

Short communication

Diagnostic value of scratch-chamber test, skin prick test, histamine release and specific IgE in birch-allergic patients with oral allergy syndrome to apple

Background: The aim of the study was to examine the diagnostic value of skin prick test (SPT), scratch-chamber test (SCT), histamine release (HR) and specific immunoglobulin E (IgE) in birch-allergic patients with oral allergy syndrome to apple.

Methods: Ten birch-allergic patients with oral allergy syndrome to apple and 10 control subjects were included. All were tested with SPT, SCT, HR and specific IgE [CAP, Pharmacia, Sweden and Magic Lite (ML), ALK-ABELLÓ, Denmark].

Results: The SPT with apple, acetone extract of apple (A72) and commercial apple extract showed sensitivities of 0.80, 0.90 and 0.10, respectively. The SCT with the same extracts showed sensitivities of 0.30, 0.50 and 0.20, respectively. The sensitivity of specific IgE to apple were 0.90 (CAP) and 0.10 (ML). The sensitivity of the HR test was 90% (A72), and 25% using the commercial extract.

Conclusion: The SPT and HR test with apple and A72 showed a good diagnostic value with a sensitivity of more than 70% and a specificity of 100%. The SCT showed a poor sensitivity to apple, A72 and commercial apple extract. The ML test was not suitable in detecting specific IgE to apple compared with the CAP test. In daily practice a detailed case history about symptoms of oral allergy syndrome combined with a SPT with fresh apple peel or A72 will be useful.

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Birch-allergic patients often report oral allergy symptoms (OAS) after ingesting cross-reacting fruits and vegetables. The oral allergy syndrome consists of itching and swelling of the lips, the oral mucosa and the soft palate after eating fresh fruits or vegetables (1–5).

The cross-reactivity is on a molecular level explained by homology between the major allergens from apple (mal d1) and Bet v 1 from birch (6–8).

It is well known that skin prick test (SPT) with fresh fruits and vegetables demonstrate a higher diagnostic sensitivity than commercial extracts (9–11). However, using fresh fruit in daily practice may be a problem as the allergen content varies between different apple strains and maturation stages (12–14).

Previous studies have investigated the value of scratch tests (15–21).

Apple tends to dry out quickly when applied to a scratch, but covering the scratch with a Finn chamber (Epitest, Helsinki, Finland) prevents drying out of the apple (22, 23).

The SCT is a controversial test, as it is no longer recommended by the American Medical Association Council of Scientific affairs because of significant number

of false positive irritative reactions, it is not as reproducible as the SPT and it may be more painful than SPT (24). Documentation is however sparse.

Determination of histamine release (HR) and specific immunoglobulin E (IgE) to apple has been examined in several studies (4, 9, 25–28), but no data on a comparison between the CAP system (Pharmacia Diagnostic, Uppsala, Sweden) and the Magic Lite (ML) test (ALK- ABELLÓ, Laboratories, Hørsholm, Denmark) in relation to apple are available.

The aims of the study were to evaluate the diagnostic value of the SPT, the SCT, histamine-release, specific IgE (CAP and ML) in birch-allergic patients with OAS to apple, and to compare different apple extracts in the skin prick, the scratch-chamber and the HR testing.

Material and methods

Patients

Ten patients with clinical allergy to birch pollen, a positive SPT to birch, and a positive case history of OAS to apple.

The controls comprised 10 nonatopics without birch pollinosis, no symptoms of OAS and a negative SPT to birch.

The presence of OAS was assessed by questioning each patient about oral itching, perioral erythema, edema of lips, tongue and throat after eating fresh apple.

Although the patients were not characterized using double-blind placebo controlled food challenge (DBPCFC), the clear cut history of OAS to fresh apple in birch allergic patients renders DBPCFC unnecessary, also because of the lack of stability in the apple allergen makes DBPCFC difficult (29). In this study, the case history thus represents the true diagnosis and sensitivity/specificity calculated accordingly.

