

IgE to food allergens are highly prevalent in patients allergic to pollens, with and without symptoms of food allergy

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Summary

Serum samples from 274 patients allergic to one or more of three pollens (birch, grass, mugwort), from 36 patients allergic to cat and/or *Dermatophagoides pteronyssinus* but not to pollen and from 55 non-allergic controls, as well as 20 cord blood samples, were examined for specific IgE to six 'pollen-associated' food allergens by using a new sensitive assay (CAP). A questionnaire asking for reactions to food was also sent to all patients.

In the pollen group, 111 patients (47%) were positive (≥ 0.71 kU/l) for a food allergen (392 positive tests). Of these, 92 were sensitive to apple, 68 to potato, 64 to carrot, 63 to celery, 61 to peach and 44 to melon. In the non-allergic group, no IgE to any of the food allergens tested was found, whereas in the group allergic to non-pollen allergens, only one individual had such an IgE. The CAP assay was found to be more sensitive than RAST for the allergens studied. A history of clinical reactions (oral symptoms in 67, rhinoconjunctivitis in 65, asthma in 42 and urticaria in 39) to the corresponding food allergen was reported mainly by patients with positive CAP.

In conclusion, we found a high prevalence of IgE to some food allergens in patients allergic to pollen and the absence of such antibodies in the control groups. The new *in vitro* assay, being more sensitive than previous ones, indicated a high prevalence of food specific IgE in pollen allergic patients, which in many cases did not correspond to clinical symptoms of food allergy.

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Introduction

In patients with pollinosis, the occurrence of allergic reactions following the intake of fresh fruits or vegetables is well recognized [1,2]. This phenomenon has been extensively characterized on the basis of clinical observations, skin and RAST tests, resulting in associations such as the birch-apple, the mugwort-celery or the ragweed-banana-melon syndromes [3–6]. Other investigations have demonstrated crossreactive carbohydrate antigenic determinants, that were present in various food, respiratory and venom allergens [7]. Recently, profilin, an ubiquitous protein present in eukaryotic cells of animals

and plants, has been identified as a major crossreactive allergen in different pollens, fruits and vegetables [8,9].

The most common clinical presentation is the so-called oral allergy syndrome consisting of itching and swelling of the lips, of the oral mucosa and of the soft palate [10,11]. Other organs are more rarely involved. However, symptoms such as rhinoconjunctivitis and asthma, angioedema and urticaria or even anaphylaxis may occur [2,12].

The diagnosis of IgE-mediated hypersensitivity to food is based on a detailed history, on the *in vivo* [13] and *in vitro* demonstration of specific IgE and, when possible, on provocation tests. Determination of specific IgE to food allergens has been found less useful in clinical practice than that to respiratory allergens. Results obtained for food specific IgE by different methods were sometimes

contradictory. Skin tests to food allergens have often been found not to reflect clinical evidence, with a tendency to being falsely negative [14]. In the present study, we investigated whether IgE antibodies to fruit or to vegetable antigens might be present in an unselected population of subjects allergic to pollens, even in the absence of symptoms of food allergy. A new sensitive assay was used for this purpose.

Materials and methods

Subjects

Serum samples from 274 patients (age range: 6–73 years, 52% women), with a diagnosis of allergic rhinoconjunctivitis and/or asthma (based on typical history and on positive skin-prick tests) were selected on the basis of a previous positive RAST test (≥ 0.71 PRU) against one of the following inhalant allergens: grass pollen mix, birch pollen, mugwort pollen, cat dander or *D. pteronyssinus*. In the study, serum samples were re-tested using the CAP system (CAP), developed by Pharmacia AB, Uppsala, Sweden, for the above mentioned inhalant allergens and separated in two groups: (a) samples from patients with a sensitization to pollens ($n=238$) and (b) from patients exclusively sensitized to cat dander or *D. pteronyssinus* ($n=36$). A third group, used as a control, consisted in 55 samples from non-atopic individuals (20–55 years, 50% women) without a history of atopy, nor positive skin-prick tests to a panel of eight inhalant allergens or positive CAP tests to the above mentioned five respiratory allergens. An additional group of 20 cord blood samples, collected at birth from unselected newborns, was equally used as control.

