 2008
DOI: 10.1111/j.1399-3038.2007.00712.x

PEDIATRIC ALLERGY AND IMMUNOLOGY

Epidemiology of atopy patch tests with food and inhalant allergens in an unselected population of children

Ronchetti R, Jesenak M, Trubacova D, Pohanka V, Villa MP. Epidemiology of atopy patch tests with food and inhalant allergens in an unselected population of children.
Pediatr Allergy Immunol 2008.

Atopy patch test (APT) has been used as a diagnostic tool in patients with suspected food or inhalant allergy. This study assessed the prevalence of positive APT with food or inhalant allergens in an unselected population of schoolchildren. We also evaluated the link between positive APT reactions and skin-prick tests (SPT) for food and inhalant allergens, circulating eosinophils and histamine skin reactivity. We studied an unselected population of 380 children aged 9 or 13 yr living in Rome, Italy. APTs were carried out with food (native or standardized) and inhalant allergens. All the children also underwent skin-prick testing with five common inhalant and four food allergens. We also measured eosinophil cell counts and histamine skin reactivity. The prevalence of positive APT reactions for foods in unselected children ranged between 4% and 11% for hen's egg, tomato, and wheat flour and was similar for both age groups studied. The prevalence of positive APT for milk was significantly lower in children aged 13 than in children aged 9 ($p = 0.013$). No concordance emerged between positive APT and SPT for foods. Conversely, APT and SPT for inhalant allergens yielded statistically significant concordance ($p < 0.001$). APT produces positive reactions for food or inhalant allergens in a significant number of subjects in the general population of schoolchildren. Age influences the prevalence of positive APTs with cow's milk to some extent. Inhalant allergens probably induce a positive APT reaction through an immunoglobulin E-linked process, while food allergens probably do not.

Roberto Ronchetti[1], Milos Jesenak[1,2], Dagmar Trubacova[1], Vladimir Pohanka[3] and Maria Pia Villa[1]

[1]Department of Paediatrics, 2nd School of Medicine, University ``La Sapienza'', Rome, Italy, [2]Department of Paediatrics, Jessenius School of Medicine, Comenius University in Bratislava, Martin, Slovak Republic, [3]Srobar's Institute for Respiratory Diseases and Tuberculosis for Children, Dolný Smokovec, Vysoké Tatry, Slovak Republic

Key words: atopy patch test; children; epidemiology; food allergens; inhalant allergens; unselected population

Professor Roberto Ronchetti, MD, Clinica Pediatrica, Ospedale Sant'Andrea, Via Grottarossa 1035/1039, 00189 Rome (RM), Italy
Tel.: +39 06 3377 5856
Fax: +39 06 3377 5941
E-mail: roberto.ronchetti@ospedalesantandrea.it

Accepted 3 December 2007

The 'atopy patch test' (APT), 'a skin reaction induced in patients with atopic dermatitis by applying food allergens or aeroallergens on non-lesional skin', was first described in 1982 (1). Thereafter, it was used in many studies in adults and children with the purpose of obtaining diagnostic results in allergology similar to those obtained by dermatologists using an array of chemical substances on the skin of patients with contact dermatitis (2). Several technical procedures, aimed at increasing the permeability of the tested skin (including abrasion, stripping and high concentrations of allergens vehiculated in special solvents), were initially widely used to facilitate positive test results (3–6). All of these procedures were later abandoned, because they proved unnecessary and difficult to standardize. The current standard APT method entails the use of commercial or naive food allergens, Finn chambers, an occlusion time of 48 h and reading at 48 and 72 h (7). APT is now a widely applied procedure, especially aimed at the diagnosis of food allergy (8–14). Some points, however, remain unresolved. The original idea

