

# Detection of Bet v1, Bet v2 and Bet v4 Specific IgE Antibodies in the Sera of Children and Adult Patients Allergic to Birch Pollen: Evaluation of Different IgE Reactivity Profiles Depending on Age and Local Sensitization

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## Key Words

Birch pollen • Bet v1 • Bet v2 • Bet v4 specific IgE • Recombinant allergens

## Abstract

**Background:** Birch pollen belongs to the major allergen triggers in the spring season in Europe. Our rapidly expanding knowledge of the allergenic molecules enables us to better recognize the individual differences between the reactivity of specific IgE antibodies of individual patients and allergic populations living in various regions of the world. **Method:** In a group of birch pollen-allergic patients living in the Czech Republic (107 children, 71 adults) we detected the presence of Bet v1, Bet v2 and Bet v4 specific IgE antibodies. **Results:** Bet v1 specific IgE antibodies were identified in most patients without any significant differences between children and adults. Bet v2 positivity was found more frequently in the group of children than in adults ( $p = 0.02$ ). In most adult patients Bet v1 monospecificity was more expressed as compared to the pediatric group. More allergic subjects reacted against minor birch allergens in the pediatric group ( $p = 0.02$ ). Specific IgE antibodies against Bet v1 were not detected in 10% of the tested patients. In this group, 5% of birch pollen-allergic patients were found to not have specific IgE

antibodies against any of the tested recombinant allergens. **Conclusion:** The investigation of specific IgE antibodies against Bet v1, Bet v2 and Bet v4 demonstrated that the specificity of allergen-induced IgE antibodies in birch pollen-allergic individuals is dependent not only on the region in which a patient lives but also on age. Especially in children, there is an increase in the number of allergic subjects who do not react exclusively against the major allergen. The question is whether some allergen-specific IgE antibodies will disappear depending on age or on the contrary whether their synthesis will be increased.

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## Introduction

In developed West European countries up to 25% of the population suffer from some form of atopy. With regard to a high rate of allergies and their impact upon the quality of life of the individual, type I hypersensitivity can be a serious medical problem and have socio-economic implications. Long-term epidemiological studies show an increasing prevalence of asthma and IgE-mediated allergy caused by inhaled allergens [1].

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Birch pollen hypersensitivity is the most frequent trigger of respiratory allergies in Europe. The prevalence of birch pollen-allergic individuals is reported to be between 5 and 54% among people with pollinosis in West Europe [2]. A number of clinical and laboratory tests are used to identify birch pollen allergy to complement a patient's history. In addition to the determination of allergen-specific IgE antibodies directed against the total allergen extract, the binding of specific IgE antibodies to particular allergen epitopes can be tested. A patient's individual sensitization profile is thus determined. It is known that differences of sensitization profiles are, to a certain extent, dependent on the areas of Europe in which the allergic patient lives [3–5].

The precise knowledge of the patient's 'allergenic profile' helps in recognizing cross-reacting specific IgE antibodies among unrelated allergen sources and choosing a better targeted immunotherapy. The selection of an appropriate allergen extract containing a precisely defined amount of an individual active molecule or molecules might lead to optimization of immunotherapy and to reduction of adverse side effects of possible new specific IgE antibodies [6]. Simultaneously, it is possible to monitor the efficacy of applied immunotherapy by means of the level of specific IgE, IgG, and IgG subclasses against recombinant allergens [7]. In the future, it might be possible to use the precisely defined recombinant allergens also for individually tailored hyposensibilization [7–10].

Bet v1 is a protein with a molecular weight of 17 kDa that was identified in Central and Northern Europe as the major allergen of the birch (*Betula pendula*). In the north of Europe more than 95% of patients hypersensitive to birch pollen react with this allergen. The percentage of birch pollen-allergic individuals reacting with Bet v1 decreases (65%) towards the south of Europe (Switzerland, Italy) [3, 4]. It is reported that 40 to 60% of birch pollen-allergic individuals react exclusively with Bet v1 [4, 11].

