

Original article

Detection of specific IgE antibodies in the sera of patients allergic to birch pollen using recombinant allergens Bet v 1, Bet v 2, Bet v 4: evaluation of different IgE reactivity profiles

Background: Birch pollen is a significant cause of immediate hypersensitivity among susceptible subjects in temperate climates, affecting 5–54% of the population in western Europe. We examined the specific serum IgE antibodies towards recombinant allergens Bet v 1, Bet v 2 and Bet v 4 in birch-sensitive patients from the province of Cuneo, north-west Italy.

Methods: Sera were obtained from 372 patients with symptomatic birch pollen-induced allergic rhinitis and/or asthma. A subgroup of these patients suffered from oral allergy syndrome after eating apple. Their sera were evaluated for specific IgE against natural birch pollen and apple extract, as well as Bet v 1, Bet v 2 and Bet v 4 using Pharmacia CAP system (Pharmacia, Uppsala, Sweden).

Results: Of 372 patients 215 (57.80%) had serum-specific IgE towards Bet v 1. A total of 166 sera (44.62%) contained serum-specific IgE to Bet v 2, while Bet v 4 IgE reactivity was documented in 35 subjects (9.41%). Moreover, 146 (39.25%) patients were monosensitized to Bet v 1; 96 (25.81%) patients were monosensitized to Bet v 2; only four sera (1.08%) contained specific IgE towards Bet v 4. Thirty-nine sera (11.02%) did not contain specific IgE to these individual birch pollen allergens. Of course, all 372 sera (100%) had specific IgE against natural birch pollen extract, of which 162 (43.55%) contained specific IgE to apple extract (75.35% of Bet v 1 positive sera).

Conclusion: In this study we observed that three birch pollen recombinant allergens alone, could sufficiently identify 90% of birch pollen-sensitive patients. Therefore, for a more precise IgE profile of patients allergic to birch, further purified birch pollen allergens (i.e. Bet v 6, Bet v 7, Bet v 8) will be required.

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There is no doubt that birch pollen is a significant cause of immediate hypersensitivity among susceptible individuals in western Europe, affecting 5–54% of the patients with pollinosis (1). The major birch pollen allergen, Bet v 1, was first reported in 1989 (2, 3). Other relevant birch pollen allergens are Bet v 2 (4) and Bet v 4 (5). Now, all these birch pollen allergens are commercially available as diagnostics. Given that the concentration of allergens in extracts can differ by several orders of magnitude, it must be difficult to reach a therapeutic dose for a less abundant allergen without inducing side-effects to others. If one were asked to choose the most important allergen in birch pollen that is linked to human allergic disease, from the current knowledge and understanding, the obvious choice would be Bet v 1. A recent study demonstrated that in patients allergic to birch the IgE reactivity profile to birch pollen allergens can significantly differ between European patients with pollinosis (6). In order to identify possible different IgE reactivity profiles in sera of patients allergic to birch pollen living in a limited geographic area, in the

present study, we have evaluated specific IgE antibody binding to three recombinant birch allergens Bet v 1, Bet v 2, Bet v 4, using Pharmacia CAP system (Pharmacia, Uppsala, Sweden).

Methods

Study population

This study used the sera of 372 patients with specific IgE towards birch pollen extract > 0.35 kUA/l. All sera were collected from the province of Cuneo, in the north-west Italy. The patients had a history of seasonal (February–April) pollen allergy with symptoms of rhinitis and/or asthma. A fraction of them suffered from oral allergy syndrome after eating apple, too. The subjects were living in or near the town of Cuneo.

Determination of allergen-specific IgE antibodies

IgE-specific antibodies for birch, apple and the birch allergens Bet v 1, Bet v 2 and Bet v 4 were evaluated using the

immunoenzymatic CAP method (Pharmacia Diagnostics, Uppsala, Sweden) according to the manufacturer's instructions. The results were expressed in classes of positivity from 0 to 6, where class 0 corresponds to <0.35 kUA/l, class 1 to 0.35–0.7 kUA/l, class 2 to 0.7–3.5 kUA/l, class 3 to 3.5–17.5 kUA/l, class 4 to 17.5–50 kUA/l, class 5 to 50–100 kUA/l and class 6 to >100 kUA/l.