that a positive APT was an 'eczematous' reaction occurring on the skin of patients with eczema, mainly because these persons had unusually reactive skin (or skin with altered permeability), naturally lead to the concept that an APT would invariably be negative in patients without eczema (15–18). This statement, supported by the results of some studies, has never been clearly addressed or contradicted by an experiment designed specifically to test it. However, several reports including groups of healthy controls or atopic patients without eczema show a considerable percentage of positive APTs in non-eczematous patients (19–20). Existing data suggest that the prevalence of positive APTs in children selected for suspected food allergy is higher in those with positive food challenges than in those with negative food challenges (9, 19, 21, 22). In general, in healthy or symptomatic subjects, APT results differ widely among tested allergens (naive or standardized food) and depend closely on technical variables (e.g. allergen concentration, size of the chamber, occlusion time and site of allergen application) (3–5, 23, 24) and on personal characteristics of the tested person, e.g. age (2, 20, 23–25). Several studies obtained similar results of APT using inhalant allergens (2–6, 15–17, 19–20). Because no study has assessed the influence of all these variables on the prevalence of positive APT in healthy or unselected children, the expected prevalence in these populations is largely unknown.

Another area of uncertainty is the relationship between APTs and immunoglobulin E (IgE) sensitization (atopy). Although an APT can yield positive reactions in non-atopic subjects with negative allergen skin-prick tests (SPT) (9, 21, 25–27), evidence showing that IgE-linked mechanisms intervene in the pathogenesis of the skin reaction during the early phases of the APT has been produced for inhalant allergens, mainly *Dermatophagoides pteronyssinus* (2–4, 6). Yet no similar information is available for APTs with food allergens. More generally, it is also not clear whether an APT is more likely to yield a positive reaction in atopic patients (19, 28, 29).

We designed this study to assess the prevalence of positive food APT reactions in unselected schoolchildren aged 9 and 13. We also evaluated the link between positive APT reactions and SPT for food and inhalant allergens, circulating eosinophils and histamine skin reactivity. We performed APTs on 380 schoolchildren aged 9 or 13 yr living in the north of Rome, Italy.

Materials and methods

Study populations

We studied an unselected population of 380 children attending two elementary schools in the north of Rome (Italy) from October 2005 to March 2006. The population consisted of two age groups. The older group, (196 children, aged 12.60 ± 0.89 yr), underwent APT with fresh food allergens (103 subjects) or standardized food allergens in petrolatum (93 subjects). The younger group (184 children, aged 8.71 ± 1.41 yr) underwent APT only with fresh food allergens. A subgroup of children (131: 43 belonging to the older group and 88 to the younger group, 58% boys) underwent atopy patch testing also with two inhalant allergens. For 1 wk before skin testing, all children were asked to refrain from antihistamine medications and from inhaled or oral corticosteroids.

The study was approved by the Ethical Committee of the Paediatric Clinic of Rome University 'La Sapienza'.

Atopy patch tests

Atopy patch tests were carried out with plastic quadratic chambers 10 mm in diameter (Finn Chambers, Haye's, the Netherlands). The allergens were placed into the chambers and were attached to an area of unaffected skin on the children's backs on both sides. As a control, physiological solution was used. In the older group, we applied APT either with fresh foods: cow's milk (containing 3.5% fat), whisked hen's egg (yolk and white of egg), tomato and wheat flour (dissolved in saline, 1 g/10 ml) (103 subjects) or with standardized food allergens (cow's milk 10%, hen's egg 10%, tomato 20%, wheat flour 10%) dissolved in petrolatum (Lofarma S.p.a., Milano, Italy) (93 subjects). In the younger group (184 subjects), we applied the four fresh food allergens. In the subgroup of 131 children from both groups (43 subjects aged 9 yr and 88 aged 13 yr), we used for APT two standardized inhalant allergen solutions for SPT (*Dermatophagoides pteronyssinus* and mixed grasses 1: *Avena sativa, Hordeum vulgare, Secale cereale, Triticum sativum*; ALK-ABELLO, Hørsholm, Denmark). The occlusion time was 48 h. The results were read at 20 min after the chambers were removed and at 72 h for the final test evaluation. The reading criteria were those recommended by the revised European Task Force on Atopic Dermatitis key for APT reading (7). Reactions were classified as positive if the test yielded erythema with infiltration, papules or

both. Erythema without palpable infiltration was considered questionable. In the final judgment, questionable results were considered negative. All test results were read by the same two well-trained operators.

