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Pollen cross-reactivity. Can we establish a link between the *in vitro* results and the clinical situation?

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It is well known that most patients allergic to birch pollen present oral allergy syndrome (OAS) when they eat fresh fruits (mainly apples), nuts (hazelnuts), and raw vegetables (celery, carrot) (1–5). Some of them may suffer from systemic symptoms such as urticaria, angioedema, asthma, or anaphylactic shock (6, 7). Bet v 1 and Bet v 2 (profilin) are the major birch-pollen allergens responsible for most cross-reactions between birch pollen and related foods (8–13). However, concomitant sensitizations to mugwort and/or grass pollen are often present (14–18) (Fig. 1). Recently, on evaluating the results of IgE determinations to rBet v 1 and rBet v 2 by the Pharmacia CAP System in 65 patients with rhinoconjunctivitis or asthma to birch pollen and OAS to apple ($n=28$), Rossi et al. (19) have suggested that sensitization to Bet v 1 is specific for birch and apple allergies, whereas sensitization to Bet v 2 is common in polysensitized patients.

The present survey was undertaken to describe what characterizes *in vitro* the clinically relevant cross-reactivities in birch-pollen-sensitized patients (absence of symptoms, isolated or polyvalent sensitivity to fruits and vegetables) and the severity of symptoms, and whether there is a correlation

between the magnitude of the IgE response to rBet v 1, rBet v 2, apple, hazelnut, celery, and carrot and the clinical situation.

Material and methods

Serum samples from 84 patients (45 females, 39 males, mean age 32 years, range 11–60, referred to the allergy unit in the period from July 1996 to June 1997) with a positive CAP result of at least class 2 to birch pollen (t3) (IgE level ≥ 0.7 kU/l) were selected for the study.

Total and specific serum IgE to birch (t3), grass (gx3, g6, or g12), mugwort pollen (w6), rBet v 1 (t215), rBet v 2 (t216), apple (f49), hazelnut (f17), celery (f85), and carrot (f31) were measured by the immunoenzymatic CAP FEIA System (Pharmacia & Upjohn Diagnostics, Uppsala, Sweden), according to the manufacturer's recommendation.

Skin prick tests (SPT) were performed with routine inhalants, food allergens (Alyostal extracts, Stallergènes, France), and fresh food (prick-prick technique) and a standardized needle (Stallerpoint, France). The results of the SPT were evaluated according to the EAACI guidelines (5, 22).

The patients were evaluated by case history, particularly of adverse reactions to food, clinical examination, and the results of the SPT. The symptoms after eating apple, hazelnut, celery, and carrot were classified as class 0 (no symptoms), class 1 (local symptoms such as OAS or contact urticaria), or class 2 (systemic symptoms such as generalized urticaria, angioedema, asthma, gastrointestinal symptoms, and anaphylactic shock).

Statistical comparisons of the data were made with the Mann–Whitney U-test for nonparametric data. Correlation coefficients were determined by Spearman’s rank correlation test. The significance level chosen was $P<0.05$. For multiple U-tests, Bonferoni’s correction was done. In the correlation tables, $P<0.01$ was chosen as significant.

Results and Discussion

Of the 84 patients in our study, 11 (13%) had a diagnosis of atopic dermatitis. Statistical analysis with the Mann–Whitney U-test showed differences in distribution for specific IgE levels of grass and rBet v 2, for total IgE level, and for pollen symptoms (Table 1). The atopic dermatitis group was excluded from further analysis.

The remaining patients, mainly affected by pollen allergy (rhinoconjunctivitis and/or pollen-induced asthma), were divided into four groups depending on the sensitization pattern of rBet v 1 and rBet v 2 (Table 2). Fig. 1 shows the distribution of the allergen-specific IgE values in these four groups; Fig. 2 the frequency of symptoms after eating the corresponding food. In both figures, the 11 patients with atopic dermatitis are also shown.