Drugs influencing test results was discontinued according to existing guidelines (30, 31).

The age of the patients in the case group varied between 23 and 53 years (median age, 42.1 years) and in the control-group between 32 and 60 years (mean 45.8 years).

Each subject reviewed and signed an informed consent form approved by the local ethics committee.

Allergens

- Commercial extracts of apple and birch Soluprick 1 : 10 w/v (ALK-ABELLÓ, Laboratories).
- Fresh apple peel and apple pulp of golden delicious bought locally.
- Low temperature acetone powder extract of apple (A72) as described by Vieths et al. (12).
- Recombinant major allergen from apple (mal d1) (32).

Skin prick test

The patients were tested with a 1 mm lancet (ALK-ABELLÓ, Laboratories) at the volar surface of the forearm with fresh apple peel, apple pulp and low temperature acetone powder of apple (A72), recombinant mal d1, commercial apple extract, birch extract, histamine standard and diluent as negative control.

The prick-prick technique was performed with fresh apple (9).

The SPT was performed in duplicate (the same forearm) according to the European Academy of Allergology and Clinical Immunology guidelines and wheals were read after 15 min (30).

The mean value of the duplicate tests was used. The wheal reactions were outlined with a marker and transferred to a paper with transparent tape.

The wheal size was measured using the formula: $(D + d)/2$, where D is the maximum diameter and d its perpendicular diameter (30). A net wheal diameter exceeding 3 mm was regarded as a positive test result.

Scratch-chamber test

The patients were tested on the volar surface of the forearm with apple peel, apple pulp, (A72), commercial apple and birch extract. The SCT was performed in duplicate on the same day as the SPT, but on the opposite forearm. The extract was first placed in a Finn chamber (12 mm; Epitest, Helsinki, Finland) and then applied on a scratch in the skin with the length of 5 mm and without bleeding made by a 1 mm lancet.

The chambers were removed after 20 min and immediate reactions were read.

A wheal-and-flare reaction was regarded as positive (22).

Histamine release

The test was performed by using a glass-fiber-based histamine assay including six allergen dilutions of each allergen in duplicate (33).

Peripheral, washed basophils obtained from the patients and control group were challenged with birch pollen, fresh apple (golden delicious), mal d1 and A72.

A HR of more than 10 ng/ml was considered as positive. The patients with nonresponding basophils, i.e. basophils releasing < 10 ng/ml of histamine following anti-IgE challenge, were classified as inconclusive.

Determination of specific IgE to apple in serum

The CAP system (Pharmacia Diagnostic) and the ML (ALK-ABELLÓ, Laboratories) test were performed according to the manufacturer's instructions.

The technical characteristics of the assays are described elsewhere (34).

The ML test is expressed in standardized units per millilitre (SU/ml) and the CAP test in kilo units of allergen specific IgE per litre (kU_A/L).

Measurable specific IgE to apple was classified as a positive test result (ML = 1.44 SU/ml, CAP = 0.35 kU_A/L).

Statistics

The outcome of the SPT, the SCT and the HR were compared to OAS to apple.

The outcome probabilities were expressed by the sensitivity (the test's ability to detect true positives) and the specificity (the test's ability to detect true negatives).

The concordance between the test result and OAS was calculated by Fisher's exact test.

Values were considered significant at the $P < 0.05$ level.

The calculations were made using the STATA PC statistical software (Stata 8.0; Stata Corporation, TX).

Results

A summary of the outcome probabilities of the SPT, the SCT, and the HR are shown in Table 1, whereas data of specific IgE (CAP and ML) are shown in Table 2.

Generally, there was a significant difference in the diagnostic sensitivity using fresh apple or A72 compared with the commercial apple extract.

The SPT showed a high sensitivity to fresh apple peel (80%), apple pulp (70%), A72 (90%), birch (100%), and low to the commercial apple extract (10%).