Skin-prick tests

HSPT were carried out in a pre-defined area on the volar side of the left forearm, with a space of at least 2.5 cm between each prick with the following inhalant allergen panel: *Dermatophagoides pteronyssinus,* mixed grasses 1 (*Avena sativa, Hordeum vulgare, Secale cereale, Triticum sativum*), mixed trees (*Betula verrucosa, Corylus avellana, Alnus glutinosa*), cat dander and *Alternaria alternata* (ALK-ABELLO). On the right volar forearm, we pricked the same four fresh food allergens used for APT (whole cow's milk, whisked hen's egg, tomato and wheat flour dissolved in 1 mg/10 ml saline). We also did a prick test with histamine dihydrochloride (10 mg/dl). As a negative control, 50% glycerine in saline was applied on the right forearm. We used 1-mm tip metallic lancets (ALK-ABELLO). The lancet was pricked vertically into the skin through each drop for 2 s with firm pressure. A new lancet was used for each prick test. Ten minutes after the procedure was finished, the wheals were outlined with a thin felt-tip pen. The contours were transferred to the record sheet with translucent tape. The size of each weal was measured as the mean of the longest diameter and the diameter perpendicular to it at its mid point. An SPT with allergens was defined as positive if the wheal was ≥3 mm in its longest dimension. All the tests were carried out by the same two well-trained operators. The children with at least one positive SPT to common inhalant or food allergens were considered as atopic.

Serum studies

Peripheral venous blood samples were drawn from 278 children. Eosinophils were measured within 2 h with an automated blood cell counter (Advia 120, Bayer®, Leverkusen, Germany).

Statistical methods

All data are expressed as mean values ± s.d. Data were analysed with the software package SPSS version 9.0 (SPSS Inc. Chicago, IL, USA). Student's two-tailed *t*-test, chi-squared (χ^2) test and Fisher's exact test were used for statistical comparison. p-Values ≤ 0.05 were considered to indicate statistical significance.

Results

In unselected children, the prevalence of positive APT reactions for foods ranged between 4% and 11% for hen's egg, tomato and wheat flour and was similar for both age groups studied (subjects aged 9 and 13 yr). Conversely, for cow's milk, the prevalence differed significantly between younger and older children (11.4% vs. 4.1%, p = 0.013, χ^2-test) (Table 1). The difference was even more significant taking into consideration only the results characterized by the presence of 'papules' (1.5% vs. 7.6%, p = 0.009, Fisher's exact test). APTs with the two inhalant allergens (*Dermatophagoides pteronyssinus* and mixed grasses) yielded significantly different rates of positive reactions in the two age groups (Table 1). Older children were more atopic and showed higher histamine skin reactivity than younger children.

Only for cow's milk and only in children aged 9 did the prevalence of positive APT reactions significant differ by sex; it was higher in boys than in girls (19.0% vs. 6.1%, p = 0.018).

No concordance emerged between positive APT and SPT for foods in either children aged 9 or those aged 13 (Table 2): none of the 100 positive APT reactions for the different food allergens concorded with SPT carried out with the same allergen. Conversely, APT and SPT for inhalant allergens yielded statistically significant concordance: about 50% of the children who had positive SPT reactions also had positive APT reactions for the same allergen (Table 2).

The mean number of positive SPT was similar in subjects with positive APT and those with negative APT with food allergens. No differences were found in histamine skin reactivity between subjects with positive and negative APT reactions. Eosinophil cell counts were also similar in subjects with positive and negative APT reactions.

Discussion

The first finding from our large epidemiological study on APT in unselected schoolchildren aged 9 and 13 is that this diagnostic procedure produces positive reactions for food or inhalant allergens in a significant number of subjects in the general population. This finding contradicts some reported statements that APT, being an 'eczematous' reaction of the skin, is positive only

