

Short communication

Diagnostic evaluation of grass- and birch-allergic patients with oral allergy syndrome

Background: Patients with birch and grass allergy often suffer from oral allergy symptoms when ingesting cross-reacting fresh fruits and vegetables. However, fruit and vegetable allergen extracts are often readily degradable or contain clinically irrelevant cross-reacting epitopes, resulting in diagnostic discrepancies when fruit and vegetable allergic reactions are evaluated. The risk of using nonstandardized fresh food extracts for skin testing may also be of concern. The objective was to compare and evaluate the clinical utility of selected recombinant grass and birch cross-reacting food allergens with fresh and commercial melon, hazelnut, and apple extracts.

Methods: Thirty-six grass- and or birch-allergic patients and 17 control subjects consented to participate in the study. All subjects were skin prick tested and had basophil histamine-release tests done with fresh fruits and various extracts of hazelnut, apple, and melon. The diagnosis of oral allergy syndrome was confirmed by oral challenges. In addition, histamine release to recombinant Bet v 1 and Bet v 2, and recombinant Phl p 1 and Phl p 2, Phl p 5 was performed.

Results: The skin prick test with fresh hazelnut, apple, and melon showed sensitivities of 0.97, 0.92, and 0.89, respectively. The corresponding specificities were 0.78, 0.72, and 0.82, respectively. In contrast, the histamine-release test with hazelnut, apple, and melon gave sensitivities of 0.87, 0.71, and 1.00, respectively. The corresponding specificities were 0.65, 0.93, and 0.43. The skin prick test showed excellent negative predictive value (>90%). No added value of recombinant allergen testing was noted. Oral challenge did not result in severe systemic reactions, and no systemic reactions were observed with skin prick tests with fresh fruits.

Conclusions: The skin prick test showed an almost optimal diagnostic value with a satisfactory sensitivity (>89%) and excellent negative predictive value with fresh fruits. When the skin prick test with fresh nut and apple cannot be performed, histamine release is a diagnostic alternative. Histamine release with melon showed lack of specificity. This was probably due to extensive IgE cross-reactivity with pollen, since these patients also responded to recombinant Phl p 1 and Bet v 1. Skin testing and challenges with fresh fruits were safe.

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Patients with allergy to birch pollen often experience oral allergy symptoms on ingestion of cross-reacting fruits and vegetables. The symptom complex is termed "oral allergy syndrome" (OAS) and consists of itching and swelling of the lips, the oral mucosa, and the soft palate immediately after ingesting the fruit or vegetable. The symptoms may sometimes develop into urticaria, conjunctivitis, asthma, anaphylaxis, nausea, and vomiting (1–3). The pathomechanism of allergy to birch pollen and concurrent allergy to fruits is explained by cross-reacting allergens (4–6). The hypothesis is that sensitization to airborne pollen allergens is the primary event leading to the induction of IgE antibodies that are

capable of cross-reacting with homologous food allergens (6). Allergens homologous to the birch-pollen allergens Bet v 1 and Bet v 2 (profilin) have been identified in several fruits and vegetables (6). For instance, the presence of a Bet v 1 homologous allergen in apple has been demonstrated by Northern blotting with the Bet v 1 cDNA by using Bet v 1-specific antibodies and serum IgE (7). Furthermore, it has been shown that allergy to hazelnuts is due to IgE antibodies that cross-react with Bet v 1 and Bet v 2 (8). The three major grass-pollen allergens (Phl p 1, 2, and 5), however, are specifically expressed in pollen, and not in other plant tissues (9, 10).

