

Tomato atopy patch test in adult atopic dermatitis: diagnostic value and comparison among different methods

E. Di Leo, E. Nettis*, F. Cardinale, C. Foti, A. Ferrannini, A. Vacca

Key words: atopic eczema/dermatitis syndrome; ready-to-use atopy patch test; repeated open food challenge; skin prick test; tomato atopy patch test.

Atopic dermatitis, defined atopic eczema/dermatitis syndrome (AEDS) according to the revised EAACI/GA²LEN nomenclature

(1), is a chronic inflammatory skin disease characterized by recurrent intense itch. The role of food allergy in promoting and maintaining the eczematous lesions in AEDS is controversial. Several studies outline that AEDS in childhood can be exacerbated by the intake of common foods such as hen's eggs, cow's milk, wheat, soy and peanuts (2), while the role of offending foods in the exacerbation of AEDS in adult patients is still unknown. Although in adolescents and in adults, the worsening of eczema is poorly influenced by food ingestion, pollen-associated food (i.e. apple, tomato, citrus fruits, tree nuts and peanuts) can play a key role in the exacerbation of AEDS in 15% of adult patients (3–5). Eczematous reactions to food can be diagnosed through accurate diagnostic procedures (*in vivo* or *in vitro* detection of specific IgE) taking into account the patient's history, degree and clinical relevance of sensitization proved by oral food challenges (6). In the last years, the atopy patch test (APT) was

considered an additional tool in the diagnosis of non-IgE-mediated food allergy in patients who experienced dermatitis after the intake of offending food (7–9). In particular, APT may play a key role in the identification of food allergy in AEDS in case of: (i) absence of significant specific IgE levels or negative response to skin prick test (SPT) in food symptomatic patients; (ii) severe-persistent AEDS without trigger factors and (iii) multiple IgE sensitizations to foods without clinical relevance (6).

The aims of our study were to assess: (i) the role of tomato ingestion correlating the results of SPT and tomato APT with the repeated open food challenge (ROFC) employing fresh tomato and (ii) the performances of APT with fresh tomato *vs* standardized commercial tomato allergens in petrolatum, in an adult population from Southern Italy affected by persistent AEDS.

A total of 98 patients with persistent mild-to-severe AEDS (42 males and 56 females) aged 24–48 years (mean age 34.7 years) were enrolled in the study. The diagnosis of atopic eczema was made according to Hanifin and Rajka criteria (10), while the severity of eczema was scored by the severity Scoring index atopic dermatitis (SCORAD) (11), which includes topography items (affected skin area), intensity criteria (erythema, oedema, crusts, excoriations, lichenification and xerosis) and subjective evaluations like intensity of itch and loss of sleep. According to severity of AEDS, 38 patients suffered from mild AEDS (SCORAD < 25 points), 48 from moderate AEDS (SCORAD between 25 and 50 points) and 12 from severe AEDS (SCORAD > 50 points).

All patients had suspended any treatment with systemic corticosteroids or cyclosporine 4 months before the enrolment, while the administration of systemic antihistamines was allowed until 7 days before the beginning of the study. Moreover, the application of topical

corticosteroids or anti-inflammatory drugs was possible until 3 weeks before the recruitment. All patients excluded from their diet tomato, apple, soy, celery, wheat, citrus fruits and nuts for 4 weeks. The evaluation of AEDS clinical status was made at the enrolment, after elimination diet and after tomato challenge. Patients with positive clinical history for immediate reactions to foods were not included.

Skin prick test with tomato was performed using commercial food extracts from Stallergènes, Milan, Italy. Positive and negative controls were histamine dihydrochloride (10 mg/ml) and glycerinated saline respectively. The readings were interpreted according to the European Academy of Allergology and Clinical Immunology-Subcommittee (EAACI) on allergen standardization and skin tests (12). The response was considered positive if the wheal diameter was more than 3 mm, bigger than that provoked by the negative control.

Tomato-specific IgE was detected using the ImmunoCAP System radioimmunoassay (Phadia, Milan, Italy). Patients with specific IgE level > 0.35 kU/l (detection limit value of the CAP System) were considered sensitized to allergen.