Table 1. Studied unselected population of children aged 9 and 13

	Group 1 (APT with foods*, age 13 years)	p (1 vs. 2)	Group 2 (APT with foods, age 9 years)	p (2 vs. 3)	Group 3‡ (APT with inhalants, age 9 years)
Population					
Number	196	–	184	–	131
Males	93 (47.4%)	n.s.	97 (52.7%)	n.s.	76 (58%)
Age (years)	12.60 ± 0.89	<0.001	8.71 ± 1.41	n.s.	9.40 ± 2.24
Height (m)	1.57 ± 0.08	<0.001	1.32 ± 0.01	n.s.	1.29 ± 0.08
Weight (kg)	50.86 ± 10.21	<0.001	32.33 ± 8.22	n.s.	29.74 ± 6.75
Atopy patch tests (prevalence of positive results)†					
Food allergens				Inhalant allergens	
Cow's milk	8/196 (4.1%)	0.013	21/184 (11.4%)	*Dermatophagoides*	39/131 (29.8%)
Hen's egg	20/196 (10.2%)	n.s.	15/184 (8.2%)	*pteronyssinus*	
Tomato	6/156 (3.8%)	n.s.	8/184 (4.3%)	*Mixed grasses 1*	5/131 (3.8%)
Wheat flour	11/196 (5.6%)	n.s.	11/184 (6.0%)		
Skin prick tests (prevalence of positive results)					
Inhalants					
Dermatophagoides pteronyssinus	45 (23%)	n.s.	31 (16.8%)	n.s.	20 (15.3%)
Mixed grasses 1	39 (19.9%)	0.003	16 (8.7%)	n.s.	6 (4.6%)
Mixed trees	16 (8.2%)	<0.001	0 (0.0%)	n.s.	1 (0.8%)
Cat dander	19 (9.7%)	0.010	(2.7%)	n.s.	2 (1.5%)
Alternaria alternata	20 (10.2%)	0.047	8 (4.3%)	n.s.	4 (3.1%)
At least 1 + SPT with inhalants	66 (33.7%)	0.003	36 (19.6%)	n.s.	24 (18.3%)
Foods					
Cow's milk	4 (2.0%)	n.s.	1 (0.5%)	n.s.	0
Hen's egg	2 (1.0%)	n.s.	0 (0.0%)	n.s.	0
Tomato	6 (3.1%)	n.s.	2 (1.1%)	n.s.	0
Wheat flour	3 (1.5%)	n.s.	1 (0.5%)	n.s.	0
At least 1 + SPT with foods	12 (6.1%)	n.s.	4 (2.2%)	n.s.	0
At least 1 + SPT with inhalants or foods	69 (35.2%)	0.002	38 (20.7%)	n.s.	24 (18.3%)
Number of positive SPT, mean ± s.d.	0.79 ± 1.31	<0.001	0.35 ± 0.76	n.s.	0.25 ± 0.57
Histamine skin reactivity (10 mg/ml wheal diameter, mean ± s.d.) (mm)	5.93 ± 1.25	<0.001	4.54 ± 1.43	n.s.	5.30 ± 1.47
Blood eosinophil cell count (10^3/1 μl)	0.26 ± 0.53	n.s.	0.31 ± 0.30	n.s.	0.31 ± 0.31

*No significant difference was found in APT performed with fresh foods (103 subjects) or with commercial food preparation (Lofarma S.p.a., Milano, Italy; 93 subjects).

†43 children belong to group 1 and 88 to group 2.

‡Results obtained with food allergens (fresh or standardized) or ihnalant allergens (preparation for skin prick test, ALK-ABELLO) in the left side of the back (include all tests which gave infiltration with erythema and/or papules).

Table 2. Concordance between the results of atopy patch tests and skin prick tests with food (fresh or standardized) or inhalant allergens

	Group 1 (APT with foods, age 13 years)	p	Group 2 (APT with foods, age 9 years)
Cow's milk			
	Left		Left
neg.	174 (88.8%)		153 (83.2%)
①?	14 (7.1%)		10 (5.4%)
②+	5 (2.6%)		7 (3.8%)
③++	1 (0.5%)	0.009	11 (6.0%)
④+++	2 (1.0%)		3 (1.6%)
pos. (②+③+④)	8/196 (4.1%)	0.013	21/184 (11.4%)

in the subjects with atopic eczema (3, 6, 7, 16, 19).

The prevalence of positive APT for milk was significantly lower in children aged 13 than in children aged 9. This age-related decline in positive APT reactions for cow's milk by the age of 13 would be even more significant if only the presence of papules were considered as a criterion of ATP positivity (APT for cow's milk elicited papules five times more frequently at the age of 9 than at the age of 13). This finding is in accordance with literature observations that APT positivity with food and inhalant allergens tends to be less prevalent in older subjects (2, 24, 20).

