

The Clinical Impact of Bet v 6 in Birch Pollen-Sensitized Patients

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Keywords

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Abstract

Background: Bet v 6, a minor birch pollen allergen, is commercially available for allergen-specific IgE antibody testing. However, there is hardly any literature on the clinical impact of Bet v 6. The aim of the present study was to analyze if testing specific IgE against Bet v 6 can add valuable benefit to the standard diagnostics of birch pollen-sensitized patients, especially in patients with unknown sensitization-eliciting allergens. **Methods:** Birch pollen-sensitized patients with missing IgE reactivity against Bet v 1, 2, and 4 were tested for specific IgE antibodies against Bet v 6. For comparison, an equal number of randomly selected patients with birch pollen sensitization, regardless of their individual sensitization patterns, were tested for specific anti-Bet v 6. **Results:** Of 57 cases with missing reactivity against the standard birch pollen allergens, 2 patients were positive for Bet v 6. In the representative sample, 3 patients showed specific IgE against Bet v 6 – resulting in a total prevalence of 5%. None of the Bet v 6-positive patients showed allergic symptoms after exposure to birch pollen or an oral allergy syndrome. An increased prevalence of asthma and a higher degree of sensi-

tization were the only distinctive clinical features in Bet v 6-positive patients. **Conclusions:** Among birch pollen-sensitized patients, the prevalence of specific IgE against Bet v 6 is low. Further, sensitization to Bet v 6, which shows characteristics of a panallergen, remains clinically silent. Therefore, determination of anti-Bet v 6 is not considered useful in the clinical routine.

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Introduction

Trees belonging to the order Fagales are recognized as one of the most potent allergen sources, at least in the temperate climate zones of Europe, North America, and Asia [1]. At present, 6 Fagales tree pollen allergens have been identified and acknowledged by the WHO/IUIS Allergen Nomenclature Sub-Committee (www.allergen.org). Besides the major allergen Bet v 1, 5 minor birch pollen allergens have been characterized as IgE-binding proteins: Bet v 2, a profilin; Bet v 3 and Bet v 4; members of the polcalcin family; Bet v 6 (formerly named Bet v 5), a homologue to the isoflavone reductase; and Bet v 7, a cyclophilin. The exact sensitization patterns vary significantly depending on the geographic location of the study population. Rossi et al. [2] reported that almost 90% of

birch pollen-sensitive patients could be identified by testing the 3 birch pollen recombinant allergens: Bet v 1, 2, and 4. However, in 11% of patients with specific IgE against natural birch pollen extract, the underlying allergen could not be identified. Hence, the authors recommended additional measurements of specific IgE against other recombinant birch pollen allergens, i.e. Bet v 6 and 7.

Bet v 6 has been cloned and characterized by Karamloo et al. [3] in 1999. Due to its relation to the isoflavone reductase family, which is involved in plant defense reactions, Bet v 6 has a high degree of amino acid sequence identity of proteins from various plants, possibly causing pollen-related oral allergy to specific foods in a minority of patients with birch pollen allergy. Already in 1996, a 35-kDa protein had already been identified in birch pollen immunologically independent from Bet v 1, but cross-reacting with proteins of comparable size from lychee, mango, banana, orange, apple, pear, and carrot [4]. This food cross-reacting 35-kDa protein probably was Bet v 6, but was not identified as such in 1996. However, the clinical relevance of Bet v 6 remains to be established. Further, at present, there is not much epidemiologic data concerning the prevalence of specific IgE against Bet v 6. Only Karamloo et al. [3] reported specific IgE binding to Bet v 6 in 9 of 76 birch pollen-allergic patients. As the aim of the study by Karamloo et al. was primarily the molecular characterization of Bet v 6 rather than its clinical relevance, we do not know to what extent Bet v 6 contributes to allergic symptoms, especially as all Bet v 6-positive patients with the exception of one single case were polysensitized.

With regard to Bet v 7, a comparable lack of data concerning epidemiology and clinical relevance is notable. However, in contrast to profilins (e.g., Bet v 2), polcalcins (e.g., Bet v 3 and 4), and isoflavone reductase homologue proteins (Bet v 6), which are vastly cross-reactive across species [5, 6], no immunological cross-reactivity of cyclophilin A members across species has been reported. Hence, specific IgE towards Bet v 7 are probably of limited clinical relevance.

Therefore, the aim of our present study was the determination of IgE reactivity against Bet v 6, especially as Bet v 6 is now commercially available for diagnostic testing. Further, our study aims to analyze the clinical features of Bet v 6-positivity and the potential value of testing Bet v 6-specific IgE antibodies in birch pollen-sensitized patients, particularly in cases with missing IgE reactivity against the well-known birch pollen components Bet v 1, 2, and 4.

Patients and Methods

Study Design

Patient data were retrospectively selected from the allergy database of the Department of Otorhinolaryngology, Head and Neck Surgery of the Ludwig-Maximilians-Universität in Munich, Germany, storing all relevant diagnostic patient results. All patients were suspected of suffering from allergic rhinitis and therefore underwent routine in vivo tests such as skin prick test (ALK-Abelló, Wedel, Germany) and/or allergen-specific provocation and in vitro tests such as total IgE and allergen-specific IgEs in serum. Further, the database includes data from a standardized clinical history questionnaire, including complaints of the upper and lower airways, demographic data, living environment, oral and gastrointestinal allergy symptoms, and medical history.