Bet v2 (profilin) is present in all eukaryotic cells of both plant and animal origins. The molecular weight of plant profilins is reported to be between 12 and 15 kDa. Due to its capacity to bind to actin, Bet v2 participates actively in the regulation of the division, growth and differentiation of cells. With regard to its biological function, profilin is a considerably conserved molecule, and so there is a high degree of structural homology between plant profilins coming from various species of plants (Bet v2 family) [12, 13].

Bet v4 is an intracellular pollen protein with a molecular weight of 9.3 kDa. Due to its biological function, it is a calcium-binding protein with two binding sites for

Ca<sup>2+</sup>. Bet v4 specific IgE antibodies are directed against these binding sites [14].

Hypersensitivity to Bet v4 is described as rather rare, occurring in 5 to 11% of birch pollen-allergic individuals, and it belongs together with Bet v2 to the minor birch pollen allergen [3, 15].

The present study summarizes the results of investigations into specific IgE antibodies against the major (Bet v1) and the minor (Bet v2 and Bet v4) birch pollen allergens performed in the years 2004–2008 in the Czech Republic. Particular attention was paid to the differences between the pediatric and adult populations of birch pollen-allergic individuals.

## Material and Methods

### Study Subjects

During the years 2004–2008 sera were tested from 178 patients with a diagnosis of any or all of the following: allergic asthma; dermatitis due to substances taken internally, and anaphylactic shock due to adverse reaction to food. The tested group comprised 85 males and 113 females of whom 107 were children aged 1–18 years (68 boys, 39 girls) and 71 adults aged 19–70 years (17 males, 54 females). Criteria for the final selection to the present study were anamnesis, positive IgE reactivity to birch pollen extract (t3) established by FEIA as measured by Phadia CAPsystem (UniCap250 Firm Phadia). Patients exhibiting a concentration of specific IgE antibodies higher than 0.35 kU/l were evaluated as positive and included in the group.

### Methods

Serum levels of allergen-specific IgE against recombinant allergens Bet v1, Bet v2, Bet v4 were measured by the FEIA method (UniCap250 Firm Phadia) according to the instructions of the manufacturer. Diagnostics with birch pollen extract, recombinant Bet v1, Bet v2 and Bet v4 are commercially available. The evaluation was performed quantitatively in kU/l or qualitatively by RAST scoring system. All 178 patients' sera were analyzed in the same laboratory in the Faculty Hospital Bulovka, Prague. All tested parameters were requested by the physicians as part of laboratory screening.

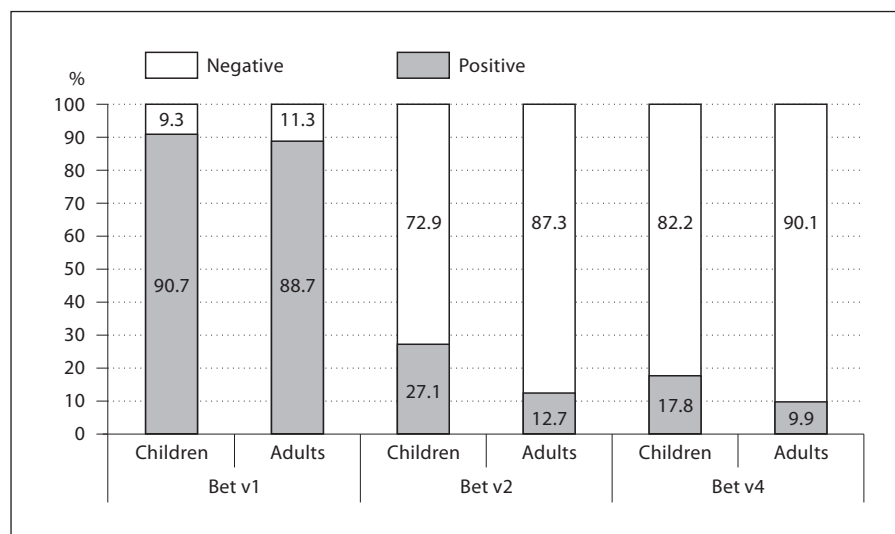
### Statistics

Statistical analysis was performed using Mann-Whitney U test.