It is an extensive problem when 47–70% of all patients with pollen allergy suffer from oral allergy symptoms (3, 11, 12). Therefore, it is important to be able to determine a specific diagnosis for each patient. The skin prick test (SPT) or various *in vitro* tests are used to determine whether a patient has IgE antibodies to specific foods. However, the diagnosis of OAS is complicated by several factors. Fruit extracts are readily degradable, leading to significant loss in potency and diagnostic sensitivity of common allergenic extracts and *in vitro* tests. Thus, although impractical and perhaps risky, the SPT with fresh fruits is recommended to improve diagnostic sensitivity. Skin tests and *in vitro* tests are poor predictors of clinical food reactivity in patients with allergy to birch and grass pollen, perhaps due to cross-reacting allergens in apple, nut, and melon. It is questioned whether panallergens, e.g., recombinant Bet v 2 (profilin) or recombinants of cross-reacting major allergens of grass and birch, may be of diagnostic value in these patients.

The objectives of this study were to compare diagnostic outcomes of fresh fruits and several new extract preparations of hazelnut, apple, and melon, and to evaluate the diagnostic use of recombinant Bet v 1, Bet v 2, and Phl p 1, 2, and 5 allergens in birch- and grass-allergic patients with oral allergy symptoms.

Material and methods

Patients

A detailed allergen-specific case history was obtained in all patients followed by an oral food challenge test and SPT with fresh fruits. The presence of OAS was assessed by questioning each patient about oral itching; perioral erythema; edema of lips, tongue, and throat; and gastrointestinal complaints or urticaria, immediately after eating hazelnut, apple, or melon or other foods. Two patients with a recently documented anaphylactic reaction to fresh fruits were not orally challenged. In these patients the case history was considered confirmatory. Patients who had a positive test with either the SPT or histamine release (HR) to the foods but regularly ate fresh fruits without symptoms of oral allergy were considered sensitized but clinically unaffected. Furthermore, blood samples were obtained from each subject for leukocyte HR to various extracts of hazelnut, apple, and melon, and to recombinant major grass allergen Phl p 1, 2, and 5 and recombinant Bet v 1 and Bet v 2.

The demographics of patients and controls are reported in Table 1. Patients were selected from a group of 67 patients with verified allergy to birch or grass pollen. Patients were classified as allergic to birch or grass pollen only when clinical findings and typical seasonal respiratory symptoms (rhinoconjunctivitis and asthma) were reported in conjunction with a positive SPT to birch or grass. Forty-four (66%) patients from this group reported symptoms of OAS to pertinent fresh fruits, and 36 consented to participate in the study. Other food allergies were not reported. Eight patients with OAS did not participate in this study for personal reasons. The distribution of birch, grass, and grass and birch allergics in this group was approximately the same as in the group of OAS patients who participated (38%, 50%, and 13%, respectively). A similar distribution of birch and grass allergy was observed in the patients who were excluded from this study, and who did not report oral allergy symptoms when ingesting the above foods.

The control group comprised five nonatopics without birch or

grass pollinosis and skin test negative to a panel of 10 inhalant allergens, and 12 birch and grass allergics with no symptoms of OAS and frequently eating the above-mentioned foods. All controls had negative SPT to pertinent fresh fruits. Among the atopic controls, five (42%) were allergic to birch, five (42%) to grass, and two (17%) were allergic to both birch and grass. Among the OAS patients, 14 (39%) were birch allergic, three (8%) were grass allergic, and 19 (53%) were allergic to both birch and grass. None of the patients were allergic to mugwort pollen.

The majority of patients were allergic to hazelnut (83%) followed by apple (67%) and melon (25%). Among hazelnut allergics, 37% were allergic to birch, 3% to grass, and 60% were allergic to both grass and birch. Among patients allergic to apple, 46% were birch allergic, 8% were grass allergic and 46% were allergic, to both grass and birch, while the distributions in the group of patients with allergy to melon were 22%, 22%, and 56% respectively.

The most common OAS symptoms were oral pruritus (80–91%), burning (40–60%), swelling (10–52%), and blisters (5–20%). Reported systemic reactions were rare. Twelve patients reported rhinoconjunctivitis when ingesting apple or nuts. Four patients experienced urticaria and two patients reported asthma when ingesting apple or nuts.

Each subject reviewed and signed an informed consent form approved by the local ethics committee. Patient criteria of exclusion were a positive skin prick reaction to the negative control (e.g., dermatographism) lack of skin test response (wheal diameter <2 mm), and treatment with antihistamines or systemic steroids within 1 week of testing.