The database was scanned for consecutive patients with a proven sensitization to natural birch extract (t3), but missing IgE reactivity to well-known recombinant birch components Bet v 1, 2, and 4. As component-resolved diagnostics were implemented as regular diagnostic tools in our clinical routine in 2007, we only searched our database for patients presenting at our department from 2007 on until 2015. Of 1,064 patients with IgE antibodies against t3, 57 met the inclusion criteria, i.e. did not show any IgE reactivity against the major component Bet v 1 nor the minor components profilin or polcalcin.

From the same sample of 1,064 patients, we selected a control group comprised of 57 patients randomly selected regardless of their reactivity pattern against different birch pollen components.

The study was approved by the local ethics committee and the local data protection commissioner. All patients provided written informed consent.

Fluorescence Enzyme Immunoassay

IgE reactivity to natural allergen extract (t3) and allergen components was measured using the fluorescence enzyme immunoassay (FEIA) method (UniCAP-FEIA, Thermo Fisher Scientific, Freiburg, Germany) with a commercially available test kit according to the manufacturer's instructions. Apart from IgE antibodies against natural allergen extracts, IgE reactivity against profilin, polcalcin, and cross-reactive carbohydrate-determinants (CCDs) was measured to avoid misinterpretation of positive results due to cross-reactivity [7, 8]. Specific IgE antibodies to Bet v 6 were not routinely measured. For the determination of IgE to Bet v 6 using the FEIA method, sera of the 57 patients fulfilling the abovementioned inclusion criteria as well as of the 57 patients of the control group were defrosted. Results were reported as concentrations (kU/L) as well as CAP classes of positivity from 0 to 6, where class 0 corresponds to <0.35 kU/L, class 1 to 0.35–0.7 kU/L, class 2 to 0.71–3.5 kU/L, class 3 to 3.51–17.5 kU/L, class 4 to 17.51–50 kU/L, class 5 50.01–100 kU/L, and class 6 to >100 kU/L. Depending on the serological results, the grade of sensitization was defined in monosensitization (IgE to 1 single allergen source), oligosensitization (IgE to 2–3 allergen sources), or polysensitization (IgE to >3 allergen sources).

Statistical Analysis

Statistical analysis was performed with SigmaStat (Jandel Corp., San Rafael, CA, USA). All of the data failed normality testing. For descriptive statistics we used median values. The paired *t* test was used for the comparison in the individual group; the Mann-Whitney rank sum test was used for the comparison of the different groups. No testing revealed a value of $p \leq 0.05$.

Table 1. Patient demographics and study results in the study group compared to the control group

Characteristics	t3+/Bet v 1/2/4– study group, (n = 57)	t3+ control group, (n = 57)
Gender		
Male	30 (53)	24 (42)
Female	27 (47)	33 (58)
Age, years	23 (15–40)	34 (21–46)
Monosensitized to birch	10 (18)	10 (18)
Oligosensitized	19 (33)	22 (38)
Polysensitized	28 (49)	25 (44)
Cosensitization against		
Grass	41 (72)	37 (65)
Ash tree	16 (28)	15 (26)
Mugwort	5 (9)	4 (7)
Domestic mites	19 (33)	26 (46)
Cat	12 (21)	18 (32)
Dog	14 (25)	13 (23)
Fungi	6 (11)	3 (5)
Allergic complaints related to birch pollen	11 (19)	28 (49)
OAS related to birch pollen sensitization (i.e., apple, nut, cherry)	3 (5)	8 (14)
Asthmatic complaints	17 (30)	22 (39)
Intrinsic	8/17 (47)	7/22 (32)
Allergic	9/17 (53)	15/22 (68)
Birch pollen related	4/17 (24)	9/22 (42)

Except for the age which is given as medians with ranges in parentheses, values are number of patients total and percent of each evaluated subgroup. OAS, oral allergy syndrome.

Results

Characterization of the Study Population

The database query with the abovementioned inclusion criteria resulted in 57 patients with a proven sensitization to birch pollen of which the sensitization-eliciting component remained unknown due to missing IgE antibodies against Bet v 1, 2, and 4. Table 1 summarizes these patients' demographics and clinical data compared to the control group comprised of 57 randomly selected birch pollen-sensitized patients.

Apart from older age of the patients, the randomly selected control group presented comparable demographic and epidemiologic data as shown in Table 1. Interestingly, the distribution of mono-, oligo-, and polysensitization – based on IgE reactivity – was very similar to the results of the initially mentioned study group, even with

Table 2. Laboratory characteristics in the study group compared to the control group

Laboratory characteristics	t3+/Bet v 1/2/4– study group, (n = 57)	t3+ control group, (n = 57)
IgE in serum, kU/L	168 (104–397)	148 (54–312)
Birch (t3) positive	57 (100)	57 (100)
CAP class	1 (1–2)	3 (3–4)
IgE level, kU/L	0.61 (0.44–1.07)	9.36 (4.69–26.50)
Bet v1 (t215) positive	0 (0)	52 (91)
CAP class	0	3 (3–4)
IgE level, kU/L	0	8.16 (3.84–22.40)
Bet v2 (t216) positive	0 (0)	6 (11)
CAP class	0	2 (2–2)
IgE level, kU/L	0	2.47 (1.89–3.72)
Bet v4 (t220) positive	0 (0)	5 (9)
CAP class	0	3 (2–4)
IgE level, kU/L	0	3.12 (1.67–27.50)
Bet v6 (t225) positive	2 (4)	3 (5)
CAP class	4 (3.5–4.5)	2 (1.5–2)
IgE level, kU/L	55.95 (33.93–77.98)	0.78 (0.68–1.40)
CCD positive	21 (40), 4 n.k.	9 (16)
CAP class	2 (1–2)	1 (1–2)
IgE level, kU/L	1.08 (0.65–2.49)	0.69 (0.54–1.50)

