ORIGINAL ARTICLE

Predictive Value of the Sulfidoleukotriene Release Assay in Oral Allergy Syndrome to Celery, Hazelnut, and Carrot

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Abstract

Background: Patients sensitized to birch pollen frequently suffer from a food allergy to plant foods such as celery, carrots, or hazelnut. One of the main manifestations of birch pollen-related food allergy is the oral allergy syndrome. Skin tests and allergen-specific immunoglobulin (Ig) E determinations are poor predictors of such reactions when assessed by double-blind placebo-controlled food challenge (DBPCFC).
Objective: To investigate whether a cellular test based on leukotriene release from basophils, the cellular antigen stimulation test in combination with enzyme-linked immunosorbent assay (CAST-ELISA), is predictive of pollen-related food allergy.
Methods: Birch pollen-sensitized patients with positive DBPCFC to celery (n = 21), hazelnut (n = 15), and carrot (n = 7) underwent skin tests along with determination of specific IgE and CAST-ELISA for the respective allergens. The results were compared with those of 24 birch pollen-sensitized patients with negative open food challenge to celery, hazelnut, and carrot.
Results: While skin prick tests had a sensitivity of 85%, 80%, and 29% for commercial extracts of celery, hazelnut, and carrot, respectively, prick testing with self-prepared extracts yielded sensitivities of 100%, 80%, and 100%, respectively. For specific IgE determinations, sensitivities were 71%, 73%, and 57%, respectively, and the respective specificities were 67%, 73%, and 60%. For CAST-ELISA with various sources and doses of allergens, the sensitivity varied from 71% to 95% for celery, 73% to 80% for hazelnut, and 43% to 86% for carrot. The respective specificities were 67% to 92%, 75% to 88%, and 77% to 91%. Analysis of the predictive value of CAST-ELISA with receiver operating characteristic curves showed that the results of the tests were more predictive of pollen-related food allergy than quantitative allergen-specific IgE determinations.
Conclusions: CAST-ELISA is more specific than routine diagnostic tests for the diagnosis of pollen-related food allergy to celery, hazelnut, and carrot.

Key words: Cellular antigen stimulation test. Sulfidoleukotriene release. Pollen-associated food allergy. Oral allergy syndrome. Double-blind placebo-controlled food challenge. Hazelnut. Celery. Carrot.

Resumen

Antecedentes: Los pacientes sensibles al polen de abedul padecen con frecuencia alergias alimentarias a determinados alimentos de origen vegetal como el apio, las zanahorias o las avellanas. Una de las manifestaciones principales de la alergia alimentaria asociada al polen de abedul es el síndrome de alergia oral. Las pruebas cutáneas y las determinaciones de inmunoglobulina (Ig) E específica al alérgeno son insuficientes para pronosticar tales reacciones, cuando se evalúan mediante una prueba de provocación alimentaria, doble ciego, controlada con placebo, (PADCCP).
Objetivo: El propósito de este estudio fue el de examinar si un análisis celular basado en la liberación de leucotrienos a partir de los basófilos que determina la producción antígeno específica de sulfidoleucotrienos mediante el enzimoinmunoanálisis de adsorción (CAST-ELISA), es pronóstico de la alergia alimentaria asociada al polen.
Métodos: Se practicaron pruebas cutáneas junto con la determinación de la IgE específica y el CAST-ELISA para los alérgenos respectivos a los pacientes sensibles al polen de abedul con un resultado positivo de la PADCCP para el apio (n = 21), las avellanas (n = 15) y las zanahorias (n = 7). Los resultados se compararon con los de 24 pacientes sensibles al polen de abedul con un resultado negativo en la prueba de provocación alimentaria abierta frente a apio, avellana y zanahoria.