Food allergens

In all samples ($n=349$), the level of specific IgE to six pollen-associated food allergens was measured: three vegetables (carrot, potato and celery root and stem) and three fruits (apple, melon and peach).

Methods

The serum samples were assayed by using the CAP system for both inhalant and food allergens. The food allergens were also subsequently re-tested at the time of the study by using the conventional RAST with a fluorescent modification [15], in order to compare CAP with RAST when applied to fruit and vegetable allergens. Both techniques were performed according to the instructions of the manufacturer.

The CAP assay differs from RAST by the introduction of a new kind of solid phase and a new conjugate, which

are expected to increase the sensitivity of the assay. Briefly, serum samples and standards were incubated at 20°C for 30 min with the Immuno-CAP, i.e. a flexible polystyrene structure encased in a capsule and coated with the allergen to be tested. After washing, a beta-galactosidase labeled mixture of a monoclonal and a polyclonal anti-IgE antibodies was added. The assay was calibrated against the WHO-standard for IgE, from 0.35 to 100 kU/l. The cut-off and the classification system of CAP is basically the same as for RAST with an extension to 6 classes (class 5: 50–100 kU/l, class 6 > 100 kU/l). In the present study, the results ≥ 0.71 kU/l ($\geq$ class 2) were considered as positive for CAP, and those ≥ 0.71 PRU ($\geq$ class 2) as positive for RAST.

Questionnaire

A questionnaire was sent to all patients ($n=274$) with questions asking for symptoms and season of the respiratory allergy, as well as for the possible occurrence and type of allergic symptoms to the six food allergens studied. Patients were also asked for possible reactions to other foods and to spices.

Cluster analysis

The analysis was performed using a programme of the SAS system (Fastclus and Candisc procedures).

Results

IgE to food allergens (CAP)

Among the 238 patients allergic to pollens, 111 (47%) had a total of 392 positive CAP results to one of the food allergens tested, whereas only one individual (2.8%) of the non-pollen allergic group had specific IgE (in this case, to peach allergen). In the non-atopic control group and in the cord blood samples, no specific IgE to any of the food allergens was detected. Patients allergic to pollens were further separated according to the particular pollen, or pollen group, they were sensitized to (Table 1). Among the 71 patients with IgE to grass alone, only three positive CAP results to one of the food allergens were found. Among the 11 with IgE to mugwort only, a single patient showed IgE against potato. In the 22 subjects reacting to birch pollen alone, 31 positive CAP results were obtained (half of them to apple). In the 71 subjects sensitized to two pollens, 97 positive results were found. Finally, in the 63 sensitized to all three pollens, the number of positive CAP results to one of the foods was 260.

Patterns of crossreactivity

The cluster analysis allowed to identify at best a number of four clusters among the 238 patients sensitive to

Table 1. Positive CAP results to 6 food allergens distributed according to pollen hypersensitivity

Specific Ig to pollen of	Number of patients	Number of positive reactions to the allergen of						Total positive tests	Total of positive patient	% of number of patients
		Carrot	Potato	Celery	Apple	Melon	Peach			
Grass	71	0	1	0	1	0	1	3	2	3
Birch	22	3	2	4	15	0	7	31	15	68
Mugwort	11	0	1	0	0	0	0	1	1	9
Grass + birch	40	10	5	12	17	1	7	52	20	50
Grass + mugwort	29	6	12	5	6	7	5	41	14	48
Birch + mugwort	2	0	0	1	1	0	2	4	2	100
Grass + birch + mugwort	63	45	47	41	52	36	39	260	57	90.5
Total	238	64	68	63	92	44	61	392	111	47