Values are number of patients total and percent of each evaluated subgroup. CAP classes and IgE levels are given as medians with ranges in parentheses. n.k.: in 4 cases anti-CCDs are not known.

similar cosensitization rates (Table 1). However, the prevalence of all registered clinical symptoms was higher in the control group compared to the Bet v 1/2/4-negative patients: more patients reported on allergic complaints during birch pollen season (49 vs. 19%), on oral allergy syndrome related to the birch pollen sensitization (14 vs. 5%), and on asthmatic symptoms (39 vs. 30%). Among asthma patients, the rate of intrinsic asthma was lower in the control group compared to the Bet v 1/2/4-negative group (32 vs. 47%).

Table 2 summarizes the laboratory characteristics of the study group compared to the control group. One interesting detail is not shown in Table 2: the concentration of Bet v 1-specific IgE antibodies was higher in patients allergic to birch pollen compared to patients without allergic complaints (9.0 vs. 7.0 kU/L). Comparably, in the subgroup of Bet v 1/2/4-negative birch pollen allergic patients, the t3-specific IgE concentration was higher than in birch pollen-sensitized patients without allergic complaints (1.0 vs. 0.6 kU/L).

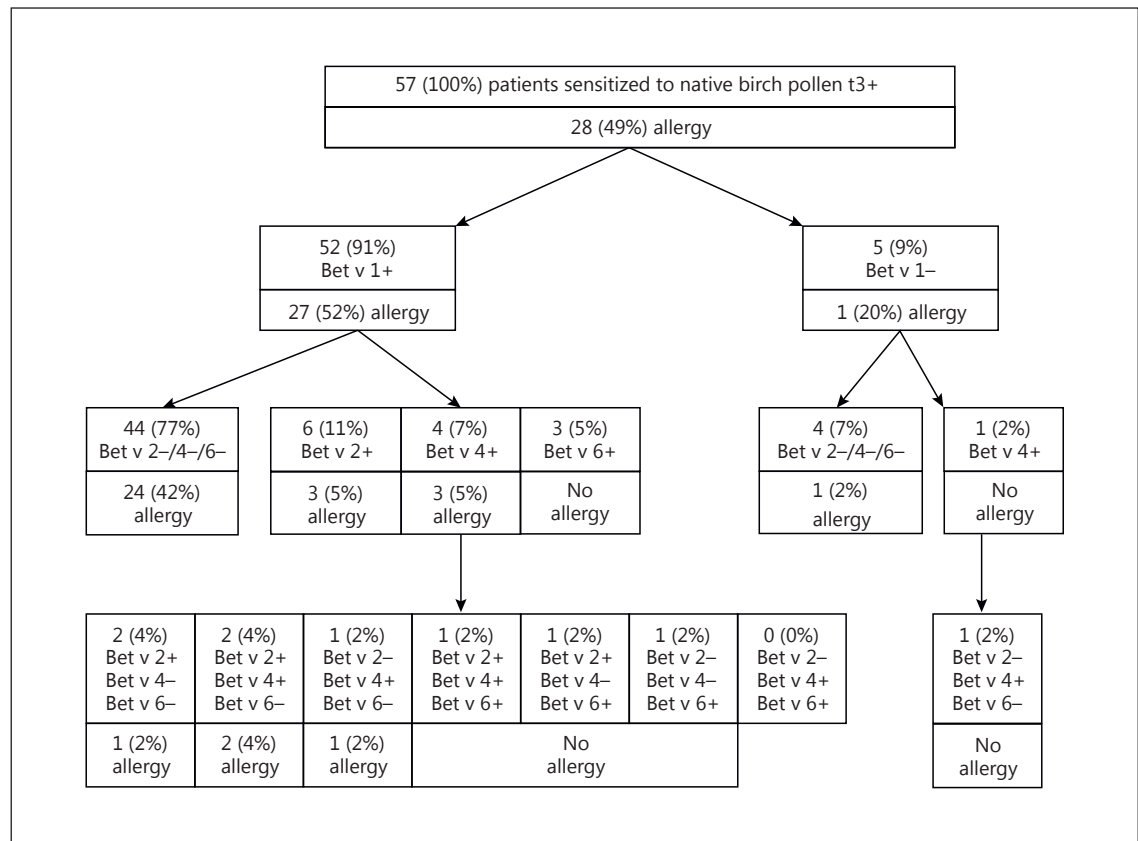


Fig. 1. IgE reactivity pattern of the control group. The IgE reactivity profiles of the 57 randomly selected birch pollen-sensitized patients display high variance. Except for 1 case, all allergic patients were sensitized against the major allergen component Bet v 1. Eighty-six percent of the birch pollen-allergic patients were even

monosensitized to Bet v 1. Four allergic patients presented additional IgE reactivity against further birch pollen components (Bet v 2 and/or 4). All 3 Bet v 6-positive patients had no birch pollen-related allergic symptoms.

IgE Reactivity Profiles

In the control group comprised of 57 randomly selected birch pollen-sensitized patients, the IgE reactivity profiles displayed a high variance as shown in Figure 1. As the patients of the study group were Bet v 1/2/4 negative per definition, their IgE reactivity profiles displayed only little variance and are, therefore, not shown.

The prevalence of specific IgE antibodies against Bet v 6 was comparable in both groups (4 and 5%, respectively). In all 5 cases, the Bet v 6 sensitization remained clinically silent.