Resultados: Las pruebas cutáneas mostraron una sensibilidad del 85 %, el 80 % y el 29 % para los extractos comerciales de apio, avellanas y zanahorias, respectivamente, mientras que la prueba cutánea con extractos propios produjo sensibilidades del 100 %, el 80 % y el 100 %, respectivamente. Para la determinación de IgE específica las sensibilidades fueron del 71 %, el 73 %, y el 57 %, respectivamente y las especificidades respectivas fueron del 67 %, el 73 %, y el 60 %. En cuanto al CAST-ELISA con alérgenos de diversos orígenes y en dosis diferentes, la sensibilidad osciló del 71 % al 95 % para el apio, del 73 % al 80 % para la avellana y del 43 % al 86 % para la zanahoria. Las especificidades respectivas fueron del 67 % al 92 %, del 75 % al 88 % y del 77 % al 91 %. El análisis del valor diagnóstico del CAST-ELISA con las curvas de eficacia diagnóstica mostró que los resultados de las pruebas eran más pronósticos de la alergia alimentaria asociada al polen que las determinaciones cuantitativas de IgE específica al alérgeno.
Conclusiones: el análisis CAST-ELISA es más específico que las pruebas de diagnóstico rutinarias para determinar la alergia alimentaria al apio, a las avellanas y a las zanahorias asociada con el polen.

Palabras clave: Producción antígeno específica de sulfidoleucotrienos. Liberación de suylfidoleucotrienos. Alergia alimentaria asociada al polen. Síndrome de alergia oral. Provocación alimentaria, doble ciego, controlada con placebo. Avellana. Apio. Zanahoria.

Introduction

Allergy to foods affects about 3% to 4% of the adult population [1]. One of its most frequent manifestations is the oral allergy syndrome (OAS), characterized by oral and pharyngeal mucosal symptoms following the ingestion of allergenic foods [2,3]. In particular, patients sensitized to birch pollen may develop oral symptoms after ingestion of apples, pears, hazelnuts, kiwi, carrots, celery, and many others [4,5].

Diagnosis of food allergy is based first on the patient's history, followed by skin prick tests (SPT) and analysis of food-specific immunoglobulin (Ig) E. Some of these tests have a relatively high sensitivity but they do not distinguish between pure sensitization and clinically relevant allergy. Thus, many test-positive patients tolerate the corresponding food without problems [6]. For that reason, the gold standard in food allergy continues to be double-blind placebo-controlled food challenge (DBPCFC) [7]. Unfortunately, DBPCFC is a tedious and time-consuming procedure that is not without risk for the patient. Consequently, there is a consensus that any in vitro diagnostic test that could replace or reduce the need for DBPCFC would be of considerable benefit in the diagnosis of food allergy. Cellular tests represent candidates to replace DBPCFC, since the stimulation of IgE-loaded basophils in vitro is, in principle, more representative of the pathophysiologic process occurring in vivo than simple determination of serum IgE concentrations. Since 1993, a new sulfidoleukotriene-release test, the cellular antigen stimulation test (CAST) in combination with enzyme-linked immunosorbent assay (ELISA), known as CAST-ELISA has been shown to be of interest in the diagnosis of IgE-mediated allergies to inhalant allergens, insect venoms, latex, and some drugs [8]. It has also been shown to display high sensitivity and specificity in IgE-mediated allergies to a number of allergenic foods [9,10].

The aim of this study was to investigate whether CAST-ELISA is useful in diagnosis of allergy to hazelnut, celery, or carrot in patients sensitized to birch pollen with or without OAS to these food allergens. In particular, we sought to establish whether CAST-ELISA exhibited predictive value and whether the results of the test were correlated with those of DBPCFC in such patients.

Materials and Methods

Patients

The study included 64 patients aged 16 to 62 years (23 male and 41 female) who were sensitized to birch pollen. All of them had positive SPT results to birch pollen extract and 96.4% also had a positive specific IgE test to t3 (birch) allergen, as measured by fluorescent enzyme immunoassay (CAP-FEIA). Twenty-one patients had a positive DBPCFC to celery, 15 to hazelnut, and 7 to carrot. In comparison, 24 birch-sensitized patients had a negative open food challenge to celery, 16 to hazelnut, and 21 to carrots.