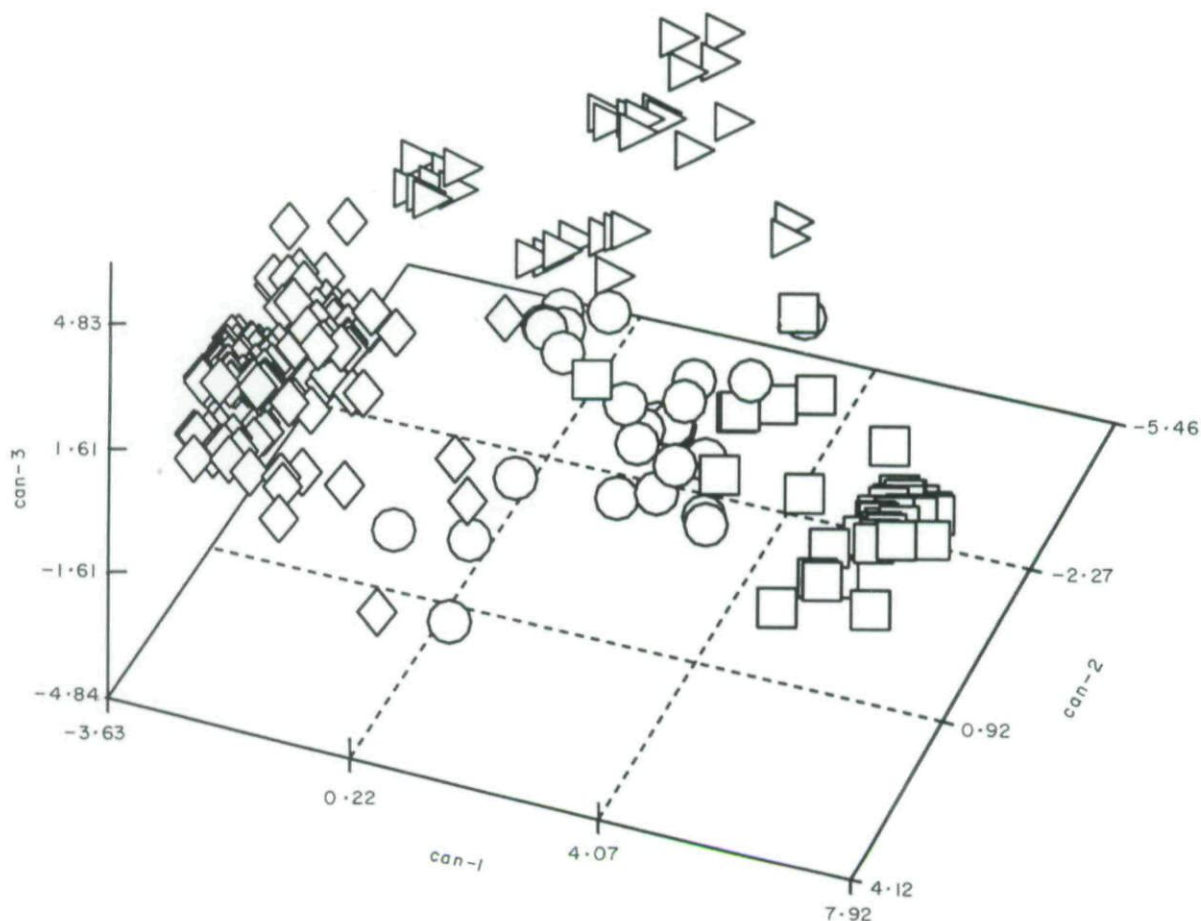


Fig. 1. Cluster analysis. Three-dimensional presentation of four clusters of sensitized patients defined by three canonical scores (axis can-1, can-2, and can-3). Cluster 1 ($\diamond$): grass alone, cluster 2 ($\square$): all pollen and food allergens, cluster 3 ($\triangle$): birch and apple and cluster 4 ($\circ$): all pollens, apple, celery and carrot.