The prevalence of IgE reactivity to CCDs was remarkably higher in the study group compared to the control group (40 vs. 16%) and differed significantly depending on the grade of sensitization: in birch pollen-monosensitized patients, reactivity against CCDs was found in 10% compared to 47% in polysensitized patients. In the con-

trol group, all 9 CCD-positive patients were polysensitized, identically to all 8 patients positive to Bet v 2 and/or 4.

The study group was characterized by the finding that the sensitization-eliciting component was unclear in all cases. In the control group, the sensitization-eliciting component could not be identified in 4 cases (7%); however, only one of these (1.7%) displayed clinical relevance. In the study group, 11 out of 57 patients (19%) – representing 1% of all 1,064 birch pollen-sensitized patients and therefore a comparable value to the control group – had a clinical relevant birch pollen sensitization of which the eliciting component remained unclear. Six of the 11 allergic patients were even negative to CCDs, indicating the potential existence of one or more allergy-eliciting birch pollen components, which are unknown yet or commercially not available for testing.

Table 3. Patient demographics and study results of Bet v 6-positive patients compared to randomly selected Bet v 6-negative patients

Characteristics	Bet v 6+					Σ Bet v 6+ (n = 5)	Σ Bet v 6– (n = 54, random)
	#1	#2	#3	#4	#5		
Gender							
Male		✓				1 (20)	24 (44)
Female	✓		✓	✓	✓	4 (80)	30 (56)
Age, years	20	70	28	54	63	54 (28–62)	34 (21–46)
Monosensitized to birch		✓				1 (20)	10 (19)
Oligosensitized			✓			1 (20)	21 (37)
Polysensitized	✓			✓	✓	3 (60)	23 (40)
Cosensitization against							
Grass	✓			✓	✓	3 (60)	39 (72)
Ash tree	✓			✓	✓	3 (60)	14 (26)
Parietaria	✓				✓	2 (40)	1 (2)
Mugwort				✓		1 (20)	4 (7)
Domestic mites				✓		1 (20)	18 (33)
Cat			✓	✓		2 (40)	11 (20)
Dog	✓		✓	✓		3 (60)	13 (24)
Fungi				✓		1 (20)	5 (9)
Allergic complaints related to birch pollen						0	28 (49)
OAS related to birch pollen sensitization (i.e., apple, nut, cherry)						0	8 (15)
Asthmatic complaints			✓	✓	✓	3 (60)	19 (35)
Intrinsic			✓	✓		2 (40)	5/19 (26)
Allergic					✓	1 (20)	14/19 (74)
Related to birch pollen						0	9/19 (47)

Except for the age which is given as medians with the ranges in parentheses, values are number of patients total and percent of each evaluated subgroup. In the second column, the characteristics of each single patient with IgE reactivity against Bet v 6 are given. The third column summarizes the data of the 5 Bet v 6-positive patients to facilitate the comparison to the 54 Bet v 6-negative patients summarized in the last column. OAS, oral allergy syndrome.

Clinical Characterization of Bet v 6-Positive Patients

To detect characteristics of Bet v 6-positive patients, Bet v 6-positive patients of both groups were compared with Bet v 6-negative patients of the randomly selected control group. In this comparison, the primary study group patients negative to Bet v 1, 2, and 4 were not considered as they do not present a representative random sample.

As shown in Table 3, the demographic and general characteristics of Bet v 6-positive patients are comparable to most characteristics of Bet v 6-negative patients. 49% of the Bet v 6-negative patients complained about allergic symptoms after exposition to birch pollen, whereas sensitization towards Bet v 6 remained clinically silent in all cases. Neither oculonasal symptoms nor oral allergy syndromes nor asthma control deterioration during birch pollen season have been reported; however, perennial asthma-like symptoms without seasonal deterioration

were more often present than in patients negative to Bet v 6 (60 vs. 35%).

As stated in the introduction, Bet v 6 is suspected to cause food allergies in birch pollen-sensitized patients, especially to Sharon fruit, kiwi, strawberry, orange, zucchini, and carrots. No patient with specific IgE against Bet v 6 reported any of these fruits as being a trigger of allergic symptoms. Two patients declared they were unable to tolerate garlic and spicy meals, respectively.

Laboratory Characterization of Bet v 6-Positive Patients

Table 4 summarizes the laboratory findings in Bet v 6-positive patients compared to Bet v 6-negative patients. Despite comparable total IgE levels in the sera of Bet v 6-positive and -negative patients, the titer of specific IgE antibodies against t3 was on average 3 times higher in Bet v 6-positive patients compared to Bet v 6-negative patients (29.5 vs. 9.13 kU/L).