Ethical Considerations

The study was reviewed and approved by the Local Ethics Committee of the University of Zurich. All subjects provided signed informed consent before enrollment in the study.

Food Challenges

Food challenges were performed and evaluated as described by Ballmer-Weber et al [4,5,11,12]. Birch pollen-allergic patients without a history of a pollen-related food allergy underwent open food challenges to confirm clinical tolerance. To exclude food allergy, those patients ingested 20 g of raw carrots, 20 g of raw celery, and 6 g of raw hazelnuts.

Skin Testing

SPT was performed with commercial extracts of birch pollen (Soluprick, ALK-Abelló, Horsholm, Denmark), celery (Allergopharma, Reinbek, Germany), hazelnut, and carrot (Stallergènes, Antony Cedex, France) on the flexor aspect of each patient's forearm with a standardized prick needle (Stallerpoint, Stallergènes). Histamine dihydrochloride (10 mg/mL) was used as a positive control, and the glycerol-containing diluent of the prick solution (Soluprick, ALK-Abéllo) was used as a negative control. Patients also underwent skin testing with self-prepared

food extracts, as described previously [4,5,11,12]. Reactions were recorded after 15 minutes. Wheals greater than 3 mm in diameter were considered positive. Skin tests were only done with the suspected culprit food allergen in DBPCFC-positive patients.

In Vitro Testing

Analysis of allergen-specific IgE to birch, celery, hazelnut, and carrots was performed by CAP-FEIA according to the manufacturer's instructions (Phadia, Uppsala, Sweden). IgE concentrations above 0.35 kIU/L were considered positive.

CAST-ELISA consists of an initial step involving in vitro incubation of isolated blood leukocytes with the putative allergen, in the presence of 2 mg/mL interleukin 3, for 40 minutes. Then, the concentration of sulfidoleukotrienes (leukotrienes C4, D4, and E4) released are analyzed by ELISA with a highly specific monoclonal antibody [8]. The results are evaluated in terms of the concentration of sulfidoleukotrienes (pg/mL). The test was performed according to the manufacturer's instructions (Bühlmann Laboratories AG, Allschwil, Switzerland) and further details are described elsewhere [8]. The celery, hazelnut, and carrot allergens used for CAST-ELISA were provided by Bühlmann Laboratories and used at final concentrations of 0.8, 4, and 20 ng/mL, respectively. The self-prepared extracts were used at final concentrations of 4, 20, and 100 ng/mL, respectively. To determine the diagnostic sensitivity and specificity of CAST-ELISA with the corresponding food allergens, 20 apparently healthy randomly selected blood donors with no history of allergy were included. CAST-ELISA reactions to birch pollen allergens were analyzed in 7 patients only. All 7 subjects showed specific stimulations of at least 1615 pg/mL sulfidoleukotrienes.

Statistical Analysis

Thresholds for optimal sensitivity and specificity of CAST-ELISA were established using receiver operating characteristic (ROC) curves according to Zweig and Campbell [13]. Sensitivity, specificity, positive predictive value, and negative predictive value were calculated according to the method of Goldman [14]. The Mann-Whitney test [15] was used to compare CAST-ELISA results between healthy controls, birch pollen-allergic patients without OAS, and DBPCFC-positive patients. A *P* value of less than .05 was regarded as statistically significant.

Results

ROC plots were prepared to determine the cutoff for positivity in CAST-ELISA to achieve optimal sensitivity and specificity in patients with a positive DBPCFC. The results for the different allergen preparations and concentrations are summarized in Table 1. The sensitivities obtained with 0.8 and 4 ng/mL final allergen concentration were too low, and therefore, these data were not considered further. For allergen concentrations between 20 and 100 ng/mL, the optimal cutoff ranged from 50 to 200 pg/mL sulfidoleukotrienes. For the sake of simplicity and direct comparison of individual results, a single cutoff of 120 pg/mL sulfidoleukotrienes was chosen.