## Results

In the group of 178 patients with confirmed specific IgE antibodies against birch pollen (t3), specific IgE antibodies were tested against the major and minor Bet v1, Bet v2 and Bet v4 birch pollen allergens.

**Fig. 1.** Comparison of occurrence of specific IgE against major and minor birch pollen allergens in the pediatric and adult populations.



#### *Rate of Occurrence of Specific IgE Antibodies against the Major and Minor Birch Pollen Allergens in Pediatric and Adult Populations*

The rate of occurrence of specific IgE antibodies against the major and minor birch pollen allergens was investigated in the sera of birch pollen-allergic patients. Antibodies against Bet v1 were found in the sera of 89.9% of patients, 21.3% of subjects reacted against Bet v2 and 14.6% of allergic patients reacted against Bet v4.

Attention was focused on investigating the differences in the rate of occurrence of specific IgE antibodies against the major and minor birch allergens in pediatric and adult populations with respect to age dependence. 107 pediatric patients aged 0–18 years and 71 adult patients aged 19–70 years were tested. No significant differences were found between the children and adults in the rate of occurrence of specific IgE antibodies against Bet v1. Bet v1 specific IgE antibodies were detected in 90.7% of children and 88.7% of adults. By contrast, a significant increase in the rate of occurrence of specific IgE antibodies against Bet v2 was found in the pediatric group ( $p = 0.02$ ). Antibodies against Bet v2 were confirmed in 27.1% of children and 12.7% of adults only. 17.8% of children and 9.9% of adults reacted against Bet v4. These differences were not found to be significant. The results are summarized in figure 1.

The patients were divided into groups by age. The percentage of patients with a positive finding of Bet v1, Bet v2 and Bet v4 specific IgE antibodies and their average concentrations are given for each category.

There is a considerable increase in the number of patients with a positive finding of Bet v1 specific IgE and also an increase in their concentration between the 2nd and 3rd years. In other age categories the concentration of Bet v1 specific IgE antibodies is increased but the percentage of patients with a positive finding is not changed too much.

It is clear that there is an increase in both the number of patients with a positive finding of Bet v2 specific IgE antibodies and in the concentration of specific IgE antibodies between the 2nd and the 3rd years again. It is also evident that children over 16 years and adults are less sensitized to Bet v2 and the concentration of specific IgE antibodies is much lower as well. This finding indicates a worsening of the state of health, and a worse prognosis of the development of allergy and for the possibilities of therapeutic interventions in allergic young children.

The percentage of birch pollen-allergic individuals having specific IgE antibodies against Bet v4 is much less dependent on age. Again the age between the 2nd and 3rd year is critical as for the increased concentration of Bet v4 specific IgE antibodies; however, such significant age dependence is not so obvious here as it is in hypersensitivity to Bet v1 and Bet v2 (fig. 2a, b).

Significant differences in the concentrations of specific IgE antibodies against Bet v1, Bet v2 and Bet v4 between children and adults can also be seen in table 1. The results were evaluated quantitatively according to RAST classes. In the pediatric age, the concentration of Bet v1 specific antibodies was evaluated by the highest classes in 40.2% of birch pollen-allergic subjects whereas in adult