Table 4. Laboratory characteristics of Bet v 6-positive patients compared to randomly selected Bet v 6-negative patients

Laboratory characteristics	Bet v 6+					Σ Bet v 6+ (n = 5)	Σ Bet v 6– (n = 54, random)
	#1	#2	#3	#4	#5		
IgE in serum, kU/L	232	81	36	2,470	148	148 (81–232)	150 (55–311)
Birch (t3) positive	✓	✓	✓	✓	✓	5 (100)	54 (100)
CAP class	4	3	3	4	4	4 (3–4)	3 (3–4)
IgE level, kU/L	29.50	4.62	8.92	44.00	36.90	29.50 (8.92–36.90)	9.13 (4.38–25.38)
Bet v1 (t215) positive			✓	✓	✓	3 (60)	49 (91)
CAP class			3	4	4	4 (3.5–4)	3 (3–4)
IgE level, kU/L			9.73	26.30	40.10	26.30 (18.00–33.20)	8.08 (3.66–20.60)
Bet v2 (t216) positive				✓	✓	2 (40)	4 (7)
CAP class				2	2	2	2 (2–2.5)
IgE level, kU/L				1.81	3.03	2.42	1.91 (1.76–3.85)
Bet v4 (t220) positive				✓		1 (20)	4 (7)
CAP-class				1		1	3 (2.5–4)
IgE level, kU/L				0.60		0.60	4.37 (3.12–50.60)
Bet v6 (t225) positive	✓	✓	✓	✓	✓	5 (100)	0 (0)
CAP class	5	3	2	1	2	2 (1–3)	
IgE level, kU/L	100.00	11.90	2.02	0.57	0.78	2.02 (0.78–11.9)	
CCD positive				✓	✓	2 (40)	7 (13)
CAP class				4	1	2.5	1 (1–2)
IgE level, kU/L				21.10	0.36	10.78	0.69 (0.60–1.29)

In the second column, the characteristics of each single patient with IgE reactivity against Bet v 6 are given. In the third and fourth columns summarizing the 5 Bet v 6-positive patients and the 54 Bet v 6-negative patients, respectively, the values are numbers of patients total and percent of each evaluated subgroup. Further, CAP classes and IgE levels are given as medians with ranges in parentheses.

Sensitization against Bet v 2 and Bet v 4 was more frequently found in patients positive to Bet v 6 (40 and 20% vs. 7 and 7%). When looking only at the 3 randomly selected Bet v 6-positive patients, the prevalence was even higher (66 and 33%). Likewise, the prevalence of IgE antibodies against CCDs was increased in patients positive to Bet v 6 compared to Bet v 6-negative patients (40 vs. 13%). This higher rate of CCD sensitization among Bet v 6-positive patients cannot be attributed to the preselection of the study group, as both CCD-positive patients derived from the control group.

When correlating laboratory characteristics of Bet v 6-positive patients with specific clinical features, a correlation between asthma and IgE antibodies against CCDs and panallergens (Bet v 2 and/or 4) becomes obvious. Both Bet v 6-positive patients with reactivity against CCDs and Bet v 2 and/or 4 complained about asthmatic symptoms, and only in one case reporting on asthmatic complaints, no reactivity against CCDs and panallergens was found. Also in the Bet v 6-negative control group, the prevalence of asthma is associated with a high degree of

sensitization; however, the correlation between asthma and reactivity against CCDs and Bet v 2 and/or 4 is less evident.

Discussion

The Relevance of Bet v 6 in Birch Pollen Sensitizations of Unknown Molecular Origin

Rossi et al. [2] reported that 89% of birch pollen-sensitized patients could be identified by testing the 3 birch pollen recombinant allergens: Bet v 1, Bet v 2, and Bet v 4. However, in 11% of patients with specific IgE against natural birch pollen extract, the underlying recombinant allergen was not identified, according to Rossi et al. In our study population of 1,064 birch pollen-sensitized patients, only 57 patients (5.4%) did not show IgE reactivity against any of the 3 birch pollen recombinant allergens: Bet v 1, 2, and 4. This discrepancy might be due to the different geographical location of the study groups in Italy and in Germany, respectively. Although the determina-

tion of Bet v 1/2/4-specific IgE failed to identify the sensitization-eliciting component in 5.4% of birch pollen-sensitized patients only, it would be desirable to fill this diagnostic gap. However, our data clearly demonstrate that the measurement of anti-Bet v 6 does not provide significant additional value as only 2 patients out of 57 showed IgE reactivity against Bet v 6. Obviously, other allergen components, which are probably unknown, seem to be relevant. One potential candidate could be the glutathione-S-transferase, a recently described minor allergen in birch pollen [9], which has not yet been acknowledged by the WHO/IUIS Allergen Nomenclature Subcommittee. Further research is required to detect unknown allergen components.

However, some confounders have to be taken into account, which might even further reduce the number of birch pollen sensitization of unknown molecular origin. In the study group of t3-positive, but Bet v 1/2/4-negative patients, FEIA results for t3 yielded 36 patients (63%) with CAP class 1, of whom 22 patients displayed IgE concentrations only a little above the cutoff value of 0.35 kU/L. As a result, one might criticize that some of these patients might have to be classified as false-positive concerning their birch pollen sensitization. However, 7 patients with CAP class 1 reported allergic symptoms, partially even despite a very low IgE concentration of <0.5 kU/L. This finding indicates that there might be a confounder by some false-positive patients of which the extent cannot be clearly assessed due to a certain gray zone around the threshold value.

Another confounder could be that some birch pollen sensitizations might be mocked by the existence of cross-reactive CCDs [7, 8], especially in the Bet v 1/2/4-negative study group. Despite a comparable ratio of mono- vs. oligo- and polysensitization, the Bet v 1/2/4-negative patients clearly showed a higher prevalence of anti-CCDs compared to the randomly selected study group (40 vs. 13%). This higher prevalence of CCD-sensitization can probably be attributed to the preselection: The Bet v 1/2/4 negative patients were preselected on being sensitized to birch pollen and on missing IgE reactivity against the main birch pollen component – making the existence of another sensitization such as against CCDs more likely.