Oral challenge test

On separate occasions, each patient and each control subject were challenged with fresh material by chewing hazelnut, apple, or melon for 60 s, and then the material was disgorged. During the next 15 min symptoms of oral itching; perioral edema; or edema of the lips, tongue, and throat were recorded. If any of these symptoms was reported, the challenge test was considered positive. Patients reporting previous systemic reactions to fresh fruits were not orally challenged. In these patients, the case history was considered confirmatory.

SPT

Patients were tested at the volar surface of the distal forearm with fresh material of hazelnut, apple, and melon by the prick plus prick technique (13). The fruit was cut and the lancet was pricked into the pulp and then immediately afterward into the skin. SPT were done in duplicate by the EAACI prick technique with a histamine standard of 10 mg/ml and the diluent as negative control. Wheals were read after 15 min and wheal reactions were outlined with a fine-point marker and transferred to paper with transparent tape. The mean value of duplicate tests was used. SPT results were compared by measuring the wheal diameter. The allergen content of fresh fruits was unknown. According to SPT results in controls and oral challenge tests, the optimal threshold value for nut and melon was 3.5 mm, while the optimal threshold value for apple was 5 mm.

Allergens

Before the study was undertaken, blood samples from nonallergic and allergic controls were incubated with the allergens to exclude cell toxicity and analytic interference in the histamine-release assay. No unspecific activity was observed. The optimal *in vitro* challenge dose was assessed before undertaking this study and adjusted by protein content to allow comparisons between the various extracts. The final concentrations were 100, 10, 1, 0.1, 0.001, and 0.0001 µg protein/ml. All allergens and extracts were stabilized and stored in 50% glycerin.

Table 1. Demographics of patient and control subjects

	Patients (n=36)	Controls (n=17)
Mean age (range)	36 (20–56) years	33 (24–62) years
Grass and birch allergics	36	12
Hazelnut OAS symptoms	33	0
Apple OAS symptoms	26	0
Melon OAS symptoms	10	0
Mean SPT wheal nut	6.6 mm	0.6 mm
Mean SPT wheal apple	6.1 mm	0.5 mm
Mean SPT wheal melon	2.4 mm	0.5 mm
Hazelnut food challenge positive	30	0
Apple food challenge positive	24	0
Melon food challenge positive	9	0

OAS: oral allergy syndrome; SPT: skin prick test.

Fruit allergens

Hazelnut Soluprick 1:10 w/v and an intermediate apple extract were kindly provided by ALK-Abelló. The apple extract was labeled Apple B1, 0.796 mg protein/vial (Bradford), batch no. 47083. The Reference Laboratory (Copenhagen, Denmark) provided fresh frozen melon extract.

Recombinant allergens

Recombinant allergens included rBet v 1, 1.6 mg/ml (ALK-Abelló, Denmark); rBet v 2, 1 mg/ml (Biomay, Austria); rPhl p 1, 1 mg/ml (Biomay, Austria); rPhl p 2, 1 mg/ml (Biomay, Austria); and rPhl p 5, 1 mg/ml (Biomay, Austria).

Leukocyte histamine release

The test was performed by using a glass-fiber-based histamine assay including six allergen dilutions of each allergen in duplicate (14). In brief, heparinized whole-blood samples were incubated with 10-fold dilutions of each allergen at the doses mentioned above. Anti-IgE (Behringwerke) at concentrations from 7875 to 52 U/ml was used as a positive cell control of leukocyte HR. The HR test was considered analytically evaluable when the patient blood sample released histamine to either anti-IgE or allergen, and the standard deviation of duplicate determinations did not exceed two times the standard deviation of repeated measurements of the zero (blank) calibrator, i.e., 15 ng/ml histamine. The amount of histamine was measured by a histamine standard curve. The area under the histamine-release curve (AUC) was computed by numeric integration and expressed in nanogram per milliliter. Previously, it has been found that the optimal threshold value for a positive test result requires an AUC of > 40 ng/ml and a release of > 20 ng/ml histamine, at least at one allergen concentration.