Table 2. Number of patients reporting symptoms after intake of the food allergens studied, out of 147 patients who answered

Allergen	Pollen allergic (<i>n</i> = 93)	Non-pollen allergic (<i>n</i> = 2)
Carrot	15 (16%)	0
Potato	7 (8%)	0
Celery	22 (24%)	0
Apple	26 (28%)	2
Melon	13 (14%)	0
Peach	21 (23%)	0
Total number of reactions reported	104	2

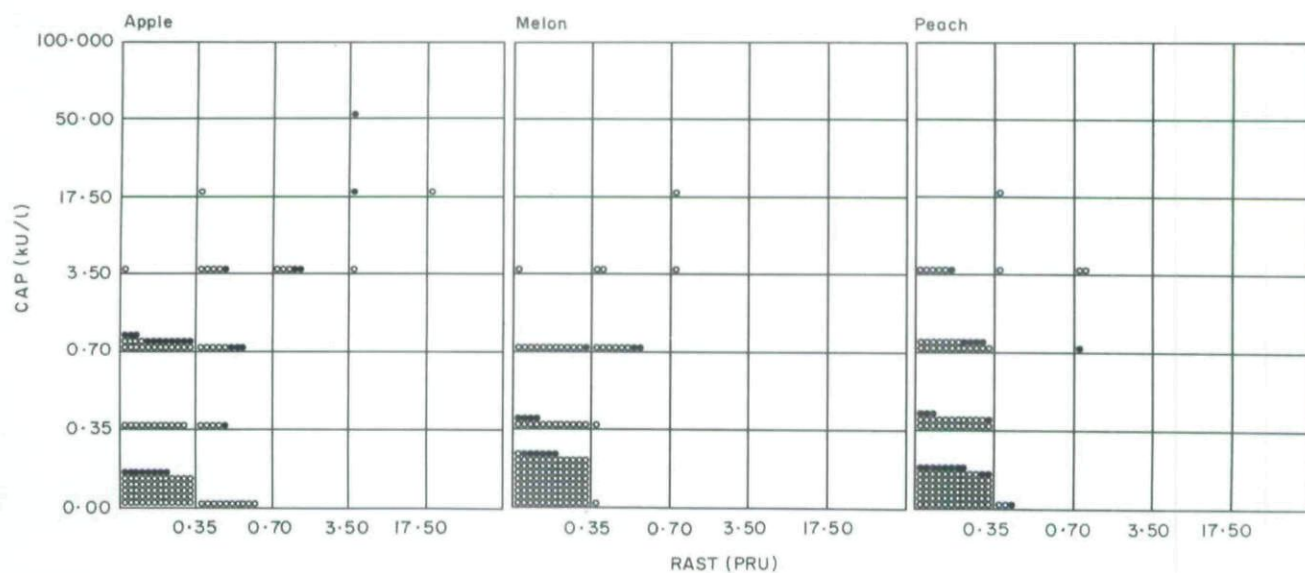
pollens. Attempts to define a smaller or larger number of clusters failed, because they lead either to too small or to meaningless groups. The four isolated clusters were the following (frequency in brackets): (1) grass alone [134], (2) all pollen and food allergens positive [45], (3) birch together with apple [33] and (4) all three pollens together with apple, carrot and celery [26]. In cluster 3, 40% of the subjects also had antibody to peach and, in cluster 4, 42% also had antibody to potato. The approximate *F*-test associated with Wilk's Lambda showed a $P < 0.0001$, indicating that the four clusters are well separated. Figure 1 displays the four clusters of patients in the three-dimensional space defined by the three canonical scores obtained from the cluster analysis.

