REPRODUCIBILITY OF ATOPY PATCH TESTS WITH FOOD AND INHALANT ALLERGENS

R. RONCHETTI[1], M. JESENAK[1,2], S. BARBERI[1], F. RONCHETTI[3], Z. RENNEROVA[1,4], D. TRUBACOVA[1,4] and M.P. VILLA[1]

[1]Department of Paediatrics, Second School of Medicine, University "Sapienza", Rome; [2]Department of Paediatrics, Jessenius School of Medicine, Comenius University in Bratislava, Martin, Slovak Republic; [3]Department of Otorhinolaryngology, Second School of Medicine, University " Sapienza", Rome, Italy; [4]Srobar's Institute for Respiratory Diseases and Tuberculosis for Children, Dolný Smokovec, Vysoké Tatry, Slovak Republic

Received April 19, 2007 – Accepted January 15, 2008

Although atopy patch tests (APT) seem a valuable additional tool in the diagnostic work-up for food allergy in children with atopic eczema/dermatitis syndrome, the immunopathology and some technical aspects of testing remain controversial. Few published data are available on the reproducibility of APT with inhalants and only two studies include fresh food allergens. In this study we therefore investigated the reproducibility of duplicate APT (left versus right side of the back) with native and commercially available food (cow's milk, hen's egg, tomato, wheat flour) and with inhalant allergens (Dermatophagoides pteronyssinus and mixed grasses) in a large unselected population of children. We tested a population of 277 Italian schoolchildren with three APT allergens: fresh food (cow's milk, hen's egg, tomato and wheat flour), standardised food allergens in petrolatum (the same four foods) and standardised inhalant allergens routinely used for skin prick testing. For the four food allergens (applied in the natural form or as the standardised commercial preparation) from one- to three quarters of the APT gave positive results on one side and negative reactions on the opposite side (Cohen's κ coefficient between 0.38, fresh tomato and 0.81, fresh cow's milk). Conversely, APT with inhalant allergens were invariably reproducible (Cohen's κ = 1.00). The possible technical and immunologic reasons explaining why reproducibility of APT differed for the two types of allergens await an answer from extensive controlled studies.

The atopy patch test (APT), an epicutaneous application of intact protein allergens followed by the evaluation of skin reactions after 48-72 hours, is widely used as a diagnostic tool in patients with symptoms elicited by aeroallergens (particularly *Dermatophagoides pteronyssinus*) (1-3) and food allergens (especially cow's milk, hen's egg and cereals) (4-9). Despite the need for standardization, especially for food APT (10-11), this test has an acknowledged clinical role, especially in the diagnosis of atopic dermatitis and food allergy (5, 12). Among several variables that can strongly influence the results of APT are allergen concentration, skin site and devices used for

Key words: atopy patch test, children, food allergens, inhalant allergens, reproducibility, unselected population

Mailing address: Prof. Roberto Ronchetti, MD., PhD.
Clinica Pediatrica, Ospedale Sant'Andrea,
Via Grottarossa 1035/1039,
00189 Rome, Italy
Tel: ++39-06-3377 5856
Fax: ++39-06-3377 5941
e-mail: roberto.ronchetti@ospedalesantandrea.it

0394-6320 (2008)

allergen application, and reading time or criteria for defining positive reactions. These variables seem to be specific for each allergen (7, 13-17).

Before any diagnostic test is applied in routine clinical practice its results must meet the criteria for reproducibility. The studies on APT reproducibility leave important unsolved issues because of peculiar study design (including retesting at variable intervals only of subjects with positive tests), small numbers of studied subjects, variable quality and concentrations of allergens, and non-homogenous criteria for reading and reporting positive tests. APT positive reactions and scores often differ in patients (especially those with atopic dermatitis) and healthy controls (1, 13, 14, 18, 19), a finding which should be considered in the many studies involving selected patients (18, 20-23). For these and many other reasons, APT performed in parallel with the same allergen in the same individual has been found reproducible for chemical substances (24-27) but the results varied from absolute agreement (20, 23, 28-29) to very poor reproducibility (18-19, 21, 30) for APT with inhalant allergens. No satisfactory data exist on the reproducibility of APT with food allergens (18, 22). Results from studies comparing two APT performed at different times in the same individual are less reproducible than tests performed at the same time in two different skin sites (18-19, 21, 31-34).