Data evaluation

Patient population data were assessed by median and range. Statistically significant differences between the patient group and controls were computed by Friedman's test. *P* values less than 0.05 were considered to be statistically significant. Skin test data were analyzed by the mean of duplicate determinations. Outcome probabilities were expressed by the sensitivity (the test's ability to detect true positives) and specificity (the test's ability to detect true negatives). Efficiency was expressed by the likelihood of the test detecting all true positives and true negatives.

Positive and negative predictive value is the likelihood that a positive and negative *in vivo* or *in vitro* test result reflects the presence or absence of oral allergy symptoms assessed by food challenge. Sensitivity and specificity pairs may vary considerably with the cutoff value established for a test. In this study, different sets of sensitivity/specificity value were calculated, and threshold values for each test were then chosen according to optimal test performance, as stated in the Material and methods section of this text. The chi-square (χ^2) test was used to test whether there was an association between the row variable (oral challenge test) and the column variable (*in vitro* or *in vivo* test) in order to determine whether the distribution of results among the categories of one variable was independent of their distribution among the categories of the other. For a small value of χ^2 , there was a significant possibility that the differences are due to chance.

Results

During our food challenges, no patients experienced severe systemic reactions. A few patients reported rhinoconjunctivitis symptoms, which were easily treated with oral antihistamines. No severe local (wheal diameter of > 8 cm) or systemic reactions to skin tests with fresh fruits were noted.

A summary of the outcome probabilities of the SPT, basophil HR, and physician evaluation, when compared to oral challenge with fresh fruits, is shown in Table 2. The diagnostic sensitivity of the SPT with fresh fruit was high for hazelnuts (97%), apple (92%), and melon (89%) with an acceptable (> 70%) specificity, as shown in Table 2. The specificities were 78%, 72%, and 82%, respectively. Basophil HR to the hazelnut extract and apple extract demonstrated a lower diagnostic sensitivity than SPT. The sensitivities were 73% and 71% with specificities of 65% and 93%, respectively. Basophil HR to the melon extract resulted in a diagnostic sensitivity of 100% with a specificity of 43%. We also evaluated OAS by

Table 2. Diagnostic outcomes when SPT, basophil HR, and allergen-specific case history (CH) were compared to oral challenge with fresh fruits. Outcomes are reported by sensitivity, specificity, efficiency positive predictive value, and negative predictive value. Numbers of challenge-positive (P) subjects and chi-square values are also reported

Test	Allergen	P	Sensitivity	Specificity	Efficiency	Positive predictive value	Negative predictive value	Chi ²
SPT	Fresh nut	30	0.97	0.78	0.89	0.85	0.95	31.78
HR	Nut	30	0.87	0.65	0.77	0.76	0.79	15.24
CH	Nut	30	1.00	0.87	0.94	0.91	1.00	41.90
SPT	Fresh apple	24	0.92	0.72	0.81	0.73	0.91	21.95
HR	Apple B1	24	0.71	0.93	0.83	0.89	0.79	23.34
CH	Fresh apple	24	1.00	0.93	0.96	0.92	1.00	45.55
SPT	Melon	9	0.89	0.82	0.83	0.50	0.97	17.72
HR	Melon	9	1.00	0.43	0.53	0.26	1.00	6.06
HC	Melon	9	0.89	0.93	0.92	0.73	0.98	30.60

traditional diagnostic means by combining the physician's evaluations of case history and SPT with fresh fruit, or by combining case history and HR. The best results were obtained for hazelnut and melon by combining case history and SPT. The diagnostic sensitivity for case history and SPT with respect to hazelnut was 97% while the specificity was 91%. For melon, the diagnostic sensitivity of combining case history and SPT was 78% and the specificity was 98%. When diagnosing oral allergy to apple, the case history alone demonstrated a diagnostic sensitivity of 100% while the specificity was 93%. In contrast, a hazelnut case history combined with HR resulted in a diagnostic sensitivity of 77%. Combining an apple case history with the results of SPT or HR gave similar diagnostic outcomes, with a sensitivity of 96% and a specificity of 93%.

