

Letter to the Editor

Is the detection of IgE to multiple Bet v 1-homologous food allergens by means of allergen microarray clinically useful?

To the Editors:

It is well known that subjects with birch pollen allergy might experience oral allergy syndrome (OAS) on ingestion of plant-derived foods, including Rosaceae, tree nuts, peanut, Apiaceae, kiwi, or soybean because of cross-reactivity between the major birch pollen allergen Bet v 1 and homologous proteins in fruits and vegetables. In these patients skin tests with fresh material, as well as specific IgE measurement, are generally able to confirm the allergic origin of the disorder. Such tests show an excellent negative predictive value,¹ which is in keeping with recent studies showing that patients with OAS react to certain specific epitopes on Bet v 1.² Unfortunately, their clinical usefulness is marred by low specificity and low positive predictive value because birch pollen-sensitized subjects frequently show cross-sensitization to plant-derived foods in the absence of any clinical symptom.³ Thus discriminating between sensitization and clinical allergy to plant-derived foods in patients with birch pollen allergy is still a problem. Although some studies found that patients with pollen-food allergy syndrome show higher birch pollen-specific IgE levels than subjects without clinical food allergy,^{1,4,5} the question remains whether *in vivo* and *in vitro* diagnostic tests have some clinical meaning in subjects with birch pollen allergy. Recently, recombinant pollen and food allergens have been introduced as diagnostic tools for specific IgE measurements. We assessed the clinical usefulness of a multiplex allergen microarray as a diagnostic and predictive method for patients with birch pollen allergy with or without associated food allergy.

The study population was selected from a large number of subjects who spontaneously presented from April 1 to June 30, 2009, at the allergy outpatient clinics of Pordenone and Paderno Dugnano, Italy, because of suspected seasonal respiratory allergy. Both centers are in the northern part of Italy, where birches are widely used in public parks and private gardens and birch pollen represents a major cause of respiratory allergy. All subjects underwent skin prick tests (SPTs) with a panel of commercial pollen extracts, including grass, ragweed, mugwort, birch, pellitory, cypress, olive, and plantain. Forty-two adult (age >14 years) patients monosensitized to birch pollen were finally included and represented the study population. Monosensitization to birch was diagnosed on the basis of the absence of skin reactivity to other seasonal airborne allergens. As a part of the routine evaluation, specific IgE levels to Bet v 1, the major birch pollen allergen, were measured by means of ImmunoCAP (Phadia, Uppsala, Sweden) in sera from all 42 patients, and all of them showed a strong Bet v 1-specific IgE reactivity. In contrast, no serum showed detectable IgE to Bet v 2 (birch profilin). Hypersensitivity to nonspecific lipid transfer protein (LTP) was excluded by a negative SPT response with a commercial peach extract (containing 30 µg of LTP/mL; ALK-Abelló, Madrid, Spain).⁶

All study patients had a history of seasonal respiratory symptoms (rhinoconjunctivitis with or without asthma) from the end of February to mid-May. Patients were thoroughly interviewed about intolerance to fruits, vegetables, or both (mainly Rosaceae, tree nuts, peanut, kiwi, soybean, and Apiaceae); those who

reported good tolerance to all fresh fruits and vegetables and their regular intake were classified as not having a pollen-food allergy syndrome ($n = 19$, group A; Table I). Those reporting typical OAS (ie, oral itching of the oral mucosa with or without angioedema of the lips immediately after the ingestion of fresh fruits, vegetables, or both but with a good tolerance for the same foods if they were processed) were classified as having pollen-food allergy syndrome ($n = 23$, group B). In these patients the clinical history of food allergy was confirmed by means of a positive SPT response with the fresh offending foods. Group B was further divided into subgroups of patients reporting allergy to Rosaceae (ie, apple, pear, peach, cherry, plum, and almond) only ($n = 7$), Rosaceae and other fruits but not vegetables ($n = 8$), and Rosaceae and other fruits and vegetables ($n = 6$, Table I). Because this was an observational study carried out within routine clinical activity, no ethical committee or institutional review board approval was required.