The APT was performed in all patients with two different methods at the same time. Each patient was tested on the right back skin without AEDS lesions with both an extemporaneous preparation of fresh whipped tomato APT (APTf) (20 mg) applied on a filter paper covered with IQ Chambers (Chemotechnique Diagnostics, Sweden) both a tomato ready-to-use APT (APT_r) (Atopy line 1 Euromedical S.r.l. Calolziocorte, Milan, Italy) placed on the IQ Chambers. IQ Chamber consists of inert square polyethylene foam chambers attached to a hypoallergenic porous tape (Scanpor tape). IQ chambers for APT_r are with already integrated tomato allergens at a standardized concentration of 20% in

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petrolatum with a variability of $\pm 3\%$ among different production lots as indicated by the manufacturer.

The patch test occlusion time and its readings followed the revised European Task Force on Atopic Dermatitis recommendations (1). A first reading was done 20 min after the application of allergens to exclude an immediate skin reaction; in case of negative response at this time, patch test was removed after 48 h. Responses to APT were evaluated 48 h (20 min after removing of the apparatus) and 72 h after the application. The contemporary presence of erythema and infiltration was considered a positive reaction (1). In this study, we considered as diagnostic the APT, response evaluated after 72 h. In addition, patch test side-effects were registered. The onset of an immediate reaction to APT (erythema, urticaria, intense itch) represented a contraindication to perform food challenge. Petrolatum and filter paper were used as negative control for APT_r and APT_f respectively.

After the elimination diet that was able to improve AEDS with the disappearance of most of skin lesions in all patients, ROFC with fresh tomato was performed the first day in hospital and at patient home during the following 6 days.

Enrolled subjects were introduced to increasing doses of tomato (0.5, 1, 2, 5, 10 g) every 30 min in hospital remaining under strict medical observation for 7 h. In the presence of clinical manifestations during the test, ROFC was immediately interrupted. A reaction occurring within 2 h from the beginning of the test was considered an immediate-onset type response, while after this period, it was defined as a delayed-type reaction (13). If the challenge test was negative at the hospital, the patients performed the test at home during the following days, reaching a total dose of 100 g of fresh tomato at 7th day. Patients were instructed to recall the medical staff, if important reactions occurred.

Repeated open food challenge was considered positive for a delayed-type food reactions in case of exacerbation of eczematous lesions (at least 10-points increase of the SCORAD score) (14). The protocol was approved by the local Ethical Committee and written consent was obtained from enrolled patients.

Two-by-two tables were used to evaluate sensitivity, specificity, positive and negative predictive values. Differences between variables were analysed using the chi-squared test. A P -value < 0.05 was considered statistically significant. The results of SPT, APT_f and APT_r were compared with those of ROFC, with calculation of sensitivity (the proportion of true-positive results detected), specificity (the proportion of true-negative results detected), positive predictive value (the proportion of symptomatic individuals among test positives) and negative predictive value (the proportion of nonsymptomatic individuals among test positives) and test accuracy (the proportion of tested individuals correctly classified by test).

Among 98 enrolled patients, SPT was positive in 12 patients (12.2%), specific tomato IgE was positive only in two subjects (2%). Tomato ready-to-use APT and APT_f were positive in nine patients (9.2%) and in 10 patients (10.2%) respectively. On the total of enrolled subjects, 10 patients (10.2%) reacted positively to ROFC, experiencing eczematous lesions 24–96 h after the intake of the first dose of fresh tomato. The SCORAD values in these patients increased in a range between 10 and 14 points. Among the 88 patients who had a negative ROFC, 79 (89.7%) showed SPT negative response, 87 (98.9%) presented negative APT_r results and APT_f was negative in 82 patients (93.2%). Among eczematous challenge-positive patients, one subject manifested also diffuse urticaria 1 h after the first administration of tomato with positive results to SPT, APT_f and APT_r. The results of SPT, APT_r and APT_f in patients related to positive and negative tomato challenge are summarized in Table 1. Among 10 ROFC-positive patients, three subjects reacted to SPT (30%), while APT_r positive response was observed in eight patients (80%) (three of them were also positive to SPT) and APT_f was positive in four patients (40%) (one of them was also SPT-positive) (Table 2). The sensitivity, specificity, positive and negative predictive values and agreement of SPT, APT_f and APT_r compared to ROFC are summarized in Table 3. SPT showed a low sensitivity (30%; $P < 0.01$ respect to APT_r, not significant respect to APT_f),

Table 1. Results of SPT, APT_f and APT_r with tomato in both ROFC-positive and -negative patients (total subjects: 98)

Skin tests	ROFC + (10 patients)	ROFC – (88 patients)
SPT +	3	9
SPT –	7	79
APT _f +	4	6
APT _f –	6	82
APT _r +	8	1
APT _r –	2	87

SPT, skin prick test; APT_f, atopy patch test with fresh tomato; APT_r, tomato ready-to-use atopy patch test; ROFC, repeated open food challenge.