The Classification of Bet v 6 as a Potential Cross-Reactive Panallergen

Some studies suggest that the sensitization to panallergens follows a sensitization to the corresponding major allergen in a chronological order [10, 11], which might allow one to draw conclusions on the origin of the pan-

allergen sensitization. As our present study reveals, specific IgE antibodies against Bet v 6 are also seen without parallel sensitization to the corresponding major allergen Bet v 1. This emphasizes the suggestion that Bet v 6 is a pan-allergen with immunological cross-reactivity across species. Consequently, serological Bet v 6 positivity might not always have its origin in birch pollen sensitization, but maybe in sensitization towards another botanic group. Hence, the sensitization to natural birch pollen extract with missing IgE reactivity to Bet v 1 might be due to cross-reactivity with panallergens.

However, the Bet v 1/2/4-negative study group contained 11 cases of symptomatic and Bet v 6-negative, but also major allergen-negative, sensitization. In one of the 11 cases, it could not be excluded that the allergic symptoms starting in April might not be due to the birch pollen sensitization, but due to a simultaneous sensitization against Oleacea. Though, in the remaining 10 allergic patients, there is no hint for any false-positive interpretation of the allergic complaints as a sign of birch pollen allergy. These 10 cases with symptomatic but major allergen-negative sensitization are in contrast to a previous study reporting that 100% of the patients with clinical complaints showed IgE against the specific major allergen component [11]. As any sole sensitization to panallergens is considered to have no clinical relevance in allergic rhinoconjunctivitis [11], our finding suggests that further clinical relevant allergen components seem to exist. This hypothesis is further emphasized by the fact that our study revealed 9 cases of monosensitization to birch pollen with no IgE antibodies against any of the tested allergen components, not even against CCDs. Former studies stated that patients monosensitized to birch pollen showed a homogenous pattern with a prevalence of antibodies against Bet v 1 in 100% [12]. In our study, the number of allergic patients with missing IgE reactivity against the major allergen component is low (1% of all birch pollen-sensitized patients); however, the fact that some of these patients show allergic symptoms indicates that the characterization of new allergen components is desirable.

The Clinical Characterization and Relevance of Bet v 6

Our study revealed Bet v 6 with a prevalence of 5% in contrast to the literature reporting a prevalence of 12 and 32%, respectively [3]. Both cited numbers derive from the same study by Karamloo et al. [3], which to our knowledge is the only existing study publishing data concerning the prevalence of Bet v 6. As Karamloo et al. state, the high prevalence of 32% was not representative for the average population with birch pollen allergy because all 28 selected

sera had high CAP classes. Indeed, our present study confirms that all Bet v 6-positive patients homogeneously display high CAP classes concerning IgE against natural pollen extract. As a result, the probability of a high prevalence of Bet v 6 increases with the average anti-t3 CAP class of the selected sera. But even in a random screening of 76 patients with birch pollen allergy, Karamloo et al. found a prevalence of 12% – maybe due to the different geographic location compared to southern Bavaria. This observation that sIgE to Bet v 6 are more frequently found in Italy than in Germany might support the hypothesis that the serological Bet v 6 positivity might not always have its origin in birch pollen sensitization as birch trees are more widely distributed across Germany compared to Italy.

Although it does not reach statistical significance, Bet v 6-positive patients – even in the one case of monosensitization – homogeneously present high CAP classes against birch pollen extract compared to randomly selected Bet v 6-negative patients. As all Bet v 6 sensitizations remain clinically silent, this finding contradicts recently published data that the level of specific IgE is the most important predictor for allergic symptoms [13]. As incidental finding, our data confirm the recent study reporting that patients with clinical relevant sensitization show higher specific IgE levels for the major allergen Bet v 1 than patients with silent sensitization [14].

As stated above, Bet v 6 is most likely to be considered as a panallergen. This finding is supported by the observation that Bet v 6-positive patients tend to be older than patients without sensitization towards Bet v 6. Component-resolved diagnostics combined with high-throughput microtechnology showed in a huge sample size that the prevalence of any specific IgE varies depending on age [15]. Specific IgE against cross-reacting allergens – as Bet v 6 is supposed to be – cannot be detected in young children and tend to appear only after the age of 6 years [16]. This immunological phenomenon of an evolution of sensitization over a lifetime has been defined as “molecular spreading” [17] and might explain the older age of Bet v 6-positive patients compared to the Bet v 6-negative control group.

To our knowledge, hardly anything is known about clinical features of Bet v 6-positive patients. Therefore, we analyzed the clinical characteristics of our Bet v 6-positive patients, although the small number of 5 patients limits the validity of interpretation. Further, 2 of the 5 Bet v 6-positive patients showed a very rare IgE reactivity pattern with missing specific IgE against the major component Bet v 1 and the well-known minor components Bet v 2 and Bet v 4. Thus, these 2 cases might not be represen-

tative of the general Bet v 6-positive population. However, in these 2 cases with isolated reactivity against Bet v 6, their clinical characteristics cannot be attributed to another well-known birch pollen component than to Bet v 6. Usually, Bet v 6-positive patients are also sensitized to Bet v 1 so that it is difficult to assess to what extent Bet v 6 contributes to the clinical features of these patients [3]. For these reasons, the clinical analysis of our five Bet v 6-positive patients appeared meaningful despite the small case number.