In this study, we investigated the reproducibility of duplicate APT (left versus right side of the back) with food (cow's milk, hen's egg, tomato, wheat flour) in its native form and as supplied by a commercial company, and with inhalant allergens (*Dermatophagoides pteronyssinus* and mixed grasses) in a large unselected population of school children.

MATERIALS AND METHODS

Study populations

We enrolled an unselected population of 277 children attending two primary schools in the north of Rome (Italy) from October 2005 to March 2006. We studied the children in 2 age groups: the younger group (184 children, 52.7% males, age 8.71 ± 1.41 years) underwent APT with fresh food allergens and the older group (93 children, 50.5% males, age 12.92 ± 0.81 years) with standardised food allergens in petrolatum vehicle. A subgroup of the same children (131 children, 58% males, age 9.40 ± 2.24 years, 88 belonging to the younger and 43 to the older group) also underwent atopy patch testing with two solutions of inhalant allergen. All the tests were applied simultaneously on each side of the back.

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

Atopy patch tests (APT)

All APTs were carried out in duplicate applying plastic quadratic chambers 10 mm in diameter (Finn Chambers, Haye's, the Netherlands) on each side of the back. One drop of each allergen (50 µL) was placed into the chambers which were attached to an area of unaffected skin on the children's backs. As a negative control, physiological solution was used. In the younger group we applied four fresh food allergens: cow's milk (containing 3.5% fat), whisked hen's egg (egg white and yolk), tomato and wheat flour (dissolved in saline, 1g/10 mL). In the older group we carried out APT with standardised food allergens (cow's milk 10%, hen's egg 10%, tomato 20%, wheat flour 10%) dissolved in petrolatum (Lofarma S.p.a., Milano, Italy). In a subgroup of children (taken from the older and younger groups) we also performed APT with two standardised inhalant allergen solutions for skin-prick testing (Dermatophagoides pteronyssinus and mixed grasses: Avena sativa, Hordeum vulgare, Secale cereale, Triticum sativum; ALK-ABELLO, Hørsholm, Denmark). The occlusion time was 48 hours. The results were read 20 min after the chambers were removed and at 72 hours for the final test evaluation. The reading criteria were those recommended by the revised European Task Force on atopic dermatitis for APT reading (11). Reactions were classified as positive if they caused either erythema with infiltration or papules. Erythema without palpable infiltration was considered as questionable. In the final judgment questionable results were considered as negative.

Statistical methods

Data were analysed with the software package SPSS version 9.0 (SPSS Inc. Chicago, IL, USA). Chi square (χ^2) test or Fisher's exact test were used for statistical comparison. *P* values less than or equal to 0.05 were considered to indicate statistical significance. The reproducibility of the APT was evaluated by assessing the percentage of agreement and by the κ statistic (Cohen's test).

RESULTS

Each of the three types of allergens tested on the two sides of the back elicited identical mean

Table I. *Prevalence of atopy patch tests with food allergens (fresh and standardised) or inhalant allergens.*