Table 2. Combined SPT–APT_r and SPT–APT_f results in both ROFC-positive and -negative patients

Skin tests	ROFC + (10 patients)	ROFC – (88 patients)
SPT +, APT _r +	3	0
SPT +, APT _r –	0	9
SPT –, APT _r +	5	1
SPT –, APT _r –	2	78
SPT +, APT _f +	1	0
SPT +, APT _f –	2	9
SPT –, APT _f +	3	6
SPT –, APT _f –	4	73

ROFC, repeated open food challenge; SPT, skin prick test; APT_f, atopy patch test with fresh tomato; APT_r, tomato ready-to-use atopy patch test.

but high specificity (89.8%); high negative predictive value (91.9%) and low positive predictive value (25%). Agreement between the positive results of the ROFC and those of the SPT was 83.7%. The APT_r compared with APT_f was characterized by a higher sensitivity (80% vs 40%, $P < 0.01$), higher specificity (98.9% vs 93.2%, $P < 0.05$), higher positive predictive value (88.9% vs 40%, $P < 0.01$), higher negative predictive value (98.9% vs 92.1% $P < 0.05$) and test accuracy (96.9% vs 87.7%, $P < 0.05$).

Although not significant, the agreement between SPT and APT_r (84.7%) was higher than that between SPT and

Table 3. Sensitivity, specificity, PPV, NPV, accuracy of skin tests related to ROFC in 98 patients (10 positive and 88 negative)

	SPT	APT _r	APT _f	Significance between		
				SPT–APT _f	SPT–APT _r	APT _r –APT _f
Sensitivity (%)	30	80	40	NS	$P < 0.01$	$P < 0.01$
Specificity (%)	89.8	98.9	93.2	NS	$P < 0.01$	$P < 0.05$
PPV (%)	25	88.9	40	$P < 0.05$	$P < 0.01$	$P < 0.01$
NPV (%)	91.9	97.7	93.2	NS	NS	NS
Accuracy (%)	83.7	96.9	87.7	NS	$P < 0.01$	$P < 0.05$

SPT, skin prick test; APT_f, atopy patch test with fresh tomato; APT_r, tomato ready-to-use atopy patch test; PPV, positive predictive value; NPV, negative predictive value; NS, not significant.

$P < 0.05$ is statistical significant.

APT_f (79.6%). No side-effects were reported by all patients with both APT techniques.

In Apulia, a region of the Southern Italy, tomato is a food frequently present in the daily diet. In this study, we investigated the role of tomato ingestion in adult patients affected by persistent AEDS in whom trigger factors were unknown. In these cases, as suggested by Werfel et al., (6) APT with foods must be performed to complete the diagnostic procedures, in addition to the SPT and serum specific IgE evaluations. The food challenge test is considered the gold standard to diagnose an eczematous reaction to food ingestion (15, 16). Most authors suggest that double blind placebo controlled food challenge (DBPCFC) represents the best diagnostic test for immediate and delayed food reactions (17–20), but currently no standardized protocols are available (21). Double blind placebo controlled food challenge is a time-consuming, expensive test, with a high risk of important adverse reactions (17). Moreover, the modality of food administration of this test does not reproduce the daily diet food intake (2). In our study, we performed fresh tomato ROFC, which is easier and safer than DBPCFC for clinical use. Furthermore, Isolauri and Turjanmaa (22) compared the ROFC with DBPCFC in children, and found a high correlation between the two tests.

In literature, several studies on children with AEDS have documented the diagnostic value of APT with food allergens, comparing its results with oral food challenge tests, after a specific elimination diet (8, 9, 13, 14). Currently, the rela-

tionship between foods sensitization and eczema has not been adequately demonstrated by SPT or serum specific IgE to foods in AEDS. In fact, if the diagnosis of immediate hypersensitivity reactions to foods is easily made through SPT (22), the same test is not helpful in identifying allergens responsible for delayed-onset-reactions to food, in particular, in AEDS (22, 23).