The results of comparisons between birch pollen-sensitized patients with either a positive DBPCFC or a negative open food challenge to the ingestion of celery, hazel, or carrot are shown in Table 2. In general, the results obtained with CAST-ELISA using a concentration of 100 ng/mL were less specific (more DBPCFC-negative patients who were CAST-ELISA positive).

With 20 ng/mL of the self-prepared extracts, CAST-ELISA

Table 1. Overall Diagnostic Sensitivity and Specificity of the Cellular Antigen Stimulation Test with Enzyme-Linked Immunosorbent Assay (CAST-ELISA) According to Cutoff and Allergen Source[a]

Allergen Source	Allergen Concentration	Cutoff, pg/mL Sulfidoleukotrienes	Sensitivity, % (OAS+)	Specificity, % (Healthy Controls)
Bühlmann (commercial)	20 ng/mL	50	83,7	93.3
		100	74.4	96.7
		120	74.4	98.3
		150	72.1	100
		200	69.8	100
Self-prepared extract	20 ng/mL	50	79.1	98.3
		100	74.4	100
		120	72.1	100
		150	69.8	100
		200	65.1	100
Self-prepared extract	100 ng/mL	50	95.3	85.0
		100	90.7	93.3
		120	88.4	96.7
		150	86.0	98.3

Abbreviations: OAS, oral allergy syndrome.
[a] The specificity of the cellular antigen stimulation test with enzyme-linked immunosorbent assay was assessed in 20 healthy controls with different sources and concentrations of celery, carrot, and hazelnut extracts, whereas 39 patients with oral allergy syndrome shown by positive double-blind placebo-controlled food challenge were tested with the extract of the culprit foods alone. The data for the 3 different food allergens were taken together and the respective sensitivities were calculated using various cutoff thresholds.

for celery was positive in 85.7% of the DBPCFC-positive patients but only in 8.3% of the challenge-negative subjects. This contrasts with the results of the specific IgE determination, since 33.3% of the challenge-negative subjects were positive. For skin tests, the respective sensitivities were 85% (SPT with commercial extracts) and 100% (SPT with self-prepared extracts). As skin-test data were only available for DBPCFC-positive patients, specificities for SPT were not assessed.

CAST-ELISA using 20 ng/mL of commercial allergen extract for hazelnut was positive in 73.3% of DBPCFC-positive patients and 12.5% of challenge-negative subjects. Analysis of specific IgE was positive in 73.3% and 27.3% of subjects, respectively. A somewhat higher sensitivity of 80% was obtained with skin tests independently of the allergen extract used.

For carrot, positive CAST-ELISA results were obtained in 85.7% of the DBPCFC-positive patients and in 9.1% of the challenge-negative patients. Analysis of specific IgE was positive in 57.1% and 40%, respectively. SPT with the self-prepared extracts gave a sensitivity of 100%, whereas SPT with the commercial extract showed only 28.6% positivity, suggesting that the commercial carrot extract was not very effective.

For all 3 food allergens, the positive predictive values and negative predictive values appeared higher for CAST-ELISA than for specific IgE determinations (Table 2). When tested in normal healthy controls, CAST-ELISA showed 95% to 100% specificity for all 3 food allergens up to a final concentration of 100 ng/mL, independently of the allergen source. However, there were significant differences between allergen sources and concentrations to be used in CAST-ELISA when tested in symptomatic patients (Table 2). The same phenomenon was also observed for the skin tests. The most appropriate allergens to be applied for CAST-ELISA seemed to be the commercial ones (except celery) at a final concentration of 20 ng/mL. The discriminative power of CAST-ELISA under those conditions is shown in Figure 1, where single CAST-ELISA results are shown for healthy controls stimulated with each of the 3 food allergens, challenge-negative patients, most of them stimulated with each of the 3 food allergens, and DBPCFC-positive patients stimulated with the culprit food allergen. The mean net stimulation was 15 pg/mL sulfidoleukotrienes for control subjects, 131 pg/mL for challenge-negative patients, and 1078 pg/mL for DBPCFC-positive patients. Significant differences were observed between the groups ($P < .001$).