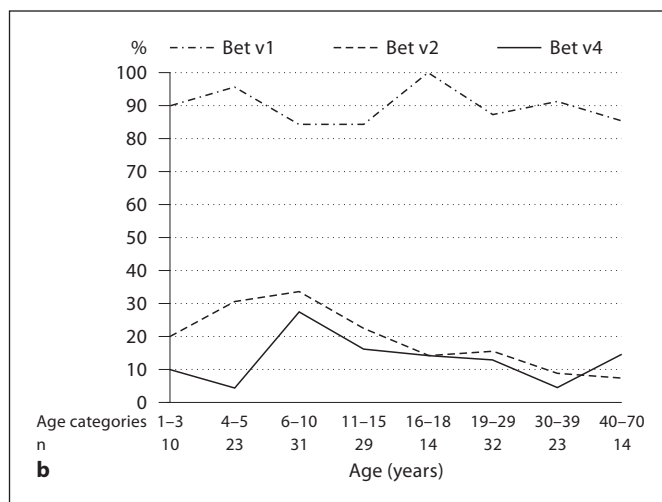
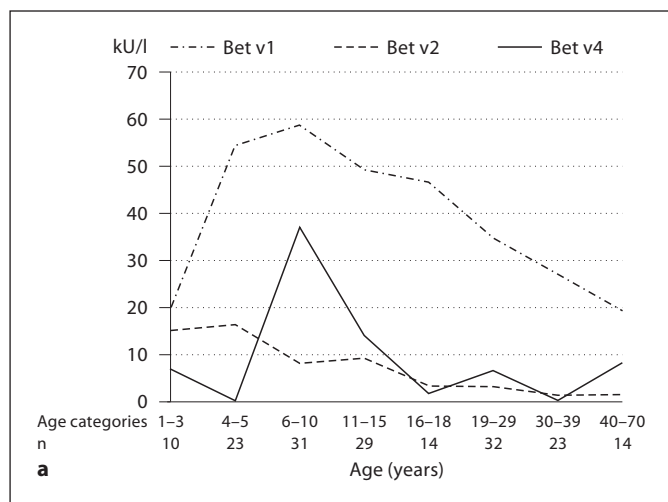


**Fig. 2. a** Average concentration of specific IgE antibodies against Bet v1, Bet v2 and Bet v4 in the sera of patients positive in the relevant allergen depending on age. **b** Rate of occurrence of Bet v1, Bet v2 and Bet v4 specific IgE antibodies depending on age.

patients it was only 21.1% of the tested samples. Differences were also found regarding specific IgE antibodies against Bet v2 and Bet v4 in which an apparent increase in the concentrations of specific IgE was determined in the pediatric population.

#### *Monosensitization and/or Polysensitization to Bet v1, Bet v2 and Bet v4 in Pediatric and Adult Populations*

We investigated how many of the tested patients reacted against one (monosensitization) or more (polysensitization) allergenic birch molecules and in what combinations. About 68% of the patients reacted exclusively against Bet v1, confirmed antibodies against minor allergens were identified in 21.9% of the Bet v1 positive allergic patients. 10.1% of the patients did not react against Bet v1, in 5.6% out of whom specific IgE against minor allergens were found. Very interesting was a 4.5% group of birch pollen-allergic individuals who did not exhibit specific IgE against any of the recombinant allergens tested.

Significant differences were found in the spectrum of specific IgE between the groups of children and adults. 62.6% of the children and 76.1% of the adults reacted exclusively against Bet v1 (n.s.). Antibodies against minor allergens in addition to Bet v1 specific IgE were detected in 28% of the children but only in 12% of the adults. The number of pediatric allergic individuals exhibiting confirmed antibodies against the major and minor birch allergens is about more than 15% higher in comparison

**Table 1.** Percentage distribution of Bet v1, Bet v2 and Bet v4 specific IgE (in RAST classes) in the sera of pediatric and adult birch pollen-allergic subjects

|             | 0    | 1   | 2    | 3    | 4    | 5    | 6    |
|-------------|------|-----|------|------|------|------|------|
| Children, % |      |     |      |      |      |      |      |
| Bet v1      | 9.4  | 0.9 | 13.1 | 14.0 | 22.4 | 15.0 | 25.2 |
| Bet v2      | 72.9 | 7.5 | 5.6  | 8.4  | 5.6  | 0.0  | 0.0  |
| Bet v4      | 82.3 | 5.6 | 3.7  | 2.8  | 2.8  | 0.9  | 1.9  |
| Adults, %   |      |     |      |      |      |      |      |
| Bet v1      | 11.3 | 5.6 | 8.5  | 22.5 | 31.0 | 11.3 | 9.8  |
| Bet v2      | 87.3 | 5.7 | 4.2  | 1.4  | 1.4  | 0.0  | 0.0  |
| Bet v4      | 90.2 | 4.2 | 2.8  | 1.4  | 1.4  | 0.0  | 0.0  |

with the adult population. This increase was found to be significant ( $p = 0.02$ ).