Atopy patch test performed							
	with fresh food allergens (n = 184)		with standardised food allergens (n = 93)			with inhalant allergens (n = 131)	
	Left	*right*	*left*	*right*		*left*	*right*
Cow's milk						***Dermatophagoides pteronyssinus***	
neg.	153 (83.2%)	154 (83.7%)	80 (86%)	85 (91.4%)		91 (69.5%)	91 (69.5%)
① ?	10 (5.4%)	10 (5.4%)	8 (8.6%)	2 (2.2%)		1 (0.8%)	1 (0.8%)
② +	7 (3.8%)	8 (4.3%)	5 (5.4%)	6 (6.5%)		0	0
③ ++	11 (6.0%)	10 (5.4%)	0	0		5 (3.8%)	4 (3.1%)
④ +++	3 (1.6%)	2 (1.1%)	0	0		34 (26%)	35 (26.7%)
pos. (②+③+④)	21 (11.4%)	20 (10.9%)	5 (5.4%)	6 (6.5%)		39 (29.8%)	39 (29.8%)
Hen's egg						**Mixed grasses**	
neg.	155 (84.2%)	161 (87.5%)	86 (92.5%)	88 (94.6%)		126 (96.2%)	126 (96.2%)
① ?	14 (7.6%)	12 (6.5%)	4 (4.3%)	2 (2.2%)		0	0
② +	6 (3.3%)	5 (2.7%)	3 (3.2%)	3 (3.2%)		0	0
③ ++	6 (3.3%)	5 (2.7%)	0	0		2 (1.5%)	3 (2.3%)
④ +++	3 (1.6%)	1 (0.5%)	0	0		3 (2.3%)	2 (1.5%)
pos. (②+③+④)	15 (8.2%)	11 (6.0%)	3 (3.2%)	3 (3.2%)		5 (3.8%)	5 (3.8%)
Tomato							
neg.	171 (92.9%)	170 (92.4%)	51 (96.2%)	51 (96.2%)			
① ?	5 (2.7%)	7 (3.8%)	1 (1.9%)	0			
② +	3 (1.6%)	1 (0.5%)	1 (1.9%)	1 (1.9%)			
③ ++	3 (1.6%)	4 (2.2%)	0	0			
④ +++	2 (1.1%)	2 (1.1%)	0	1 (1.9%)			
pos. (②+③+④)	8 (4.3%)	7 (3.8%)	1 (1.1%)	2 (3.8%)			
Wheat flour							
neg.	165 (89.7%)	163 (88.6%)	83 (89.2%)	85 (91.4%)			
① ?	8 (4.3%)	9 (4.9%)	5 (5.4%)	4 (4.3%)			
② +	2 (1.1%)	2 (1.1%)	3 (3.2%)	3 (3.2%)			
③ ++	7 (3.8%)	8 (4.3%)	1 (1.1%)	1 (1.1%)			
④ +++	2 (1.1%)	2 (1.1%)	1 (1.1%)	0			
pos. (②+③+④)	11 (6.0%)	12 (6.5%)	5 (5.4%)	4 (4.3%)			

neg. - negative reaction; ① ? – erythema without infiltration; ② + - erythema with palpable infiltration; ③ ++ - erythema and less than 3 papules; ④ +++ - erythema and 4 or more or spreading papules; pos. – positive reaction.

APT positive reactions – we observed the same frequencies of positive APT results on both sides of the back for each allergen in the studied population (p non significant; χ^2 test or Fisher's exact, Table I). The prevalence of positive APT for cow's milk tended to be lower in older children (standardised food allergens). The mean number of positive reactions on the two sides remained unchanged regardless of the criteria used to define positivity (Table I).

The four food allergens applied as standardised commercial preparations yielded reproducible results in 50-57% of the tests (Cohen's κ between 0.65 and 0.71). Among fresh food allergens, tomato (Cohen's κ = 0.375) and wheat flour (Cohen's κ = 0.490) gave reproducible results in 25-32% of the tests, whereas fresh cow's milk yielded reproducible results in 70% of positive reactions and achieved the highest Cohen's κ (0.81) (Table II).

Conversely, inhalant allergens invariably gave optimally reproducible results. All the positive APT reactions were positive on the opposite side of the back and negative reactions were negative also on the other side (Cohen's κ = 1.00 for both allergens) (Table II).