Table 1. Frequencies of clinical and serologic characteristics of birch-pollen-sensitive patients (no. of patients). Significance level for Mann–Whitney U-test: $P<0.05$

	No atopic dermatitis	Atopic dermatitis	Total	U-test
Patients	73	11	84	—
Sensitization (IgE ≥ 0.35 kU/l)				
Birch	73	11	84	0.49
rBet v 1	60	9	69	0.99
rBet v 2	14	6	20	0.01
Alder	72	11	83	0.66
Hazel	72	9	81	0.52
Mugwort	38	9	47	0.10
Grass	50	10	60	0.01
Apple	63	9	72	0.60
Hazelnut	56	7	63	0.81
Celery	39	5	44	0.74
Carrot	49	8	57	0.28
Total IgE (median)	210	1622	246	<0.0001
Symptoms (local or systemic)				
Apple	38	2	40	0.03
Hazelnut	31	2	33	0.09
Celery	12	0	12	0.15
Carrot	15	2	17	0.90
Pollen	68	5	73	0.001

In Spearman ranking, the CAP results for all measured allergens were correlated with each other (Table 3). In Table 4, the same was done with food symptoms.

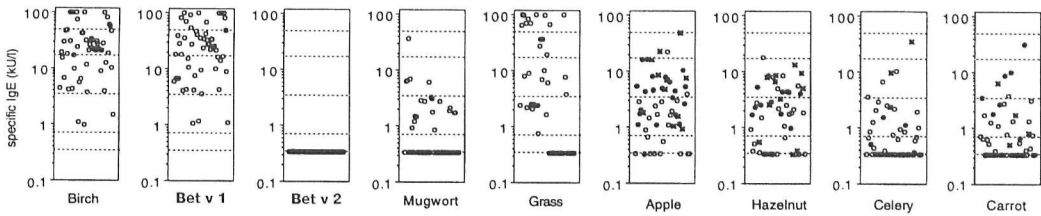
Fig. 3 shows box-plots of allergen-specific IgE levels in comparison with symptom classes for apple, hazelnut, celery, and carrot. In Table 5, the relationship among them is statistically analyzed by the Mann–Whitney U-test.

Comparison of patients with diagnosed atopic dermatitis with patients with mainly pollen allergy

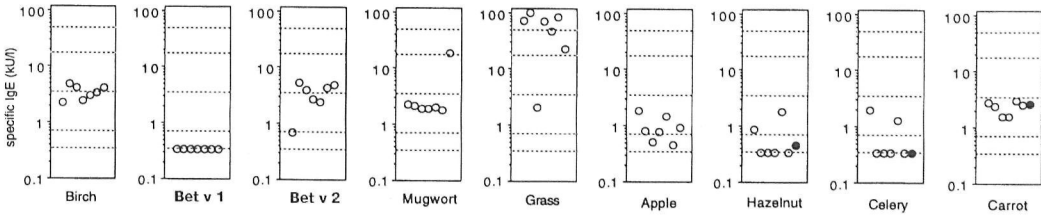
Table 2. Frequencies of sensitization and symptoms in all patients without atopic dermatitis (no. of patients)

	rBet v 1 + rBet v 2 –	rBet v 1 – rBet v 2 +	rBet v 1 + rBet v 2 +	rBet v 1 – rBet v 2 –	Total
Total patients	53 (73%)	7 (9.5%)	7 (9.5%)	6 (8%)	73 (100%)
Sensitization (IgE ≥ 0.35 kU/l)					
Birch	53	7	7	6	73
rBet v 1	53	0	7	0	60
rBet v 2	0	7	7	0	14
Alder	52	7	7	6	72
Hazel	52	7	7	6	72
Mugwort	20	7	6	5	38
Grass	31	7	7	5	50
Apple	45	7	5	6	63
Hazelnut	41	3	6	6	56
Celery	27	2	4	6	39
Carrot	29	7	7	6	49
Symptoms (local or systemic)					
Apple	32	0	4	2	38
Hazelnut	23	1	5	2	31
Celery	9	1	0	2	12
Carrot	11	1	1	2	15
Pollen	52	6	7	3	68

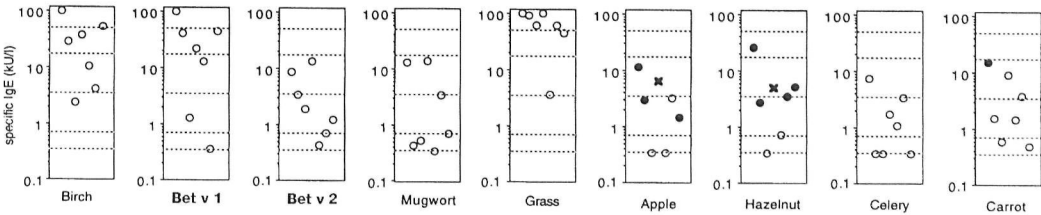
Group 1: Bet v 1 $\geq$ 0.35 kU/I IgE, Bet v 2 $<$ 0.35 kU/I IgE ($n = 53$)