In our study, the comparably high prevalence of asthmatic symptoms among Bet v 6-positive patients is remarkable, especially as none of the Bet v 6-sensitized patients complained about any allergic symptoms when exposed to birch pollen. Two of the 3 patients with asthma-like symptoms were polysensitized, the third patient was oligosensitized. As sensitization to multiple allergens is associated with a high prevalence of asthma [18], the increased prevalence of asthma among Bet v 6-positive patients can probably more likely be attributed to the higher degree of sensitization than to the Bet v 6 sensitization itself. Indoor allergen sensitization appears to be more pivotal for asthma development than exclusive sensitization to pollens, which is primarily associated with rhinitis [19]. This observation can be confirmed by our data. Considering the higher degree of sensitization as the reason for a high prevalence of asthma in Bet v 6-positive patients, the higher prevalence based on our data might even be underestimated. The 2 patients without asthmatic symptoms are possibly not representative as they were part of the study group characterized by rare IgE reactivity patterns. Consequently, the prevalence of asthma in Bet v 6-positive patients might even be higher in reality than calculated on the basis of our data.

A few studies suggest a cross-reactivity of Bet v 6 with plants as a potential trigger of food allergies [3, 4, 20, 21]. However, following an overview on cross-reactivity of food allergy and pollen by Vieths et al. [22] from 2002, it still remains unclear to which degree the occurrence of cross-reactive IgE antibodies against Bet v 6-related proteins is correlated with a clinically apparent food allergy. According to a single study on 2 patients with Sharon fruit allergy, Bet v 6 seems to be related to allergy to persimmon [23]. IgE-binding proteins related to Bet v 6-like proteins have been found in kiwi, orange, strawberry, and zucchini as well [22, 24]. In our study, though, none of these potential food allergies have been found in Bet v 6-positive patients. However, when interpreting our clinical data, the small number of only 5 Bet v 6-positive patients has to be taken into account.

Future Aspects

Recently, a trend from in vivo diagnosis of IgE-mediated allergies towards in vitro tests has been anticipated in the European Union because of increased regulatory demands [25]. Due to EU directives 89/342/EEC and 2001/83/EC, diagnostic test allergens used in the EU have to be authorized for each test method in each EU member state. This development leads to a decrease of availability of allergen-specific in vivo tests although in vitro assays cannot completely substitute the diagnostic value of in vivo tests. In the context of this recent trend towards in vitro tests, allergen component-resolved diagnostics have garnered increased attention. Our present data confirm that component resolved diagnosis – despite its high diagnostic value – cannot fully substitute testing with natural pollen extracts: in 57 of the 1,064 birch pollen-sensitized patients, the sensitization-eliciting protein could not be identified by testing the standard components Bet v 1, 2, and 4. The percentage of patients in which component resolved diagnostics failed to detect the underlying allergen could be reduced from 5.4 to 5.2% by widening the component resolved diagnostics-portfolio by the additional determination of Bet v 6. In our opinion, this total improvement of detection rate by 0.2% does not justify the increased investment of testing Bet v 6 in clinical routine, especially as Bet v 6-sensitization did not display any clinically relevant characteristics.

Conclusion

The aim of the present study was to analyze the diagnostic and clinical impact of determining Bet v 6 sensitization in birch pollen-sensitized patients.

In our study cohort of 114 birch pollen-sensitized patients, the prevalence of specific IgE antibodies against Bet v 6 was 5%. None of the Bet v 6-positive patients complained about allergic symptoms when exposed to birch pollen. However, compared to birch pollen-sensitized patients without IgE reactivity against Bet v 6, Bet v 6-positive patients were more often affected by asthma. This increased prevalence of asthma is associated with a higher degree of sensitization, which is probably more crucial for the development of asthma than the Bet v 6 sensitization itself. In contrast to 2 case reports found in the literature, food allergies to tropical fruits were not registered among our Bet v 6-positive patients. However, the small sample size of five Bet v 6-positive cases has to be taken into account when interpreting the clinical data.

Enlarging the standard component-resolved diagnostics portfolio by the determination of anti-Bet v 6 improved the detection rate of the sensitization-eliciting components by 0.2%.

In summary, the determination of sensitization against Bet v 6 does not seem to add sufficient benefit in the clinical routine to justify the additional investment of routine Bet v 6 testing.

Disclosure Statement

The authors declare no conflict of interest.

References

- ▶ 1 Asam C, Hofer H, Wolf M, Aglas L, Wallner M: Tree pollen allergens – an update from a molecular perspective. *Allergy* 2015;70:1201–1211.
- ▶ 2 Rossi RE, Monasterolo G, Monasterolo S: 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. *Allergy* 2003;58:929–932.
- ▶ 3 Karamloo F, Schmitz N, Scheurer S, Foetisch K, Hoffmann A, Haustein D, Vieths S: Molecular cloning and characterization of a birch pollen minor allergen, Bet v 5, belonging to a family of isoflavone reductase-related proteins. *J Allergy Clin Immunol* 1999;104:991–999.
- ▶ 4 Wellhausen A, Schoning B, Petersen A, Vieths S: IgE binding to a new cross-reactive structure: a 35 kDa protein in birch pollen, exotic fruit and other plant foods. *Z Ernährungswiss* 1996;35:348–355.
- ▶ 5 Hauser M, Roulias A, Ferreira F, Egger M: Panallergens and their impact on the allergic patient. *Allergy Asthma Clin Immunol* 2010;6:1.
- ▶ 6 Santos A, Van Ree R: Profilins: Mimickers of allergy or relevant allergens? *Int Arch Allergy Immunol* 2011;155:191–204.
- ▶ 7 Ebo DG, Hagendorens MM, Bridts CH, De Clerck LS, Stevens WJ: Sensitization to cross-reactive carbohydrate determinants and the ubiquitous protein profilin: mimickers of allergy. *Clin Exp Allergy* 2004;34:137–144.
- ▶ 8 Schmid-Grendelmeier P: Recombinant allergens. For routine use or still only science? (in German). *Hautarzt* 2010;61:946–953.
- ▶ 9 Deiff S, Zwicker C, Vejvar E, Kitzmuller C, Gadermaier G, Nagl B, Vrtala S, Briza P, Zlabinger GJ, Jahn-Schmid B, Ferreira F, Bohle B: Glutathione-S-transferase: a minor allergen in birch pollen due to limited release from hydrated pollen. *PloS One* 2014;9:e109075.
- ▶ 10 Barber D, de la Torre F, Feo F, Florido F, Guardia P, Moreno C, Quiralte J, Lombardero M, Villalba M, Salcedo G, Rodriguez R: Understanding patient sensitization profiles in complex pollen areas: a molecular epidemiological study. *Allergy* 2008;63:1550–1558.
- ▶ 11 San Nicoló M, Braun T, Eder K, Berghaus A, Groger M: Clinical relevance of IgE to profilin and/or polcalcin in pollen-sensitized patients. *Int Arch Allergy Immunol* 2016;169:101–107.
- ▶ 12 Muehlmeier G, Maier H: Polysensitisation to pollen due to profilin and calcium-binding protein: distribution of IgE antibodies to marker allergens in grass and birch pollen allergic rhinitis patients in southern Germany. *Eur Arch Otorhinolaryngol* 2014;271:719–725.