The capacity of CAST-ELISA to discriminate between DBPCFC-positive and challenge-negative patients is shown in Figure 2 for the individual food allergens at 20 ng/mL and in Figure 3, where all DBPCFC challenges are considered together and compared to the alternative test methods.

Considering all tested individuals case by case, SPT and analysis of specific IgE were frequently positive in patients without OAS, whereas CAST-ELISA seemed to coincide with clinical reactivity in most cases.

It was recently claimed that quantitative determination of

Table 2. Sensitivity, Specificity, and Positive and Negative Predictive Value for the Different Diagnostic Tests[a]

DBPCFC	Test	Allergen Source	Allergen Concentration	Sensitivity, %	Specificity, %	PPV,%	NPV,%
Celery	CAST-ELISA	Commercial	20 ng/mL	71.4	91.7	88.2	78.6
21 positive		Self-prepared	20 ng/mL	85.7	91.7	90.0	88.0
24 negative		Self-prepared	100 ng/mL	95.2	66.7	71.4	94.1
	CAP-FEIA			71.4	66.7	75.0	62.5
	SPT-1	Allergopharma	44 µg/mL	85.0	nd	na	na
	SPT-2	Self-prepared	1 mg/mL	100	nd	na	na
Hazelnut	CAST-ELISA	Commercial	20 ng/mL	73.3	87.5	84.6	77.8
15 positive		Self-prepared	20 ng/mL	66.7	87.5	83.3	73.7
16 negative		Self-prepared	100 ng/mL	80.0	75.0	75.0	80.0
	CAP-FEIA			73.3	72.7	78.6	66.7
	SPT-1	Stallergènes	6.26 mg/mL	80.0	nd	na	na
	SPT-2	Self-prepared	1 mg/mL	80.0	nd	na	na
Carrot	CAST-ELISA	Commercial	20 ng/mL	85.7	90.9	75.0	95.2
7 positive		Self-prepared	20 ng/mL	42.9	86.4	50.0	82.6
21 negative		Self-prepared	100 ng/mL	85.7	77.3	54.5	94.4
	CAP-FEIA			57.1	60.0	40.0	75.0
	SPT-1	Stallergènes		28.6	nd	na	na

Abbreviations: CAP-FEIA, fluorescent enzime immunoassay; CAST-ELISA, cellular antigen stimulation test with enzyme-linked immunosorbent assay; DBPCFC, double-blind placebo-controlled food challenge; na, not assessed; nd, not determined; NPV, negative predictive value; PPV, positive predictive value; SPT, skin prick test.

[a] Tests were performed with commercial food extracts and self-prepared food extracts in patients with positive and negative food challenges to hazelnut, carrot, and celery. DBPFC was used as the gold standard.

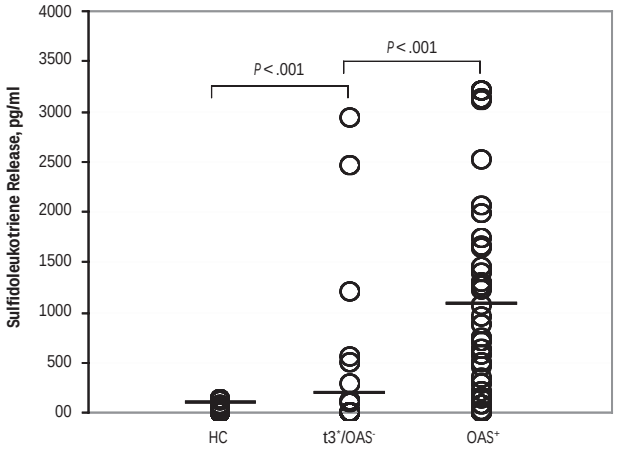

Figure 1. Results of the cellular antigen stimulation test in combination with enzyme-linked immunosorbent assay (CAST-ELISA) in healthy controls (HC), birch pollen-allergic patients without oral allergy syndrome ($t3^+/OAS^-$), and birch pollen-allergic patients with a positive response to double-blind placebo-controlled food challenge (OAS^+). Horizontal bars indicate mean values.