All the reported reproducibility rates were the same in males and females (p non significant; χ^2 test

Table II. *Atopy patch test reproducibility (comparison of the two sides of the back tested).*

	Atopy patch tests with fresh food allergens (n = 184)				Atopy patch tests with standardised food allergens (n = 93)			
	Left +/ Right +	**Left -/ Right-**	**Left +/Right- Left-/Right+**	**Cohen's kappa**	**Left +/ Right +**	**Left -/ Right-**	**Left +/Right- Left-/Right+**	**Cohen's kappa**
Cow's milk	17 (9.2%)	160 (87%)	7 (3.8%)	0.808	4 (4.3%)	86 (92.5%)	3 (3.2%)	0.710
Hen's egg	9 (4.9%)	167 (90.8%)	8 (4.3%)	0.670	2 (2.2%)	89 (95.7%)	2 (2.2%)	0.656
Tomato	3 (1.6%)	172 (93.5%)	9 (4.9%)	0.375	1 (1.9%)	51 (96.2%)	1 (1.9%)	0.658
Wheat flour	6 (3.3%)	167 (90.8%)	11 (6%)	0.490	3 (3.2%)	87 (93.5%)	3 (3.3%)	0.650

	Atopy patch tests with inhalant allergens (n = 131)			
	Left +/ Right +	**Left -/ Right-**	**Left +/Right- Left-/Right+**	**Cohen's kappa**
Dermatophagoides pteronyssinus	39 (29.8%)	92 (70.2%)	0	1.000
Mixed grasses *(Avena sativa, Hordeum vulgare, Secale cereale, Triticum sativum)*	5 (3.8%)	126 (96.2%)	0	1.000

or Fisher's exact test).

DISCUSSION

In this study, carrying out APT on both sides of the back with food allergens (fresh or standardised) in two unselected groups of schoolchildren aged 9 or 13, we achieved poor reproducibility between the two sides of the back: 25-75% of positive APT resulted negative when repeated on the opposite side. Conversely, APT with standardised inhalant allergens (*Dermatophagoides pteronyssinus* and mixed grasses) yielded optimal overall reproducibility with 100% of the tests giving identical results in the two sides.

The much better APT reproducibility obtained with inhalant allergens than with food allergens cannot be added to current knowledge because of insufficient information concerning food allergens (18, 22). Although reproducibility might have differed owing to technical aspects of the procedure (allergen origin, diameter and form of the chamber, duration of application and time of reading, and criteria for defining positive reactions) (7, 13-17, 20), we think this unlikely considering our standardised technique produced perfectly reproducible results for the two inhalant allergens. With fresh foods we achieved rather better reproducibility, especially with fresh cow's milk (the highest Cohen's kappa) than with standardised food allergens. Canani et al observed the differences in the diagnostic accuracy of APT with the same food allergen in two different forms (native foods and freeze-dried purified food extracts in petrolatum). The better results were achieved with fresh food allergens. These differences could be due to various factors, including protein purification procedure, antigen concentration or capability of penetrating the skin (9). Hence, on the basis of our findings and of the convincing literature data showing that patch tests with chemical substances yield good reproducibility rates (discordance less than 10%) (24-27) we conclude that APT results strongly depend on the tested allergens, with food allergens being at the bottom of reproducibility

scale. No doubt, also for inhalant allergens a certain variability of results has been reported in literature: using inhalant allergens, Weissenbacher et al (23) found a concordance of only 69% by comparing the two arms of the same individual at the same time. In their study, Heinemann et al (21), taking into consideration inhalant allergens obtained from two different commercial sources, two sites of application and results obtained at an interval of 4-12 weeks, observed a reproducibility of only 56%. Using inhalant allergens from two different commercial sources, two sites of application (back and arms) and results in eczematous and healthy subjects re-tested after 2 to several weeks, Bygum et al (18) and Ingordo et al (19) report an APT reproducibility of less than 70% corresponding to a Cohen's kappa of 0.60- 0.85). Although Cohen's kappa statistic optimally takes into account all the outcomes of a repeated diagnostic test (concordant and discordant positive and negative results), it is influenced by the prevalence of positive as well as of negative results: if the prevalence of positive test lies between 1 and 11%, a "satisfactory" (19) kappa value (>0.60) corresponds to a reproducibility of positive APT reactions in only about 50% of cases and a "highly satisfactory" (19) Cohen's kappa (>0.81) corresponds to the reproducibility of only about 66% of positive tests. The fact that certain allergens elicit dissimilar positive reactions in two different skin sites and that the variability of the results increases significantly when the test is repeated after a certain time, even for patch tests performed with chemical allergens (31-34), certain individual characteristics relevant for the APT result can differ in different parts of the body or can change over time.