Specific IgE against Bet v1 was not determined in 9.4% of the children and 11.2% of the adult patients out of whom specific IgE against minor allergens was confirmed in 5.6% of the children and adult allergic patients as well. Specific IgE against any of the tested recombinant allergens were not confirmed in 3.7% of the children and 5.6% of the adults (fig. 3).

Since an allergen extract used for immunotherapy is primarily standardized only in the content of the major allergen, it is possible that hyposensitization does not have a positive effect on the attenuation of allergic disorder.

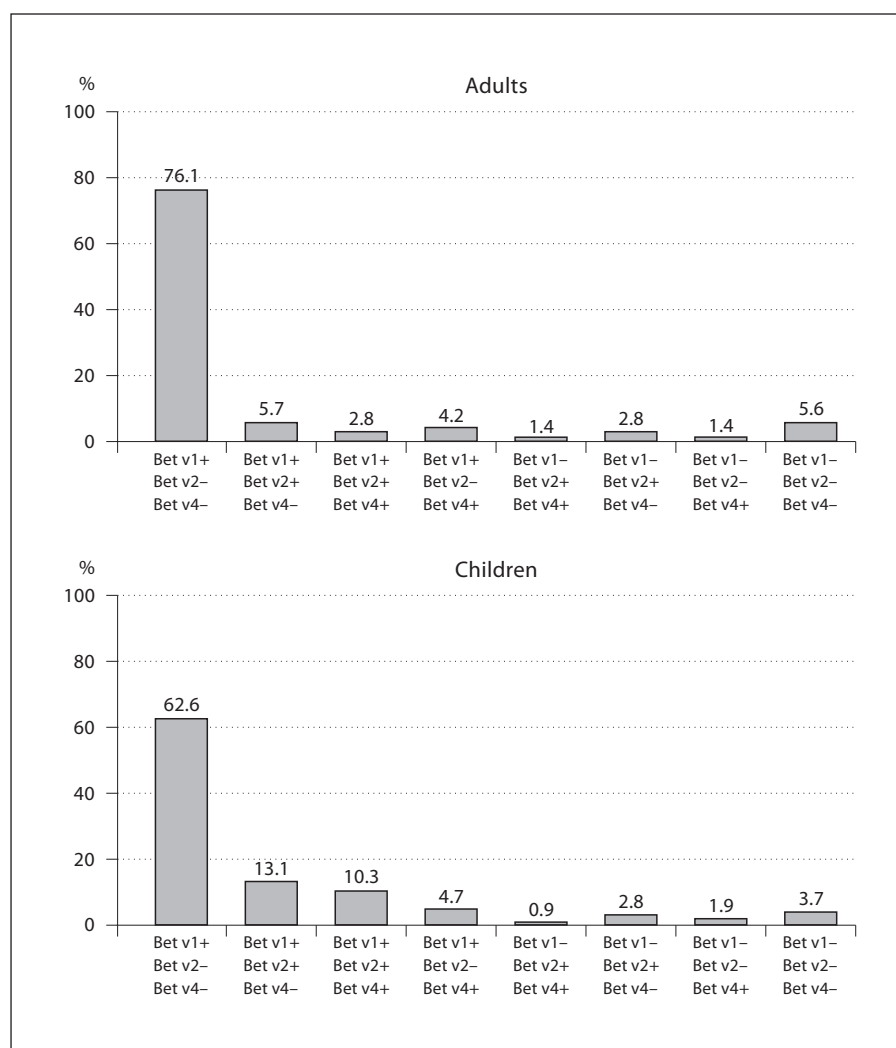


**Fig. 3.** Monosensitization and/or polysensitization in the population of pediatric and adult birch pollen-allergic individuals.

ders in patients reacting against Bet v2 and Bet v4, especially if Bet v1 specific IgE are not confirmed at all. In our group of patients, 37.4% of the children and 23.9% of the adult birch pollen-allergic patients either exhibited antibodies against minor allergens in addition to the major allergen or they did not carry any antibodies against the major allergen at all.