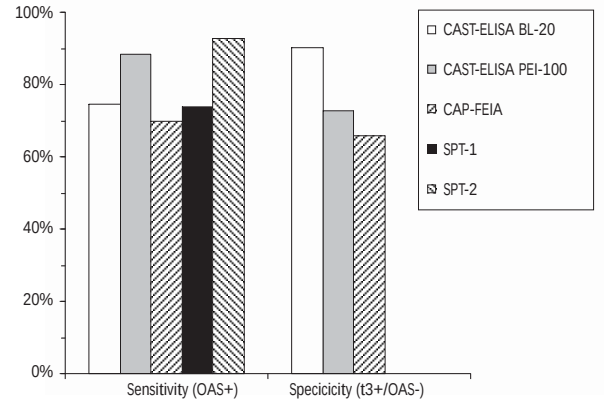

Figure 3. Overall sensitivity and specificity (celery, hazelnut, and carrot) of the cellular antigen stimulation test in combination with enzyme-linked immunosorbent assay (CAST-ELISA), analysis of specific immunoglobulin E by fluorescent enzyme immunoassay (CAP-FEIA) and skin prick tests (SPT) with commercial (SPT-1) and self-prepared (SPT-2) extracts using double-blind placebo-controlled food challenge (DBPCFC) as the gold standard. OAS^+ indicates patients with oral allergy syndrome, determined by DBPCFC; $t3^+/OAS^-$, birch pollen-allergic patients without OAS; BL-20, commercial allergens (Bühlmann) at final concentrations of 20 ng/mL; PEI-100, allergen extracts prepared by the Paul Ehrlich Institute at final concentrations of 100 ng/mL.

specific IgE has some predictive value in terms of the outcome of DBPCFC [16,17]. The correlation between the results of analysis of specific IgE and DBPCFC to birch and foods (celery, hazelnut and carrot) in our cases is shown in Figure 4. Although a weak correlation was observed for the food allergens, it was clearly weaker than the correlations observed for the qualitative (positive or negative CAST-ELISA) or quantitative measures of CAST-ELISA with DBPCFC.

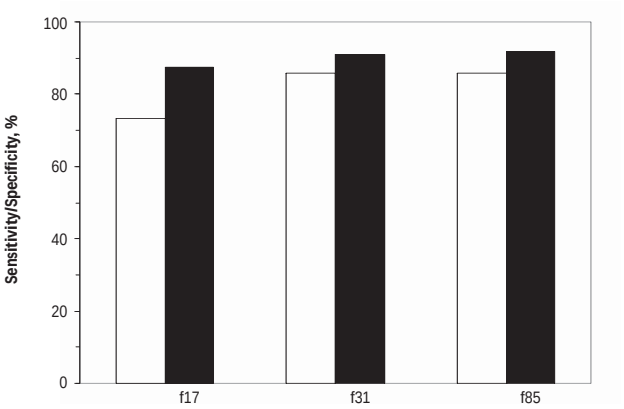

Figure 2. Sensitivity and specificity of the cellular antigen stimulation test in combination with enzyme-linked immunosorbent assay (CAST-ELISA) for the individual food allergens using double-blind placebo-controlled food challenge (DBPCFC) as the gold standard. CAST-ELISA was performed with the commercial allergens f17 (hazelnut) and f31 (carrot) and with a self-prepared extract of raw celery (f85), all at a concentration of 20 ng/mL. Open bars indicate DBPCFC-positive patients (sensitivity); closed bars, birch pollen-allergic patients without oral allergy syndrome (specificity).