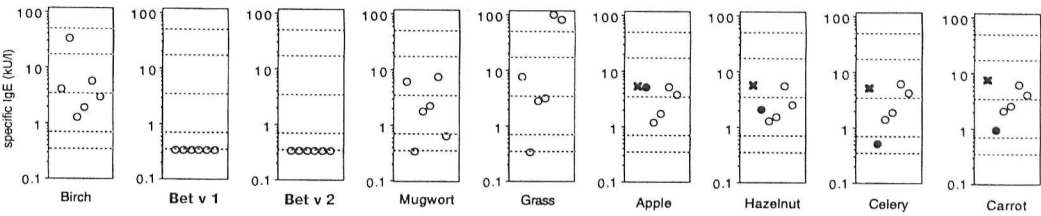
Group 2: Bet v 1 $<$ 0.35 kU/I IgE, Bet v 2 $\geq$ 0.35 kU/I IgE ($n = 7$)



Group 3: Bet v 1 $\geq$ 0.35 kU/I IgE, Bet v 2 $\geq$ 0.35 kU/I IgE ($n = 7$)



Group 4: Bet v 1 $<$ 0.35 kU/I IgE, Bet v 2 $<$ 0.35 kU/I IgE ($n = 6$)



Patients with atopic dermatitis ($n = 11$)

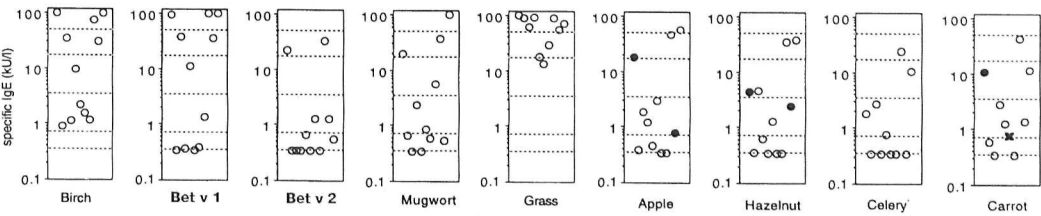


Fig. 1. Scatter-plots of allergen-specific IgE values in all four patient groups and in atopic dermatitis group. Dotted lines indicate CAP classes according to Pharmacia & Upjohn Diagnostics. In plots with specific IgE against food, ○=no symptoms, ●=local symptoms, ×=systemic symptoms.