We conjecture that local skin conditions such as permeability, perfusion, humidity or transpiration can be naturally heterogeneous or subtly modulated by environmental factors including micro-trauma, quality of clothing, environmental temperature, duration and timing of physical activity, and emotional and psychological factors. These and other appropriate factors could be sufficient to modify locally the status of the skin and significantly influence the degree of allergen contact with the skin and its penetrance. Presumably these factors would be relevant for food allergens but far less relevant for chemical or inhalant allergens.

In conclusion, our study shows that APT performed simultaneously in two back sites in unselected children with the recommended standardised technique yield absolutely reproducible results with inhalant allergens but reproducible results in only about 33% (tomato and wheat flour) to 71% (cow's milk) of tests with food allergens. We need further extensive studies investigating and developing the optimal testing material (optimal allergen source and form, sufficient concentration of main allergens, procedure of its preparation) for APT which could lead to better reproducibility of the results. In particular, APT should be standardised not only for the amount of antigen deposited in the Finn Chamber but also for the amount of antigen able to reach the reactive cells in the skin (9). Reaching good reproducibility of APT re-testing at the same time, the clinical validity of APT with food allergens will become more stable and evident. Our study supplies sound data suitable for opening the discussion on children regarding the clinical mean of these tests widely used in practice.

REFERENCES

1. 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; 69:201-208.
2. Ingordo V, D'Andria GD, D'Andria CD, Tortora A. Results of atopy patch tests with house dust mites in adults with "intrinsic" and "extrinsic" atopic dermatitis. J Eur Acad Dermatol Venereol 2002; 16: 450-461.
3. 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; 17:346-351.
4. Isolauri E, Turjanmaa K. Combined skin prick and patch testing enhances identification of food allergy in infants with atopic dermatitis. J Allergy Clin Immunol 1996; 97:9-16.
5. Niggemann B, Reibel S, Wahn U. The atopy patch test (APT) - a useful tool for the diagnosis of food allergy in children with atopic dermatitis. Allergy 2000; 55:281-287.
6. Giusti F, Seidenari S. Patch testing with egg