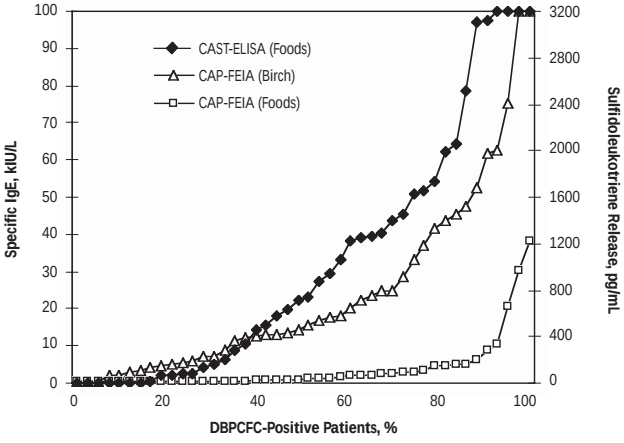

Figure 4. Correlation between the percentage of patients with a positive double-blind placebo-controlled food challenge (DBPCFC) and the results of the cellular antigen stimulation test in combination with enzyme-linked immunosorbent assay (CAST-ELISA) and fluorescent enzyme immunoassay (CAP-FEIA)..

Discussion

Since its introduction around 1993, CAST-ELISA has been used relatively infrequently in food allergy [8-10,18-19], despite its sensitivity (81%-85%) and specificity (95%-100%) having been reported to be quite high, often higher than that of SPT or analysis of specific IgE [9,10,18,19]. Specificities, however, have mostly been determined against a panel of

healthy controls, and neither of the clinically validated studies that have been published [9,18] addressed whether CAST-ELISA discriminates between immunologically sensitized patients–based on SPT results or analysis of specific IgE–who have a positive result in food challenge and those in whom food challenge is negative.

The results of this study show that, in the majority of cases, CAST-ELISA with celery, hazelnut, and carrot allergens discriminates between birch pollen-sensitized patients who show positive or negative results in challenges with these allergenic foods. The greatest discriminatory power lies within a limited range of allergen concentrations. If the concentration is too low (less than 20 ng/mL) the test is not sufficiently sensitive, whereas if the concentration is too high (greater than 100 ng/mL), a positive result may be obtained in some birch-sensitized patients in whom the results of food challenge are negative, even though the results of CAST-ELISA are still negative in healthy nonsensitized controls. Accordingly, the optimal allergen concentration to discriminate prospectively between challenge-positive and challenge-negative patients appears to be around 20 ng/mL of total protein for celery, hazelnut, and carrot, and this concentration may also be valid for other allergenic foods. Apart from the optimal allergen concentration, the quality of the allergen extract seems to be crucial and has to be thoroughly controlled for each new batch, as is also true of the commercial CAST allergens. It is apparent, however, that once the appropriate allergen concentration has been determined, CAST-ELISA correlates better with clinical reactivity than SPT or determination of specific IgE concentration.

All currently used test methods are influenced by the phenomenon of immunological cross-reactivity between birch pollen allergens and the related food allergens, and as a result, patients with a positive SPT to the cross-reactive foods may not be clinically allergic to those foods. Thus, it is not surprising that a test based on basophil reactivity might correlate better with clinical reactivity than does determination of IgE concentrations. In addition to the presence of IgE antibodies, this test also takes into account the individual cellular reactivity of each patient [8]. Recently, it has been shown in a similar situation of birch pollen-sensitized patients with plant-related allergy that the flow cytometric basophil activation test can discriminate between patients with and without food allergy [20,21], with a sensitivity of 88% and a specificity of 75% [21].

Further confirmation of the discriminatory power of CAST-ELISA should be obtained through multicenter studies including additional allergenic foods and more severe manifestations of IgE-mediated allergy, such as generalized urticaria, respiratory symptoms, gastrointestinal symptoms, or even anaphylactic reactions. SPT and specific IgE determinations may continue to be first-line diagnostic tools in food allergy, and DBPCFC the gold standard, but CAST-ELISA offers significant advantages as a complementary test that will remove the need for DBPCFC, at least in part, in the routine management of food-allergic patients.

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