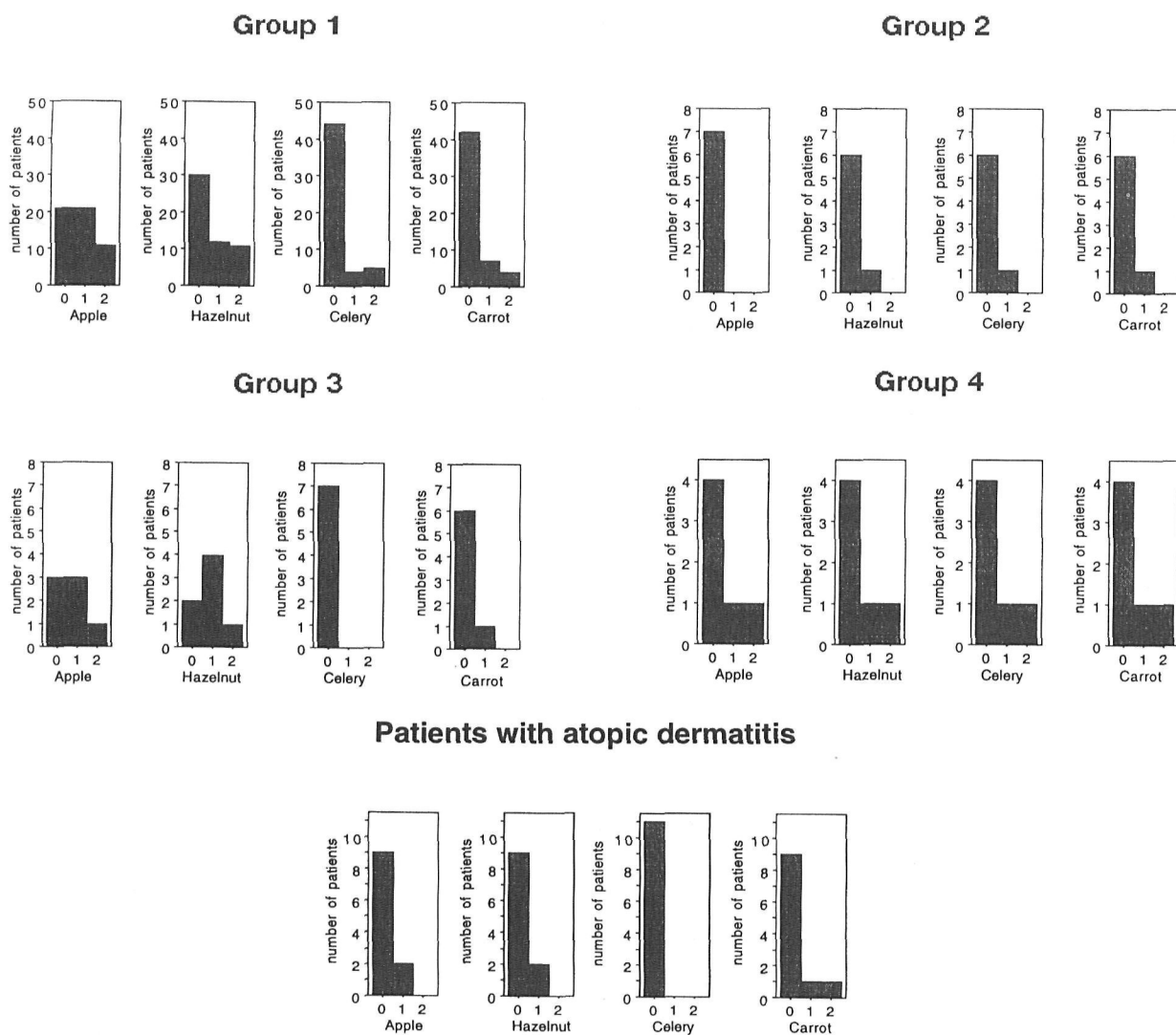


Fig. 2. Frequency of symptoms after eating corresponding food in all four patient groups and in atopic dermatitis group (0 = no symptoms; 1 = local symptoms; 2 = systemic systems).

showed significant differences in distribution for the total IgE levels, for specific IgE levels of grass and rBet v 2, and for pollen as well as apple symptoms (Table 1). However, our patients with atopic dermatitis and birch-pollen sensitization did not have more frequent or severe symptoms from birch pollen-related food than patients with birch allergy. Nevertheless, the atopic dermatitis group was excluded from further analysis.

The division of the remaining 73 patients into four groups according to their sensitization to the recombinant allergens rBet v 1 and rBet v 2 differs from other recent studies. Rossi et al. found more patients with sensitization to rBet v 2 (37/65=57%) and none without sensitization to rBet v 1 and/or rBet v 2 (19). In contrast, Scheiner et al. had no patients with sensitization to rBet v 2 exclusively, but they had 2/34 (6%) without sensitization to either rBet v 1 or rBet v 2 (21). In a paper by Pauli

et al., one out of 51 patients was sensitized to neither rBet v 1 nor rBet v 2 (23). All these differences may be due to nonuniform selection of study patients and to geographic differences in the study group (24).

Recently, a third group of proteins, other than Bet v 1, Bet v 2, and their homologs, displaying IgE cross-reactivity shared by birch, mugwort pollen, and celery, has been identified (20). These proteins have a molecular range of 46–60 kDa. Our patients in group IV may illustrate such a cross-reacting allergen. With one exception, all patients were sensitized to grass, mugwort pollen, apple, hazelnut, celery, and carrot. They may also have suffered from local or systemic reactions after eating fruits or vegetables.