## Discussion

In this report, we assessed the rate of occurrence of Bet v1, Bet v2 and Bet v4 specific IgE antibodies and their concentrations in a group of birch pollen-allergic individuals in the Czech Republic. Special attention was focused on differences between pediatric and adult patients

regarding their capacity to react with particular allergens of the birch.

With respect to the facts reported in the literature that a decrease in the number of Bet v1 positive allergic individuals is observed from the north towards the south of Europe, we were interested to find out in which part of the continent the Czech Republic, being situated in the middle of Europe, should be included. In Sweden and Finland, Bet v1 specific IgE antibodies are detected in up to 98% of birch pollen-allergic subjects whereas in Switzerland and Italy the number of Bet v1 positive patients decreases below 60% [3, 4]. In our group, we have demonstrated the presence of specific IgE antibodies directed against Bet v1 in 90% of the sera tested. The differences concerning the number of birch pollen-allergic individuals sensitized to particular birch pollen allergens and

living in various areas of Europe can be induced by local climatic conditions and hence by exposure of the population to a different pollen load. The length of the pollen season of individual species of plants in various territories of Europe may also have an effect on the induction of allergy. In addition, the character of a landscape is affected by cultural factors, high traffic emissions and by climatic changes [2, 26]. Another factor that can have an effect on the differences in allergenicity of the Bet v1 molecule is the finding that Bet v1 exhibits a certain degree of variability (isoform). These isoforms have different allergenic efficacy. Single isoforms with tiny differences in their amino acid composition react with the patient's sera with a different intensity [17–19]. It is likely that all the above mentioned factors affect the ability of birch pollen-allergic individuals coming from various areas of Europe to react with particular allergen molecules.

When comparing children and adult patients we have not found significant differences in the number of Bet v1 positive patients depending on age. Higher concentrations of Bet v1 specific IgE antibodies were detected in pediatric patients.

We have come to different conclusions when evaluating the results referring to Bet v2 and Bet v4 positive birch pollen-allergic individuals. Bet v2 is classified as the second major birch allergen. The number of birch pollen-allergic individuals producing specific IgE antibodies against Bet v2 has a reversed increase trend as compared with patients with confirmed Bet v1 positivity. In southern Europe, specific IgE antibodies against Bet v2 are detected in 22–44% of birch pollen-allergic individuals [3–5], whereas in the north their number decreases significantly. In Switzerland and Finland, Bet v2 positive IgE antibodies have been identified only in 12 and 2% of patients, respectively. In our group, Bet v2 specific IgE antibodies have been found in 21% of birch pollen-allergic subjects. In Austria, which is also located in the middle of Europe, 30% of patients are Bet v2 positive [3].

When comparing the pediatric and adult birch pollen-allergic individuals, we have found a significant increase in the number of Bet v2 positive findings in children. An increasing amount of patients sensitized to Bet v2 is reported also in northern Europe [20].

The results obtained by the detection of Bet v4 specific IgE antibodies have not been so unambiguous. Bet v4 positive IgE antibodies were confirmed in 14.6% of birch pollen-allergic individuals. When analyzing the differences between children and adults, we have again observed almost a 10% increase in positive findings in the pediatric population. However, the evaluation of the re-

sults obtained in individual age categories have shown that neither the number of patients with a positive finding of Bet v4 specific IgE antibodies nor their concentrations are fully dependent on age. With respect to the fact that hypersensitivity to Bet v4 is reported in the literature as being rather rare (5–11%), the increasing number of children found capable of reacting with this minor allergen is therefore surprising [3, 15].