When we evaluated the link between positive APT reactions and SPTs, we found no association for food allergens, but a statistically significant association for inhalant allergens. It appears therefore that the results of APT differ according to the type of allergen tested. Given that SPT with inhalant allergens evoke a positive reaction by mean of a mechanism linked to IgE, our finding suggests that APT reactions with inhalants are

also in some way produced by this mechanism, but that positive APTs with food take place using other immunological mechanisms. This finding is in agreement with pathological findings reported in the literature. Several studies show that APT with *Dermatophagoides* are largely dependent on an IgE-mediated mechanism (2, 4, 5, 27), whereas in positive APT with foods, skin biopsies detected immuno elements attributable to all four Gell and Combs reactions (27).

In conclusion, our robust epidemiological approach to APT with food allergens, evaluating two large groups of unselected children at different ages with food allergens and one similar age group with inhalant allergens, shows that APT with both types of allergens can be found positive in about 4–11% for food allergens and in about 4–30% for inhalant allergens of an unselected children population. In our study, age influenced the prevalence of positive APTs to some extent. Inhalant allergens probably induce a positive APT reaction through an IgE-linked process. Conversely, the absence of a link between the results of APTs with food allergens and SPTs with the same allergen suggests that food allergens do not.

References

1. Mitchell EB, Crow J, Chapman MD, Jouhal SS, Pope FM, Platts-Mills TA. Basophils in allergen-induced patch test sites in atopic dermatitis. Lancet 1982: 1: 127–30.
2. Darsow U, Laifaoui J, Kerchenlohr K, et al. The prevalence of positive reactions in the atopy patch test with aeroallergens and food allergens in subjects with atopic eczema: a European multicenter study. Allergy 2004: 59: 1318–25.
3. Darsow U, Vieluf D, Ring J. Atopy patch test with different vehicles and allergen concentrations: an approach to standardisation. J Allergy Clin Immunol 1995: 95: 677–84.
4. Heinemann C, Schliemann-Willers S, Kelterer D, et al. The atopy patch test – reproducibility and comparison of different evaluation methods. Allergy 2002: 57: 641–5.
5. Oldhoff JM, Bihari IC, Knol EF, Bruijnzeel-Koomen CAFM, de Bruin-Weller MS. Atopy patch test in patients with atopic eczema/dermatitis syndrome: comparison of petrolatum and aqueous solution as a vehicle. Allergy 2004: 59: 451–6.
6. Seidenari S, Manzini BM, Danese P, Giannetti A. Positive patch test to whole mite culture and purified mite extracts in patients with atopic dermatitis, asthma and rhinitis. Ann Allergy 1992: 69: 201–6.
7. Turjanmaa K, Darsow U, Niggemann B, Rancé F, Vanto T, Werfel T. EAACI/GA^{2}LEN position paper: present status of atopy patch test. Allergy 2006: 61: 1377–84.
8. Heine RG, Verstege A, Mehl A, Staden U, Rolinck-Werninghaus C, Niggemann B. Proposal for a standardized interpretation of the atopy patch test in children with atopic dermatitis and suspected food allergy. Pediatr Allergy Immunol 2006: 17: 213–7.
9. Majamaa H, Moisio P, Holm K, Kautianen H, Turjanmaa K. Cow's milk allergy: diagnostic accuracy of skin prick and patch tests and specific IgE. Allergy 1999: 54: 346–51.
10. Mehl A, Rolinck-Werninghaus C, Staden U, et al. The atopy patch test in the diagnostic workup of suspected food-related symptoms in children. J Allergy Clin Immunol 2006: 118: 923–9.
11. Niggemann B. The role of atopy patch test (APT) in the diagnosis of food allergy in infants and children with atopic dermatitis. Pediatr Allergy Immunol 2001: 12 (Suppl. 14): 37–40.