In our patients, sensitization to rBet v 2 (birch profilin) was not associated with more frequent or more severe reactions to fruits or vegetables, in

Table 3. Spearman rank correlations for IgE levels of all patients without atopic dermatitis. Correlations with significance below $P < 0.01$ are in boldface

	Birch	rBet v 1	rBet v 2	Alder	Hazel	Mugwort	Grass	Apple	Hazelnut	Celery	Carrot
Birch											
rBet v 1	0.92										
rBet v 2	-0.21	-0.29									
Alder	0.96	0.90	-0.19								
Hazel	0.96	0.90	-0.16	0.95							
Mugwort	-0.03	-0.14	0.37	0.01	0.00						
Grass	-0.08	-0.14	0.44	-0.04	-0.01	0.68					
Apple	0.69	0.61	-0.20	0.75	0.71	0.17	0.04				
Hazelnut	0.70	0.63	-0.14	0.73	0.67	0.16	0.03	0.80			
Celery	0.29	0.22	-0.07	0.35	0.34	0.45	0.36	0.69	0.54		
Carrot	0.11	0.00	0.36	0.20	0.21	0.61	0.52	0.54	0.36	0.80	

Table 4. Spearman rank correlations for symptoms of all patients without atopic dermatitis. Correlations with significance below $P < 0.01$ are in boldface

	Apple	Hazelnut	Celery	Carrot
Apple				
Hazelnut	0.70			
Celery	0.02	0.36		
Carrot	0.41	0.39	0.38	

contrast to the findings of other authors (8, 10, 12, 18, 19, 23).

Statistical analysis of the CAP data mostly confirmed the results of previous studies (9, 11, 12, 19)

(Table 3). There was good correlation between rBet v 1, birch, alder, and hazel pollen. Moreover, apple and hazelnut were associated with them and with each other.

Specific IgE to rBet v 2 showed a weak correlation with mugwort, grass pollen, and carrot but no significant correlation with tree pollen, apple, hazelnut, and celery. These findings have to be treated with caution since the lowness of the number of patients with rBet v 2 sensitization (IgE ≥ 0.35 kU/l) might influence the results of Spearman's rank correlation.

Mugwort pollen showed weak correlation to celery, carrot, and grass pollen ($r = 0.37$ – 0.68),