Statistical analysis

The statistical analyses were performed using the software Graph Pad Prism version 2.0 (Graph Pad Software, San Diego, CA, USA).

Results

Of 372 sera with positive CAP results for natural birch pollen extract, 215 (57.80%) had specific IgE towards Bet v 1; 166 sera (44.62%) contained specific IgE against Bet v 2. Bet v 4 IgE reactivity was documented in 35 subjects (9.41%) (Fig. 1). A total of 146 patients (39.25%) were monosensitized to Bet v 1, 96 patients (25.81%) were monosensitized to Bet v 2 and only four sera (1.08%) contained specific IgE towards Bet v 4. Thirty-nine sera (11.02%) did not contain specific IgE to these three individual birch pollen allergens. Of 215 sera with specific IgE to Bet v 1, 162 (75.35%) had specific IgE to natural apple extract. In birch pollen-sensitive pollinosis patients of this geographic area eight different IgE reactivity profiles were observed (Table 1). Finally, the specific IgE levels to natural birch pollen extract, natural apple extract, rBet v 1, rBet v 2, rBet v 4, in patients with birch-pollen pollinosis are indicated on Fig. 1.

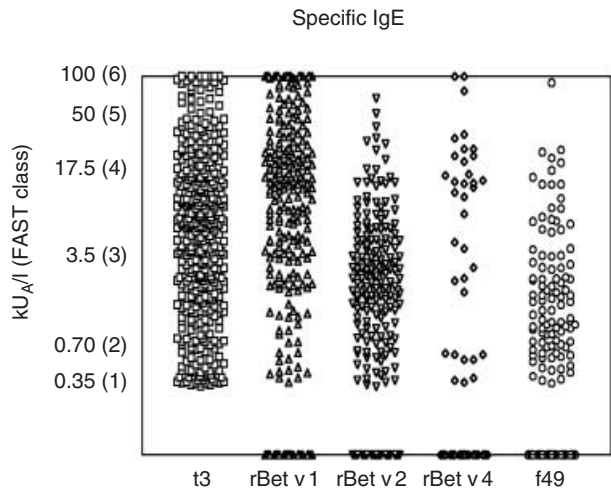


Figure 1. Specific IgE levels to natural birch pollen extract (t3), natural apple extract (F49), and recombinant Bet v 1, Bet v 2, Bet v 4, in 372 patients with pollinosis in the province of Cuneo, north-west Italy. All sera were analysed by the Pharmacia CAP System. The specific IgE levels corresponding to the lower limits of the FAST classes are indicated on the logarithmic y-scale.

Table 1. Percentage of allergic patients with IgE profiles to different combinations of recombinant Bet v 1, Bet v 2 and Bet v 4 and their serum levels of specific IgE to individual birch recombinant allergens as measured by Pharmacia CAP System

	n = 146 (39.25%)	n = 96 (25.81%)	n = 54 (14.52%)	n = 41 (10.9%)	n = 16 (4.2%)	n = 9 (2.5%)	n = 6 (1.7%)	n = 4 (1.08%)
	Bet v 1+ Bet v 2 – Bet v 4 –	Bet v 1 – Bet v 2 + Bet v 4 –	Bet v 1 + Bet v 2 + Bet v 4 –	Bet v 1 – Bet v 2 – Bet v 4 –	Bet v 1 – Bet v 2 + Bet v 4 +	Bet v 1 + Bet v 2 + Bet v 4 +	Bet v 1 + Bet v 2 – Bet v 4 +	Bet v 1 – Bet v 2 – Bet v 4 +
Bet v 1	14.7	<0.35	<0.35	<0.35	<0.35	3.64	3.16	<0.35
Bet v 2	<0.35	<0.35	15.6	<0.35	<0.35	(0.58–96.80)	(0.65–35.90)	<0.35
Bet v 4	<0.35	2.56	2.85	<0.35	3.76	6.16	<0.35	<0.35
Age (years)	34	24	22	30	14.7	4.35	8.19	7.85
	(22–42)	(16–30.5)	(16.5–27)	(18–44)	(4.86–21.20)	(0.57–26.80)	(23–32)	(1.51–24.15)
					29	29	27.5	22.5
					(16–40)	(22–41)	(23–32)	(12–37.5)

The given values are median values (kUA/l) (25th–75th percentile).