12. Roehr CC, Reibel S, Ziegert M, Sommerfeld C, Wahn U, Niggemann B. Atopy patch tests, together with determination of specific IgE levels, reduce the need for oral food challenges in children with atopic dermatitis. J Allergy Clin Immunol 2001: 107: 548–53.
13. Fogg MI, Brown-Whitehom TA, Pawlowski NA, Spergel JM. Atopy patch test for the diagnosis of food protein-induced enterocolitis syndrome. Pediatr Allergy Immunol 2006: 17: 351–5.
14. Hansen TK, Host A, Bindslev-Jensen C. An evaluation of the diagnostic value of different skin tests with egg in clinically egg-allergic children having atopic dermatitis. Pediatr Allergy Immunol 2004: 15: 428–34.
15. Beltrani V, Hanifin J. Atopic dermatitis, house dust mites, and patch testing. Am J Contact Dermat 2002: 13: 80–2.
16. Bruijnzeel PLB, Kuijper PHM, Kapp A, Warringa RAJ, Betz S, Bruijnzeel-Koomen CAFM. The involvement of eosinophils in the patch test reaction to aeroallergens in atopic dermatitis: its relevance for the pathogenesis of atopic dermatitis. Clin Exp Allergy 1993: 23: 97–109.
17. Czarnecka-Operacz M, Bator-Wegner M, Silny W. Atopy patch test reaction to airborne allergens in the diagnosis of atopic dermatitis. Acta Dermatovenerol Croat 2005: 13: 3–16.
18. Osterballe M, Andersen KE, Bindslev-Jensen C. The diagnostic accuracy of the atopy patch test in diagnosing hypersensitivity to cow's milk and hen's egg in unselected children with and without atopic dermatitis. J Am Acad Dermatol 2004: 51: 556–62.
19. Ingordo V, Nogare RD, Colecchia B, D'Andria C. Is the atopy patch test with house dust mites specific for atopic dermatitis? Dermatology 2004: 209: 276–83.
20. Seidenari S, Giusti F, Pellacani G, Bertoni L. Frequency and intensity of responses to mite patch tests are lower in nonatopic subjects with respect to patients with atopic dermatitis. Allergy 2003: 58: 426–9.
21. Kekki OM, Turjanmaa K, Isoulari E. Differences in skin-prick and patch-test reactivity are related to the heterogenity of atopic eczema in infants. Allergy 1997: 52: 755–9.
22. Strömberg L. Diagnostic accurancy of the atopy patch test and the skin-prick test for the diagnosis of food allergy in young children with atopic eczema/dermatitis syndrome. Acta Paediatr 2002: 91: 1044–9.
23. Niggemann B, Ziegert M, Reibel S. Importance of chamber size for the outcome of atopy patch testing in children with atopic dermatitis and food allergy. J Allergy Clin Immunol 2002: 110: 515–6.
24. Rancé F. What is the optimal occlusion time for the atopy patch test in the diagnosis of food allergies in

children with atopic dermatitis? Pediatr Allergy Immunol 2004: 15: 93–6.
25. Rokaitè R, Labanauskas L, Vaidelienè L. Role of the skin patch test in diagnosing food allergy in children with atopic dermatitis. Medicina (Kaunas) 2004: 40: 1081–6.
26. Fuiano N, Incorvaia C. Confronto tra *skin prick test* e *atopy patch test* nella diagnosi di allergia a *Dermatophagoides* in soggetti con patologia respiratoria e/o dermatite atopica. Minerva Pediatr 2004: 56: 537–40.
27. Holm L, Matuseviciene G, Scheynius A, Tengvall Linder M. Atopy patch test with house dust mite allergen an IgE-mediated reaction? Allergy 2004: 59: 874–82.
28. De Boissieu D, Waguet JC, Dupont C. The atopy patch test for detection of cow's milk allergy with digestive symptoms. J Pediatr 2003: 142: 203–5.
29. Guler N, Kirerleri E, Tamay Z, Ones U. Atopy patch testing in children with asthma and rhinitis symptoms allergic to house dust mite. Pediatr Allergy Immunol 2006: 17: 346–50.
30. Kerschenlohr K, Darsow U, Burgdorf WHC, Ring J, Wollenberg A. Lessons from atopy patch testing in atopic dermatitis. Curr Allergy Asthma Rep 2004: 4: 285–9.