Questionnaire

The questionnaire was returned by 147 patients, 130 (55%) from the pollen group and 17 (47%) from the non-

Table 3. Comparison between information obtained by questionnaire (Q) and results of CAP (C) (n = nb of answers to questionnaire)

Allergen	n	Concordance			Discordance		
		Q+/C+	Q-/C-	Total (%)	Q+/C-	Q-/C+	Total (%)
Carrot	146	9	107	116 (79)	6	24	30 (21)
Potato	145	4	108	112 (77)	3	30	33 (23)
Celery	147	8	102	110 (75)	14	23	37 (25)
Apple	145	19	85	104 (72)	9	32	41 (28)
Melon	146	3	111	114 (78)	10	22	32 (22)
Peach	144	6	95	101 (70)	15	28	43 (30)
Total	873	49	608	657 (75)	57	159	216 (25)

**Fig. 2.** Comparison of specific IgE to fruits (apple, melon, peach) measured by CAP and RAST in patients allergic to pollen. No symptoms to fruits reported (○), Symptoms to fruits reported (●).

pollen allergic group. Oral symptoms were reported by 67 persons, rhinoconjunctivitis by 65, asthma by 42, urticaria by 39 and anaphylactic reactions by none of them. All these reactions were reported by patients belonging to the pollen group except for two reactions to apple reported by subjects of the non-pollen allergic group. Ninety-three of the pollen group and two of the non-pollen group reported one or several symptoms to one or several foods.

Table 2 shows that among the foods tested, those most commonly believed to elicit symptoms were apple, celery and peach. Other foods not studied here, as well as spices, were also made responsible by the patients for eliciting symptoms: nuts (reported by five patients), strawberry and tomato (four patients), pear and avocado (three

patients); grapes, kiwi, citrus fruits, fennel and cherries (two patients), orange, mango, peanut, basilisk, Lima bean, cabbage, turnips, pepper and honey (one patient).

Comparison of the results of CAP to those of RAST

The concordance between the results of CAP and those of RAST was 82% on the average, varying between 75 and 89% depending on the allergen tested (data not shown). Of the 18% which were discordant, 17.1% were CAP positive/RAST negative and 0.9% CAP negative/RAST positive, indicating a higher yield of specific IgE by the CAP test.