The question arises whether there is a relationship between the increasing number of Bet v2 and Bet v4 positive birch pollen-allergic children and the ever increasing number of allergies and the generally increasing tendency to pathologically react with nonpathogenic antigens (higher occurrence of allergic as well as autoimmune diseases) or whether there is a disappearance of Bet v2 and Bet v4 specific IgE antibodies with advancing age. The increasing polyreactivity of pediatric patients can be due to spreading of decorative plants and trees into areas where they were previously only rarely seen, which causes increased allergen load at early ages [21]. The already mentioned changes of the climate and industrialization of the environment may be due to other factors contributing to the increase of antigenic load of the organism as well. On the other hand, insufficient stimulation by formerly common antigens, e.g. of the infection origin and the increasing load of the child organism by chemical substances from nature and from foods, has a negative influence on the development of the immune system.

It is likely that the rapidly increasing number of pediatric patients reacting to greater number of birch allergen molecules might affect the suitability of hyposensibilization [22]. Simultaneously, in patients in whom hyposensibilization lacks a long-term beneficial therapeutic effect, the reason might be simply the low concentration of allergen-active molecules in the extract applied. For this reason, we have concentrated on the determination of an individual spectrum of specific IgE antibodies against the major and minor allergens of the birch in children and adults. Bet v1 monosensitization was found in most birch pollen-allergic individuals. The remaining patients are divided into two groups. The first group comprises patients producing, in addition to Bet v1 specific IgE antibodies, antibodies against the minor allergens Bet v2 and Bet v4. The second group is composed of the patients without confirmed Bet v1 specific IgE antibodies. In this group, it is questionable whether immunotherapy should be initiated. It is discussed whether the application of an extract with a high content of Bet v1 could be effective for attenuation of allergic disorders and, simultaneously, whether it could induce a novel sensitization to the ma-

for birch allergen and worsen the patient's state of health. The possibility of sensitization of the patient to novel allergen molecules has been demonstrated in the course of hyposensitization of the patients with detected Bet v1 positive and negative Bet v2 and Bet v4 antibodies by an allergen birch pollen extract. Within one year after the initiation of immunotherapy, antibodies were detected against Bet v2 and Bet v4 [6]. On the other hand, other studies have shown that even a long-term immunotherapy does not affect the development of sensitization to new allergen molecules [23, 24].

An analysis of age dependence has shown that adult patients preferably produce IgE antibodies against Bet v1. In the pediatric group, the number of children sensitized only to Bet v1 is significantly lower and the children also carry specific IgE antibodies against minor birch pollen allergens. Our results are indicative of a higher ability of children today to react with a broader spectrum of antigens currently occurring in nature.

We consider these findings to be of particular relevance with regard to the fact that specific IgE antibodies against the major and minor allergens of the birch are responsible for cross-reactions between pollens and foods [25, 26].

The examination of specific IgE antibodies directed against Bet v1, Bet v2 and Bet v4 covers about 90–99% of sensibilizations to birch pollen. 4.5% of patients with identified birch pollen allergy are lacking antibodies against any of allergens tested and it is possible to assume that specific IgE antibodies are directed against other allergen molecules. In this group, both pediatric and adult subjects were represented equally. In this respect our

results correspond to the data reported in the literature [3, 4].

A higher concentration of specific IgE directed against all the allergens tested also indicates a growing reactivity of the pediatric population born during the last decade (table 1). It is a question for the future as to what development of allergy can be envisaged in these children: whether the concentration of allergen-specific IgE antibodies will further increase or whether there will be an attenuation of allergy with advancing age. For the time being, our experience suggests that there is an increase in concentration of allergen-specific antibodies. This observation is also supported by the growing amount of birch pollen-allergic children suffering from food allergies demonstrated by skin prick tests, specific IgE and by a test of the activation of basophiles (data are being processed). Long-term studies dealing with the prevalence of asthma and IgE-mediated allergies show a growing number of allergies and asthmatic individuals in developed countries. Association of 'the Western lifestyle' with higher prevalence of allergy in children [27, 28] and adults [29–31] is assumed.

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