Generally, good concordance (test efficiency of >80%) was found between case history, SPT, and oral challenge tests. The negative predictive value of the SPT was excellent (>90%). In contrast, a positive HR or SPT result with melon was not reliable (positive predictive value less than 50% for all allergens). However, a combination of case history of OAS with a SPT or basophil HR with fresh hazelnut or apple resulted in an excellent positive predictive value (>90%, data not shown).

Finally, we compared the HR response of the recombinant major allergens with the diagnostic outcomes of food challenges to determine whether such measurements would help to distinguish clinical food allergy from IgE sensitization related to cross-reactivity. The diagnostic concordance of recombinant allergen-induced HR and oral challenges ranged from 42% to 81%. The best concordance (81%) was observed with rBet v 1 and nut challenges. The lowest concordances (42-49%) were noted for melon-allergic patients tested with recombinant grass allergens. A number of grass- and birch-allergic controls without oral allergy symptoms also demonstrated significant HR to recombinants with a varying degree of specificity. The specificity of recombinant allergen testing ranged from 36% to 78%. The opposite was also observed, resulting in a sensitivity of the recombinant allergens ranging from 44% to 93%.

Discussion

The diagnosis of oral allergy has been complicated by readily degradable fruit extracts, leading to significant loss in potency and diagnostic sensitivity. The objective of this study was to compare and evaluate the clinical utility of selected recombinant grass and birch cross-reacting food allergens with fresh and newly developed commercial melon, hazelnut, and apple extracts.

This study showed that in birch- and grass-allergic patients with oral allergy to hazelnut, apple, and melon a diagnosis may be safely obtained by an explicit physician evaluation, which includes a complete allergen-specific

case history and SPT with fresh fruits. When skin tests are negative to fresh hazelnut, apple, and melon, it is unlikely (negative predictive value of >90%) that subjects will suffer from oral allergy symptoms to the fruits studied. However, when SPT with fresh fruit cannot be performed, basophil HR may be a valuable diagnostic alternative when diagnosing oral allergy to hazelnut and apple. Previously, *in vitro* tests and SPT with commercial extracts showed questionable diagnostic performance. This study indicates that the extracts have improved considerably compared to earlier studies (15). The intermediate apple extract B1 used in this study seems especially promising.

Interestingly, the case history alone in this study population was the optimal predictor for apple allergy, showing negative and positive predictive values of 100% and 92%, respectively. The difference between diagnosing oral allergy to hazelnut and apple may be due to the fact that hazelnuts often are used as one ingredient among several others, and that they are often processed in some way. Consequently, it might be difficult for patients to determine whether they are allergic to hazelnut. On the other hand, apples are usually eaten raw, and it is therefore obvious when a patient experiences symptoms and is allergic to this fruit. This would explain why the case history is such a reliable predictor for oral allergy to apple. However, in assessing hazelnut allergy, the specificity is improved considerably if we use combination of the case history and SPT.

HR to melon showed lack of specificity. This is probably due to extensive IgE cross-reactivity with pollen, since these patients also responded to rPhl p 1 and rBet v 1.

We were not able to identify a reaction pattern to recombinants that could help us to identify grass- or birch-allergic patients who had symptoms of oral allergy. Thus, no diagnostic value with respect to assessing clinical food allergy by recombinant Bet v 2 or the other recombinant major allergens was observed.

In conclusion, most apple-, hazelnut-, or melon-sensitized patients are easily and safely diagnosed by a thorough case history and brief local oral exposure followed by disgorging the suspected fruits. If this method is not practicable, oral allergy may be accurately diagnosed by combining an allergen-specific case history with SPT using fresh fruits. When fresh fruits are not available, SPT or basophil HR tests may be performed with commercial fruit extracts. However, this requires careful consideration of extract quality and potency.

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