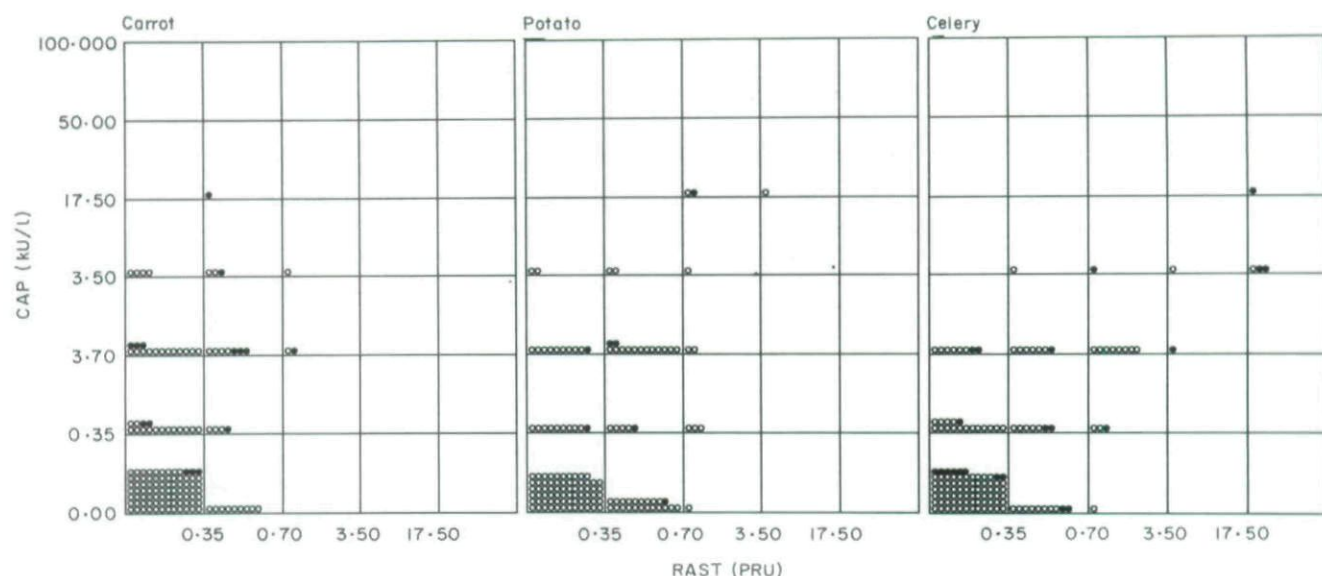


Fig. 3. Comparison of specific IgE to vegetables (carrot, celery, potato) measured by CAP and RAST in patients allergic to pollen. No symptoms to vegetables reported (○), Symptoms to vegetables reported (●).

Table 4. Percentage of positive CAP results according to total IgE levels

	<i>n</i> patients (total = 236)	IgE ≤ 80	IgE 81–180	IgE 181–500	IgE 501–850	IgE > 850
	<i>n</i> per IgE range	57	60	56	39	24
Allergen	<i>n</i> with pos CAP	%	%	%	%	%
Grass	200	70	82	91	92	100
Birch	125	44	40	57	72	67
Mugwort	103	26	33	50	72	50
Carrot	63	7	20	30	49	46
Potato	66	3.5	22	30	56	50
Celery	62	7	20	32	41	50
Apple	91	23	30	45	56	54
Melon	44	3.5	13	18	41	33
Peach	60	12	20	18	46	54

Results of the *in vitro* tests compared with those of the questionnaire

The comparison of the CAP results with the answers to the questionnaire revealed a mean concordance of 75%. The discordant results consisted mainly of positive CAP results without a corresponding reported history (Table 3). When the comparison was made with RAST instead of CAP, the concordance was 86%, but, since it consisted for 98% of negative results, this concordance should not be over-interpreted.

A comparison between the results of CAP and RAST distributed by classes and including the answers of the

questionnaire is given in Fig 2 and 3. It also shows a better yield of the CAP. Many results were 'questionnaire positive/specific IgE negative', appearing thus either as 'false negative' for the *in vitro* methods or 'false positive' for the questionnaire.

Total IgE levels

Total IgE values from 236 patients were separated according to the five following ranges: < 80, 81–180, 181–500, 501–850 and > 850 kU/l, corresponding to the limits given by the percentiles 25, 50, 75 and 90 of the 238

patients sensitive to pollens. Table 4 shows, for each of these IgE ranges and for each allergen, the percentage of positive CAP results.

Discussion

Different fruit and vegetable allergens, the so-called 'pollen associated' food allergens, may elicit in pollen-sensitive subjects an immediate type allergic reaction of the skin, the nose, the upper gastrointestinal tract or of the conjunctiva. The diagnosis of this condition is usually made by the patient's history. *In vitro* tests to food allergens have often been negative in this condition, as well as in food allergy in general.

In the present study, we took advantage of a new *in vitro* assay of specific IgE, offering an increased sensitivity [16–18], in order to test the presence of specific IgE to some fruits and vegetables in pollen allergic subjects in general and, in particular, in those reporting symptoms after intake of these foods. The purpose was to investigate whether the crossreaction existed subclinically in a broader proportion of the pollen allergic subjects than just in those reporting symptoms to food.

We found a surprisingly high prevalence of specific IgE to food in pollen allergic subjects, no such IgE in the nonallergic subjects and none (but one exception) in the subjects with non-pollen respiratory allergy. The prevalence of food IgE differed according to the pollen to which the patients were sensitive. This prevalence was found high for birch, or for birch associated with one or the other, or for both other respiratory allergens. It was low for grass or mugwort when alone, but surprisingly high when grass and mugwort were associated (Table 1). This curious finding is difficult to explain: probably the risk of crossreactivity increases with the number of sensitivities to pollens.