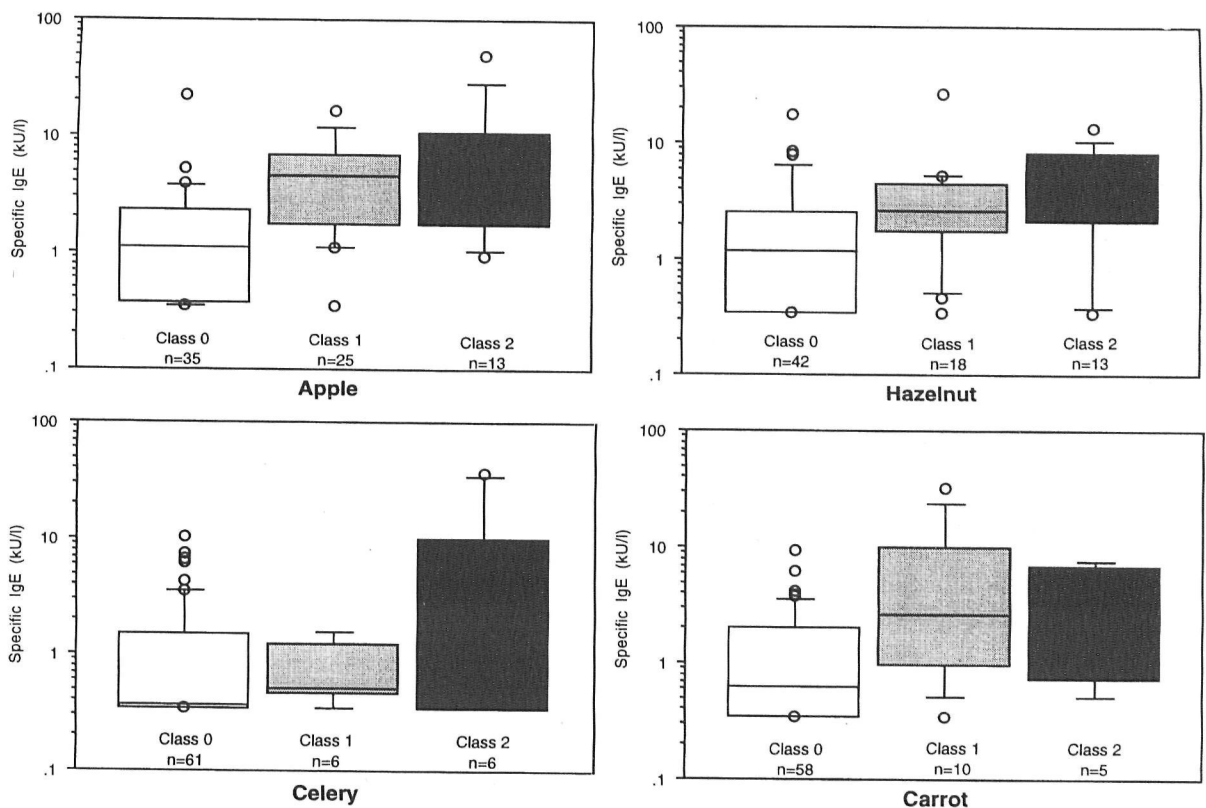


Fig. 3. Box-plot of allergen-specific IgE levels compared with symptom classes from corresponding food.

Table 5. Mann-Whitney U-test for IgE levels grouped by symptom classes. Significance level after Bonferroni correction $P < 0.013$

	Apple	Hazelnut	Celery	Carrot
Class 0 vs class 1	$P < 0.0001$	$P = 0.02$	$P = 0.65$	$P = 0.007$
Class 0 vs class 2	$P = 0.0002$	$P = 0.006$	$P = 0.07$	$P = 0.07$
Class 1 vs class 2	$P = 0.28$	$P = 0.15$	$P = 0.27$	$P = 0.39$
Class 0 vs classes 1 + 2	$P < 0.0001$	$P = 0.002$	$P = 0.12$	$P = 0.002$

whereas no significant association with tree pollen, rBet v 1, apple, and hazelnut could be found. Celery and carrot sensitizations have been shown to be strongly associated (25).

From the clinical point of view, a good correlation between symptoms to apple and hazelnut could be determined. All other correlations were weak or not statistically significant (Table 4).

Birch-pollen-allergic patients with symptoms after eating apple, hazelnut, and carrot showed significantly higher IgE levels to the respective food items than asymptomatic patients. There were no significant differences in the IgE level between patients with localized or systemic symptoms. For patients with celery sensitization, no significant differences could be found in the distribution of IgE levels between patients with and without symptoms after eating celery (Fig. 3, Table 5).

Of the 11 patients with symptoms after eating celery, three (27%) had a negative CAP result to celery. Moreover, in general, in symptomatic celery-allergic patients, the CAP to celery showed lower IgE levels than to apple, hazelnut, or carrot (Fig. 4). This could be due to the lack of relevant allergens in the commercial celery CAP (a mixture of celery stick and celery root).

Conclusions

In our study, serum samples from 85 patients sensitized to birch pollen were examined for specific IgE to rBet v 1 and rBet v 2, tree pollen (alder, hazel), grass, mugwort, and foods (apple, hazelnut, celery, and carrot). Evaluation of symptoms based on the patients' case histories was compared to the *in vitro* data. Patients with atopic dermatitis did not have more frequent or severe symptoms to birch-pollen-related food than the rest of our study group. Among patients without atopic dermatitis, 82.5% reacted to rBet v 1, 19% to rBet v 2, 9.5% to both, and 8% to neither. Sensitization to rBet v 2 was not associated with more frequent or more severe reactions to foods. Patients with symptoms after eating apple, hazelnut, and/or carrot showed significantly higher IgE levels to the respective food items than asymptomatic patients. This was

not true for patients with clinical manifestations after eating celery.

Taken together, our study failed to demonstrate a clear correlation between the pattern of sensitization to rBet v 1 and rBet v 2 and specific IgE to conventional allergen extracts. Therefore, the routine determination of IgE to rBet v 1 and rBet v 2 in birch allergy and birch-pollen-related food allergy seems to be of little diagnostic value for clinical work.

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