In the last years, many authors have suggested that the APT may be a diagnostic tool to understand better the pathogenesis of AEDS (24, 25). Although the APT reaction is believed to be an allergen specific IgE-mediated reaction via the Langerhans' cells in epidermis, there is a subgroup of AEDS patients in which the serum specific IgE is absent instead of the APT positivity (25). In fact, if about 80% of adult patients with AEDS present increased serum IgE levels (> 150 kU/l) and aeroallergens and food allergens sensitization (26, 27), the residue 20% have normal serum IgE levels. For this form of AEDS, characterized by a late onset (> 20 years of life) and a lack of IgE sensitization against inhalant or food allergens (26, 27), a diagnosis of 'non-IgE-associated AEDS' can be made according to the novel nomenclature for allergy by European Academy of Allergy and Clinical Immunology (28). In our study, patients with persistent AEDS and negative of most *in vivo* (SPT) and/or *in vitro* (specific IgE) test were considered as having an 'intrinsic' form of eczema. In fact, in our population, only two enrolled patients (2% of total subjects) affected by AEDS presented specific tomato IgE with a titre < 0.75 kUA/l without a clinical relevance. Our findings show that among

98 AEDS patients, the percentage of subjects who had a positive tomato SPT (12.2%) was greater than that of patients with positive response to tomato APT_r (9.2%) and APT_f (10.2%). However, the interpretation of our data must be related to the results of the ROFC, which represented the reference test in this study. In fact, when we evaluated the SPT, the APT_f and the APT_r results in the subgroups of patients with different reactions to the tomato challenge, SPT showed a lower sensitivity (30%) together APT_f (40%) respect to APT_r (80%), but high specificity (89.8%); high negative predictive value (91.9%) and low positive predictive value (25%). These results are at least partially confirmed by Seidenari et al. (2) in a study on 132 patients (aged 3–28 years) affected by AEDS, where the role of peanuts intake in eczema exacerbation was investigated with SPT, APT and ROFC. The Authors documented that there was no significant difference between SPT and APT sensitivity (50%) for examined food in a population of subjects older than 12 years (2). In this study, the Authors used fresh whipped peanuts for patients' testing and their results were similar to those observed in our study when the tomato APT_f (40% of sensitivity) was used. On the contrary, our data show that the sensitivity of APT_r was higher than that of both tests, APT_f and SPT. Thus, we conclude that the APT_r seems to be a useful tool to identify the AEDS patients who will have a positive ROFC and the subjects truly negative to the challenge (only one patient had a false negative response). Moreover, APT_r showed higher specificity, positive predictive value, negative predictive value and a better test accuracy than APT_f, when both tests were correlated to ROFC.

Probably, the standardization in terms of quantity and concentration of the allergen in the APT_r may explain the better performance of this test compared with the APT_f. In fact, the amount of tomato deposited on the IQ chamber in the APT_r can be easily evaluated and the allergen dilution in petrolatum corresponds to 20%, with a variation of $\pm 3\%$ among different production lots.

Considering that in our study we used the same support to perform the APT, the different test performances should be

attributed to the different allergen availability on the skin that was able to induce the cutaneous reaction. In fact, it was difficult to evaluate the exact amount of fresh tomato that reacted with the skin, as an indeterminate quantity of the food remained inside the blotting paper. This was the reason for a high percentage of false negative responses to the APTf among patients with a positive ROFC.

Finally, APT_r showed a higher concordance with SPT (84.7%) compared with that of APT_f (79.6%). We conclude that the APT_r, rather than APT_f, can be a useful and safe diagnostic test, in addition to SPT, for the detection of offending foods in adult patients with AEDS, in whom trigger factors remain unknown. In addition, considering the high concordance between APT_r and ROFC, we might speculate that in case of AEDS exacerbated by tomato intake and in the presence of a positive APT_r, the food challenge test may not be performed. We underline the importance of the tomato ingestion in the determinism of adult AEDS in a population with a diet consumption of this vegetable high.

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*Cattedra di Allergologia ed Immunologia Clinica
Università degli Studi di Bari
Policlinico
Piazza Giulio Cesare
1170124 Bari
Italy
Tel.: 0039 080 5592821
Fax: 0039 080 5593576
E-mail: e.nettis@allergy.uniba.it

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