Specific IgE levels to Bet v 1, as well as to Mal d 1, Pru p 1, Gly m 4, Ara h 8, Api g 1, Dau c 1, and Act d 8, the Bet v 1-homologous proteins from apple, peach, soybean, peanut, celery, carrot, and kiwi, respectively, were measured by using a commercial allergen microarray immunoassay (ISAC; Phadia) according to the manufacturer's recommendations. Reaction sites were incubated with 20 µL of patients' sera for 2 hours. After rinsing, washing, and drying, allergen-specific IgE complexes were stained with a fluorescence-labeled anti-human IgE for 1 hour. After further washings, a laser scanner took fluorescence readings, and results were transformed into numeric data by comparison with a reference serum standardized against ImmunoCAP IgE. As a consequence, the results, expressed as ISAC standardized units (ISU/L), are indirectly linked to the World Health Organization IRP 75/502 IgE standard. Levels greater than 0.3 ISU/L were regarded as positive, according to the manufacturer's recommendations. Prevalences were compared by using the χ^2 test with the Yates correction. Specific IgE levels measured in the different subgroups were compared by nonparametric means with the Mann-Whitney *U* test. The simultaneous comparison of several subgroups of patients was performed by using the nonparametric Kruskal-Wallis test. Probability values of less than 5% were considered statistically significant. Statistical analysis was carried out with SPSS for Windows, version 11.5 (SPSS, Inc, Chicago, Ill), and MedCalc for Windows, version 7.4.1.0 (MedCalc Software, MariaKerke, Belgium).

Specific IgE levels found in all 42 patients are shown in Table I. The prevalence of subjects showing detectable circulating IgE specific for Mal d 1 was similar in the 2 groups without or with clinical food allergy (15/19 [79%] vs 21/23 [91%], respectively; $P =$ not significant), as was the prevalence of IgE specific for Pru p 1 (18/19 [95%] vs 22/23 [96%]), Gly m 4 (4/19 [21%] vs 6/23 [26%]), Ara h 8 (8/19 [42%] vs 10/23 [43%]), Api g 1 (4/19 [21%] vs 8/23 [35%]), Dau c 1 (1/19 [5%] vs 2/23 [9%]), and Act c 8 (4/19 [21%] vs 3/23 [13%]). The 2 groups showed similar levels of IgE to Bet v 1 and to all other homologous food allergens on the Mann-Whitney *U* test ($P =$ not significant). Similarly, the 4 groups of patients without (group A) or with (subgroups B1, B2, and B3) OAS did not differ in levels of IgE specific for Bet v 1-homologous foods by using the Kruskal-Wallis test ($P =$ not significant).

By analyzing the generated data in light of the clinical history of food allergy, it turned out that 8 of 8 and 5 of 6 patients with

TABLE I. Reportedly offending foods and specific IgE levels to several PR-10 proteins in 42 patients with birch pollen allergy

Patient no.	Offending foods	Bet v 1	Mal d 1	Pru p 1	Gly m 4	Ara h 8	Api g 1	Dau c 1	Act d 8
1	–	P	P	P	0	0	0	0	0
2	–	P	P	P	0	P	P	0	0
3	–	P	P	P	0	P	0	0	P
4	–	P	P	P	0	P	0	0	0
5	–	P	P	P	0	0	0	0	0
6	–	P	P	P	0	P	0	0	0
7	–	P	0	P	0	0	0	0	0
8	–	P	P	P	0	0	P	P	0
9	–	P	P	P	0	P	0	0	0
10	–	P	0	P	P	P	0	0	0
11	–	P	P	P	0	0	0	0	0
12	–	P	0	P	0	0	0	0	0
13	–	P	P	P	P	P	P	0	P
14	–	P	P	0	0	0	0	0	0
15	–	P	P	P	0	0	0	0	0
16	–	P	P	P	0	0	P	0	P
17	–	P	P	P	P	0	0	0	0
18	–	P	P	P	P	P	0	0	P
19	–	P	0	P	0	0	0	0	0
20	A, P	P	P	P	0	0	0	0	0
21	A	P	P	P	P	P	P	0	P
22	A, Pr, C	P	0	P	0	0	0	0	0
23	Pr, C	P	P	P	0	0	0	0	0
24	A, Pr	P	P	P	0	0	0	0	0
25	A, P, At	P	P	P	P	P	P	0	P
26	A, P, Pr	P	P	P	P	0	0	0	P
27	Pr, P, Al	P	P	P	0	P	0	0	0
28	Pr, H	P	P	P	0	0	0	0	0
29	A, H	P	P	P	0	0	0	0	0
30	A, Pr, P, K, Pa	P	P	P	0	0	0	0	0
31	P, H, K	P	P	P	0	0	0	0	0
32	A, K	P	P	P	0	0	P	0	0
33	A, C, H	P	P	P	0	0	0	0	0
34	A, P, K, H	P	P	P	0	0	P	0	0
35	A, W	P	0	0	0	P	0	0	0
36	A, H, W, B, Ca	P	P	P	0	P	P	0	0
37	A, At, P, H, Al, PN, F	P	P	P	P	P	0	P	0
38	A, At, P, C, Pr, At, K, Pl, H, W, Al Ca	P	P	P	0	P	P	0	0
39	A, C, H, K, Ca, F	P	P	P	P	0	0	0	0
40	A, C, H, P, At, Ca	P	P	P	0	P	0	0	0
41	A, C, P, K, W, F, Ca	P	P	P	P	P	P	P	0
42	A, Pr, P, C, At, K, H, W, Pe, F, Ce, Ca, L	P	P	P	0	P	P	0	0

Patients 1 to 19 = group A; patients 20 to 42 = group B; B subgroups: patients 20 to 27 = subgroup 1; patients 28 to 35 = subgroup 2; patients 36 to 42 = subgroup 3. Specific IgE levels are expressed as *P* (positive) or *0* (negative).