The SCT showed a low sensitivity to fresh apple peel, apple pulp (30%), A72 (50%), and the commercial apple extract (20%).

The HR test showed a high sensitivity to birch (90%), A72 (90%), mal d1 (70%) and low to the commercial apple extract (25%).

The efficiency was good between the case history and the SPT and HR with A72, apple peel and apple pulp, whereas this was not the case using the SCT no matter which extract was used. Furthermore, the efficiency was

Table 1. Diagnostic outcomes of skin prick test (SPT), scratch-chamber test (SCT) and histamine release (HR)

Test	Allergen	Sensitivity	Specificity	Efficiency	P-value
SPT	Apple (commercial extract)	0.10	1.00	0.55	>0.05
SPT	Apple peel	0.80	1.00	0.90	<0.05
SPT	Apple pulp	0.70	1.00	0.85	<0.05
SPT	A72	0.90	1.00	0.95	<0.05
SPT	Birch	1.00	1.00	1.00	<0.05
SCT	Apple (commercial extract)	0.20	1.00	0.60	>0.05
SCT	Apple peel	0.30	1.00	0.65	>0.05
SCT	Apple pulp	0.30	0.90	0.60	>0.05
SCT	A72	0.50	1.00	0.75	<0.05
SCT	Birch	0.60	0.90	0.75	>0.05
HR	Apple	0.25	0.80	0.40	>0.05
HR	A72	0.90	0.90	0.80	<0.05
HR	Mal d1	0.70	1.00	0.80	<0.05
HR	Birch	0.90	0.80	0.80	<0.05

Table 2. Patients with oral allergy symptoms to apple, evaluated by specific immunoglobulin E [CAP and Magic Lite (ML)]

	CAP	ML	
Positive	10	1	11
Negative	0	9	9
	10	10	20

not good in the SCT no matter type of extract. Overall the efficiency was poor with the commercial apple extract in all methods.

The CAP test showed a higher diagnostic sensitivity (100%) than the ML test (10%) for apple.

Discussion

The diagnosis of oral allergy syndrome to apple has been complicated by unstable apple extracts and questionable diagnostic methods leading to a loss in the diagnostic sensitivity. The diagnostic value of a test depends on the materials used, the test technique, the disease prevalence and the number of the patients included. In order to enable a direct comparison between the ability of various

diagnostic test methods, we included patients with a clear cut case history of OAS to apple and birch allergy.

The diagnostic sensitivity of the SPT with fresh apple peel and A72 was better compared to the commercial apple extract in agreement with other studies (9, 26). Although, the diagnostic value of the prick-prick technique with fresh apple is useful, it is still very problematic that the expression of the major allergen mal d1 varies between different apple strains and maturation stages (12). A standardization of the apple extract would therefore be very suitable to get a good and reliable diagnostic value of the tests.

The apple extract A72 may be an alternative to fresh apple, especially when a standardization is mandatory as in clinical trials. Unfortunately, the preparation of A72 is complicated (12). A more simple way forward might be preparation of freeze-dried apple powder as described by Skamstrup et al. (29).

The study showed that the diagnostic outcome of the SCT was uncertain compared with the SPT for all types of apple extracts.

The HR test with A72 was better compared with mal d1 and the commercial apple extract.

The ML test was not suitable for detecting specific IgE to apple in this study.

However, it may be a problem if the CAP test is too sensitive compared with the ML test, which has not been evaluated in this study.

The case story regarding OAS was used as the gold standard. However, the gold standard of the diagnosis should be the DBPCFC. In previous tests, the challenges with apple have been performed openly, mostly because of the instability of the apple allergens together with a lack of a valid DBPCFC model.

In conclusion the SCT cannot be recommended as a method in evaluation of birch-allergic patients with OAS to apple.

In daily practice the SPT is a useful method when using fresh apple or A72.

The HR test or the CAP test may be an alternative, when the SPT cannot be performed.

In future more investigation of a standardized apple extract is needed.

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