- ▶13 Olivieri M, Heinrich J, Schlunssen V, Anto JM, Forsberg B, Janson C, Leynaert B, Norback D, Sigsgaard T, Svanes C, Tischer C, Villani S, Jarvis D, Verlato G; European Community Respiratory Health Survey IIV, Pavia INGADBSU, Uppsala SPFBN, London UK: The risk of respiratory symptoms on allergen exposure increases with increasing specific IgE levels. *Allergy* 2016;71:859–868.
- ▶14 Ciprandi G, Comite P, Ferrero F, Minale P, Voltolini S, Bignardi D, Fontana V, Bruzzzone M, Troise C, Mussap M: Can serum white birch (*Betula verrucosa*) pollen antigen (Bet v 1) immunoglobulin E measurement distinguish between sensitization and allergy? *Int Forum Allergy Rhinol* 2015;5:1151–1155.
- ▶15 Scala E, Alessandri C, Bernardi ML, Ferrara R, Palazzo P, Pomponi D, Quarantino D, Rasi C, Zaffiro A, Zennaro D, Mari A: Cross-sectional survey on immunoglobulin E reactivity in 23,077 subjects using an allergenic molecule-based microarray detection system. *Clin Exp Allergy* 2010;40:911–921.
- ▶16 Melioli G, Marcomini L, Agazzi A, Bazurro G, Tosca M, Rossi GA, Minale P, Rossi R, Reggiardo G, Canonica GW, Passalacqua G: The IgE repertoire in children and adolescents resolved at component level: a cross-sectional study. *Pediatr Allergy Immunol* 2012;23:433–440.
- ▶17 Matricardi PM: Molecular profile clustering of ige responses and potential implications for specific immunotherapy. *Curr Opin Allergy Clin Immunol* 2013;13:438–445.
- ▶18 Pallasaho P, Ronmark E, Haahtela T, Sovijarvi AR, Lundback B: Degree and clinical relevance of sensitization to common allergens among adults: a population study in Helsinki, Finland. *Clin Exp Allergy* 2006;36:503–509.
- ▶19 Boulet LP, Turcotte H, Laprise C, Lavertu C, Bedard PM, Lavoie A, Hebert J: Comparative degree and type of sensitization to common indoor and outdoor allergens in subjects with allergic rhinitis and/or asthma. *Clin Exp Allergy* 1997;27:52–59.
- ▶20 Vieths S, Frank E, Scheurer S, Meyer HE, Hrazdina G, Hausteiner D: Characterization of a new IgE-binding 35-kDa protein from birch pollen with cross-reacting homologues in various plant foods. *Scand J Immunol* 1998;47:263–272.
- ▶21 Mahler V, Fischer S, Heiss S, Duchene M, Kraft D, Valenta R: cDNA cloning and characterization of a cross-reactive birch pollen allergen: identification as a pectin esterase. *Int Arch Allergy Immunol* 2001;124:64–66.
- ▶22 Vieths S, Scheurer S, Ballmer-Weber B: Current understanding of cross-reactivity of food allergens and pollen. *Ann NY Acad Sci* 2002;964:47–68.
- ▶23 Bolhaar ST, van Ree R, Ma Y, Bruijnzeel-Koomen CA, Vieths S, Hoffmann-Sommergruber K, Knulst AC, Zuidmeer L: Severe allergy to Sharon fruit caused by birch pollen. *Int Arch Allergy Immunol* 2005;136:45–52.
- ▶24 Ciardiello MA, Tamburrini M, Tuppo L, Caratore V, Giovane A, Mattei B, Camardella L: Pectin methylesterase from kiwi and kaki fruits: purification, characterization, and role of pH in the enzyme regulation and interaction with the kiwi proteinaceous inhibitor. *J Agric Food Chem* 2004;52:7700–7703.
- ▶25 Klimek L, Hoffmann HJ, Renz H, Demoly P, Werfel T, Matricardi PM, Muraro A, Schmid-Grendelmeier P, Cardona V, Papadopoulos NG: Diagnostic test allergens used for in vivo diagnosis of allergic diseases are at risk: a European perspective. *Allergy* 2015;70:1329–1331.