–, None; A, apple; Al, almond; At, apricot; B, banana; C, cherry; Ca, carrot; Ce, celery; F, fennel; H, hazelnut; K, kiwi; L, lettuce; P, peach; Pa, pineapple; Pe, peanut; Pl, plum; PN, pine nut; Pr, pear; W, walnut.

a history of OAS after the ingestion of kiwi and carrot, respectively, did not react to the Bet v 1-homologous allergens in kiwi (Act d 8) or carrot (Dau c 1). Reactivity to allergens other than Act c 8 and Dau c 1 cannot be ruled out but seems unlikely because all sera scored negative for Act d 1, Act d 2, and Act d 5 and profilins on the microarray assay; therefore it seems that the negative results with these foods are due to lack of sensitivity of the Bet v 1-homologous kiwi and carrot allergens. On the opposite side, 7 and 2 patients without a history of OAS induced by kiwi and carrot, respectively, showed detectable specific IgE levels for these proteins. Specific IgE was detected in 1 of 1 patients with celery allergy and 1 of 1 patients with peanut allergy; however, a further 12 and 17 patients who tolerated these foods, respectively, had positive results as well. Ten patients showed IgE specific for

Gly m 4, the soybean Bet v 1 homologue, but none reported soybean intolerance. Finally, 18 of 19 patients with apple allergy showed detectable IgE levels to Mal d 1, but their levels did not differ from levels in those without apple allergy (*P* = not significant); the same situation was observed among patients with peach allergy. Interestingly, no serum showed IgE reactivity to nAna c 2, bromelain, a molecular marker of reactivity to cross-reactive carbohydrate determinants.

High sensitivity and specificity are essential for every *in vivo* and *in vitro* diagnostic test to be regarded as useful in clinical practice. This study shows that in patients primarily sensitized to birch pollen, the problem of discriminating between true allergy and clinically irrelevant sensitization to plant-derived foods, both caused by cross-reactivity to Bet v 1-homologous proteins,

is far from being solved. In our series most patients without clinical allergy to plant foods showed IgE specific for Bet v 1-homologous proteins in apple and peach, and patients with and without food allergy did not show any difference in the prevalence of positive *in vitro* results for all allergen proteins considered. These findings are in keeping with the results of a very recent Belgian study⁷ but are even worse because in our hands IgE reactivity to carrot (Dau c 1) and kiwi (Act d 8) PR-10 proteins did not show differences between allergic and nonallergic subjects. Furthermore, all patients with kiwi allergy and 5 of 6 patients with carrot allergy had negative results on the allergen microarray. In our patients we did not confirm the diagnosis of food allergy using double-blind, placebo-controlled challenges; however, we believe that this fact in no way hampered the results of this study because the problem with the diagnostic method examined here regarded in most cases poor specificity (ie, positive test results in food-tolerant patients) rather than poor sensitivity (negative test results in allergic subjects), and reportedly tolerant patients are normally not submitted to oral food challenges.

In patients with pollen allergy, IgE reactivity to foods in the total absence of clinical allergy has been already observed³ and is frequently observed also *in vivo* when SPTs with fresh material are carried out. Thus it is possible to conclude that because of poor specificity, currently available *in vitro* and *in vivo* diagnostic tests do not seem to have any predictive value and hence are presently of little clinical value. The allergen microarray immunoassay seems to show problems also in terms of sensitivity, particularly for some allergens, such as Act d 8 and Dau c 1.

In this situation, to detect the relevant allergen or allergens in a patient with a history of OAS induced by fruits/vegetables confirmed by a positive SPT response with the fresh offending food, it is probably much better to use a limited number of sensitive markers of reactivity to different allergens, using them properly also as an exclusion test. For instance, measuring IgE levels to rBet v 1, rBet v 2 (or alternatively skin testing with date palm profilin extract), rPru p 3 (or alternatively skin testing with a peach extract containing uniquely LTP), and Act c 1/Act c 2⁸ (or alternatively skin testing with a commercial extract containing uniquely kiwi-specific allergens⁹) and investigating reactivity to some storage proteins of peanut (Ara h 1-3), Brazil nut (Ber e 1), sesame (Ses i 1), and/or walnut (eg, performing an SPT

with a commercial walnut extract containing uniquely stable allergens^{6,10,11}) probably provides a much better picture of the patient's allergy than carrying out a large series of expensive examinations showing little or no specificity, irrespective of the plant-derived food or foods causing clinical allergy.

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