The crossreactivity described between birch pollens and apple allergens was also clearly shown here by the cluster analysis [5,6]. In some of these cases, antibody to peach was also present. This might correspond to a crossreaction to *Bet v* I [4]. The analysis further identified the existence of a broader cluster including all three pollens and three of the foods: apple, carrot and celery, representing perhaps a crossreaction due to profilin [8,9,19]. The broadest cluster—reaction including all pollens and all foods tested—might also correspond to crossreactivity to profilin [9,19] or to crossreactive carbohydrate determinants (CCD) [4,7]. The pure grass group shown by the analysis has no significance as far as cross-reactive patterns are concerned. The association between allergy to mugwort pollen and to celery was not found here, perhaps because the celery mix used as antigen was less recognized by the antibodies of this particular group

of patients [20], or because our number of patients allergic to mugwort only was too small.

The observation that only one positive CAP was obtained in the non-pollen allergic group and none in the non-atopic group indicates, first that the high prevalence of IgE to the fruits and vegetables is actually restricted to subjects with pollinosis and, second, that the CAP assay is specific since there were no false positive in the controls and since it correctly identified specific IgE to the particular food allergens. The new assay appeared clearly more sensitive than RAST for detecting the IgE to the food allergens studied here.

Analysis of the total IgE results showed that the percentage of specific IgE positive results increased progressively with the total IgE levels (Table 4). This increase was roughly the same for all pollen and food allergens studied. This finding is in accordance with the fact that patients with IgE-mediated reactions often have moderately elevated total IgE levels. For most allergens studied here, there was, however, no more increase of positive CAP results between the last two categories of IgE levels. This indicates that high levels of non-specific IgE probably did not induce false positive results in the specific IgE assay.

The interpretation becomes more difficult when *in vitro* results are compared with those of the questionnaire. It is clear that there was an over-reporting tendency in the answers to this questionnaire, like in others and like in clinical practice. However, a marked difference between the number of reactions after food intake reported by pollen allergic person and by non-pollen allergic persons was found (Table 2), which suggests that at least part of the reactions were correctly reported.

The data indicate that the discordances 'CAP positive/questionnaire negative' resulted from the correct *in vitro* detection of antibodies to epitopes existing in pollen as well as in food allergens, in cases that were not clinically apparent [3,4]. There are thus subclinical cases of pollen-associated food allergy. Such cases do not result from a lack of specificity, since CAP results were negative in the control groups and since the number of positive CAP to pollen correlated, in each patient, with the number of CAP found positive to food allergens. The cases 'CAP negative/questionnaire positive', which were less frequent, might be either due to over-reporting or to the assay that is not sufficiently sensitive.

If the indications given by the questionnaire were compared with the results of the *in vitro* assays, the calculated sensitivity of CAP would be comprised between 23 and 68% according to the allergen, its specificity between 73 and 83% and its accuracy between 70 and 79%. Similarly, the sensitivity of the RAST would be between 0 and 27%, its specificity between 90 and 98

and its accuracy between 80 and 90%. However, one should be cautious in judging here RAST specificity and accuracy since it was mainly calculated on negative results. In addition, one should also be cautious when defining sensitivity or specificity of a test in allergic diseases, since the presence of specific IgE does not imply clinical disease [17].

In conclusion, we found a high prevalence of IgE to some fruits and vegetables in patients allergic to pollens, including patients who did not report symptoms after the intake of such foods. Such antibodies were absent in the control groups.