represents a useful integration to diagnosis of egg allergy in children with atopic dermatitis. Pediatr Dermatol 2005; 22:109-117.
7. 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.
8. Mehl A, Rolinck-Werninghaus C, Staden U, Verstege A, Wahn U, Beyer K, Niggemann B. The atopy patch test in the diagnostic workup of suspected food-related symptoms in children. J Allergy Clin Immunol 2006; 118:923-930.
9. Canani Berni R, Ruotolo S, Auricchio L, Caldore M, Porcaro F, Manguso F, Terrin G, Troncone R. Diagnostic accuracy of the atopy patch test in children with food allergy-related gastrointestinal symptoms. Allergy 2007; 62:738-743.
10. Kerchenlohr K, Darsow U, Burgdorf WHC, Ring J, Wollenberg A. Lessons from atopy patch testing in atopic dermatitis. Curr Allergy Asthma Rep 2004; 4: 285-292.
11. Turjanmaa K, Darsow U, Niggemann B, Rancé F, Vanto T, Werfel T. EAACI/GA²LEN Position paper: Present status of atopy patch test. Allergy 2006; 61: 1377-84.
12. De Bruin-Weller MS, Knol EF, Bruijnzeel-Koomen CAFM. Atopy patch testing – a diagnostic tool? Allergy 1999; 54:784-789.
13. Darsow U, Vieluf D, Ring J. Atopy patch test with different vehicles and allergen concentrations: An approach to standardization. J. Allergy Clin Immunol 1995; 95:677-682.
14. Langeveld-Wildschut EG, Bruijnzeel PLB, Mudde GC, Versluis C, Van Ieperen-Van Dijk AG, Bihari IC, Knoll EF, Thepen T, Bruijnzeel-Koomen CA, van Reijsen FC. Clinical and immunological variables in skin of patients with atopic eczema and either positive or negative atopy patch test reactions. J Allergy Clin Immunol 2000; 105:1008-14.
15. 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; 110:515-521.
16. 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; 15:93-98.
17. 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; 59:451-458.
18. Bygum A, Mortz CG, Andersen KE. Atopy patch tests in young adult patients with atopic dermatitis and controls: dose-response relationship, objective reading, reproducibility and clinical interpretation. Acta Derm Venereol 2003; 83:18-26.
19. Ingordo V, Nogare RD, Colecchia B, D'Andria C. Is the atopy patch test with house dust mites specific for atopic dermatitis? Dermatol. 2004; 209:276-280.
20. Langeveld-Wildschut EG, van Marion AMW, Thepen T, Muddle GC, Bruijnzeel PLB, Bruijnzeel-Koomen CAFM. Evaluation of variables influencing the outcome of atopy patch test. J Allergy Clin Immunol 1995; 96:66-70.
21. Heinemann C, Schliemann-Willers S, Kelterer D, Metzner U, Kluge K, Wigger-Alberti W, Elsner P. The atopy patch test – reproducibility and comparison of different evaluation methods. Allergy 2002; 57:641-646.
22. Hansen TK, Høst A, Bidslev-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; 15:428-434.
23. Weissenbacher S, Bacon T, Targett D, Behrendt H, Ring J, Darsow U. Atopy patch test – Reproducibility and elicitation of itch in different application sites. Acta Derm Venereol 2005; 85:147-153.
24. Lindelöf B. A left versus right side comparative study of Finn Chamber™ patch tests in 220 consecutive patients. Contact Dermatitis 1990; 22:288-293.
25. Bousema MT, Geursen AM, van Joost TH. High reproducibility of patch tests [letter]. J Am Acad Dermatol 1991; 24:322-328.
26. Memon AA, Friedmann PS. Studies on the reproducibility of allergic contact dermatitis. Br J Dermatol 1996; 134:208-212.
27. Schiessl C, Wolber C, Strohal R. Reproducibility of patch tests: comparison of identical test allergens from different commercial sources. Contact

Dermatitis 2004; 50:27-33.
28. Seidenari S, Giusti F. Reproducibility of APT. Allergy 2002; 57:1082-87.
29. Giusti F, Seidenari S. Reproducibility of atopy patch tests with Dermatophagoides: a study on 85 patients with atopic dermatitis. Contact Dermatitis 2004; 50: 18-24.
30. Ingordo V, D'Andria G, Cannata AT. Reproducibility of the atopy patch test with whole house dust mite bodies in atopic dermatitis. Contact Dermatitis 2000; 42:174-180.
31. Mitchell JC. The angry back syndrome: eczema creates eczema. Contact Dermatitis 1975; 1:193-97.
32. Maibach H, Fregert S, Magnusson B, Pirilä V, Hjorth N, Wilkinson D, Malten K, Lachapelle JM, Calnan C, Cronin E. Quantification of the excited skin syndromes (the "angry back"). Retesting one patch at a time. Contact Dermatitis 1982; 8:78-83.
33. Bruynzeel DP, van Ketel WG, von Blomberg der Flier M, Scheper RJ. Angry back or excited skin syndrome. A prospective study. J Am Acad Dermatol 1983; 8:392-97.
34. Gollhausen R, Przybilla B, Ring J. Reproducibility of patch tests. J Am Acad Dermatol 1989; 21:1196-1201.