Discussion

In the present study, we have examined the IgE binding and diagnostic potential of three birch recombinant allergens, Bet v 1, Bet v 2 and Bet v 4 and compared the results with a natural birch-pollen extract. Our findings seem to indicate that additional recombinant allergens are needed to reach the diagnostic accuracy obtained with natural birch extract. In fact, 39 (11%) sera did not contain specific IgE towards birch recombinant allergens, but all sera had specific IgE binding to birch pollen extract. In other words, if the appropriate allergens are not used in the assays, antibody to a particular purified antigen may be lacking in the sera resulting in a false-negative reaction. Previous studies reported that Bet v 3 is recognized by IgE from about 10% of pollen-allergic patients (7), as well as Bet v 6 (recognized by IgE from 12% of patients) (8). Moreover, other novel birch pollen allergens Bet v 7 and Bet v 8, have been purified (9, 10). Interestingly, Bet v 7 is recognized by serum IgE from up to 21% of birch pollen-allergic patients (9). As showed in the study of Moverare (6), 25% of sera from Swiss birch allergic patients did not have specific IgE to Bet v 1, Bet v 2 and Bet v 4. This high percentage may be because of a low number of subjects in study. In the same paper, 98–100% of patients from Finland, Sweden and Austria had specific IgE towards Bet v 1. In the present study, only 57.8% of patients with specific IgE towards birch pollen extract, had serum-specific IgE to Bet v 1, and 44.62% of them had serum-specific IgE to Bet v 2 [2% of sera from Finland had specific IgE to Bet v 2 (6)]. The lower frequency of Bet v 1 and higher frequency of Bet v 2 reactivity in the patients from the north-west Italy (province of Cuneo) might indicate that these subjects, probably, have primarily been sensitized to allergens from other sources, such as grass pollen. In fact, our data significantly differ from results obtained in patients from Finland, Sweden and France (6). This might reflect the mixed climate of the province of Cuneo that experiences sub-continental, fresh temperate, cold temperate climates

because of varying orographic features. This contributes to local distribution of vegetation in prevalence composed of Gramineae and trees (order Fagales). In fact, in this geographic area, the main sensitization sources are the Gramineae, the Betulaceae and Corylaceae. However, the Gramineae are largely the primary cause of pollen sensitization, in the province of Cuneo.

Cross-reactive specific IgE to Bet-v 1 and apple is a common feature of birch-pollen allergic patients (11, 12). Our finding that 75.35% of positive sera towards Bet v 1, also contained specific IgE to natural apple extract, seems to confirm that sensitization is primarily because of Bet v 1.

As allergen extracts currently used for specific immunotherapy are standardized regarding their content of major, but not minor, allergens components, it is possible, or even more likely that patients who are sensitized against minor allergens may benefit less from immunotherapy than patients allergic to the major allergen. According to the arguments outlined by Kazemi-Shirazi (13) patients containing IgE antibodies specific for Bet v 1, are considered suited for birch pollen extract-based immunotherapy. Patients who, in addition to Bet v 1, have IgE reactivity towards Bet v 2 and/or Bet v 4 are considered less suitable for that treatment. Finally, those patients with specific IgE to Bet v 2 and Bet v 4 but not to Bet v 1, are not suitable for birch pollen immunotherapy. Curiously, this last subgroup of patients could be treated with a grass-pollen extract containing relevant amounts of cross-reactive minor birch pollen allergens (i.e. Phl p 7, Phl p 12) whether these patients are also co-sensitized to other major grass-pollen allergens. However, this assumption must be proven at this moment.

In conclusion, our findings confirm the previous report of Moverare (6) of highly variable sensitization IgE profiles in patients from various European areas. Moreover, given that 10% of patients with specific IgE to birch extract lack IgE to Bet v 1, Bet v 2 and Bet v 4, for more precise diagnosis other individual birch purified allergens (i.e Bet v 6, Bet v 7, Bet v 8) will be required.

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