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References

- Lahti A, Hannuksela M. Hypersensitivity to apple and carrot can be reliably detected with fresh material. *Allergy* 1978; 33:143-6.
- Dreborg S. Food allergy in pollen-sensitive patients. *Ann Allergy* 1988; 61:41-6.
- Halmepuro L, Vuontela K, Kalimo K, Björkstén F. Cross-reactivity of IgE antibodies with allergens in birch pollen fruits and vegetables. *Int Arch Allergy Appl Immunol* 1984; 74:235-40.
- Calkhoven PG, Aalbers M, Koshte VL et al. Cross-reactivity among birch pollen vegetables and fruits as detected by IgE antibodies is due to at least three distinct cross-reactive structures. *Allergy* 1987; 42:382-90.
- Berrens I, van Dijk AG, Houben GF, Hagemans MLD, Koers WJ. Cross-reactivity among the pollen proteins of birch and apple trees. *Allerg Immunol* 1990; 36:147-56.
- Ebner C, Birkner T, Valenta R et al. Common epitopes of birch pollen and apples-Studies by Western and Northern blot. *J Allergy Clin Immunol* 1991; 88:588-94.
- Aalberse RC, Koshte V, Clemens JGJ. Immunoglobulin E antibodies that crossreact with vegetable foods, pollen, and Hymenoptera venom. *J Allergy Clin Immunol* 1981; 68:356-64.
- Vallier P, Dechamp C, Valenta R, Vial O, Deviller P. Purification and characterization of an allergen from celery immunochemically related to an allergen present in several other plant species. Identification of profilin. *Clin Exp Allergy* 1992; 22:774-82.
- van Ree R, Voitenko V, van Leeuwen WA, Aalberse RC. Profilin is a cross-reactive allergen in pollen and vegetable foods. *Int Arch Allergy Appl Immunol* 1992; 98:97-104.
- Amlot PL, Kemeny DM, Zachary C, Parkes P, Lessof MH. Oral allergy syndrome (OAS): symptoms of IgE-mediated hypersensitivity to foods. *Clin Allergy* 1987; 17:33-42.
- Ortolani C, Ispano M, Pastorello EA, Ausaloni R, Magri GC. Comparison of results of skin prick tests (with fresh foods and commercial food extracts) and RAST in 100 patients with oral allergy syndrome. *J Allergy Clin Immunol* 1989; 83:683-90.
- Wüthrich B. Birkenpollen-Allergie in Verbindung mit Unverträglichkeiten gegen verschiedene Obstsorten. *Hautarzt* 1990; 41:583-4.
- Dreborg S. Skin test in diagnosis of food allergy. *Allergy Proc* 1991; 12:251-4.
- Moneret-Vautrin DA, Gueant JL, Abdel-Ghani A, Maria Y, Nicolas JP. Comparative evaluation between two immunochemical techniques (FAST and Phadezym) and the Phadebas RAST in food allergy. *Allergy* 1990; 45:104-8.
- Pécoud A, Peitrequin R, Duc J et al. Application of microtitre plates and fluorescence reading to shorten handling of Phadezym RAST® and Phadezym IgE PRIST®. *Clin Allergy* 1986; 16:231-9.
- Bousquet J, Chanez P, Chanal I, Michel FB. Comparison between RAST and Pharmacia CAP system: a new automated specific IgE assay. *J Allergy Clin Immunol* 1990; 85:1039-43.
- Ewan PW, Coote D. Evaluation of a capsulated hydrophilic carrier polymer (the ImmunoCAP) for measurement of specific IgE antibodies. *Allergy* 1990; 45:22-9.
- Leimgruber A, Mosimann B, Claeys M et al. Clinical evaluation of a new *in vitro* assay for specific IgE the Immuno CAP System. *Clin Exp Allergy* 1991; 21: 127-31.
- Valenta R, Duchene M, Ebner C et al. Profilins constitute a novel family of functional plant pan-allergens. *J Exp Med* 1992; 175:377-85.
- Wüthrich B, Stäger J, Johansson SGO. Celery allergy associated with birch and mugwort pollinosis. *Allergy* 1990; 45: 566-71.

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