



Different clinical effect of several types of airborne allergens on the severity of bronchial hyperreactivity

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Summary

Background Atopic sensitization belongs to the most common risk factors for bronchial asthma. However, in clinical practice, it is not clear whether sensitization against pollen and perennial allergens is differently associated with the severity of bronchial hyperresponsiveness (BHR).

Aim To find out whether patients sensitized to perennial allergens differ in severity of bronchial hyperresponsiveness from patients sensitized to pollen allergens.

Methods The study includes 109 patients. Based on the results of skin prick tests, patients were divided into three groups: sensitivity to pollen allergens – group A; sensitivity to perennial allergens – group B; sensitivity to both pollen and perennial allergens – group C. Based on the histamine bronchoprovocation test, we compared the values of histamine provocative concentration causing a 20% drop in FEV₁ (PC₂₀) among particular groups of patients. Mild bronchial

hyperresponsiveness was determined if the value of PC₂₀ was >4 mg/ml, while if the value of PC₂₀ was <4 mg/ml, the bronchial hyperresponsiveness was considered as moderate/severe.

Results A statistically significant difference was found in the degrees of bronchial hyperresponsiveness between the three groups of patients, namely, group A with the patients sensitized only to the pollen allergens, group B comprising patients sensitized to the perennial allergens only, and group C, involving patients sensitized to the combination of both pollen and perennial allergens. The PC₂₀ values were higher among the patients from the group A (7.46 mg/ml) compared to group B (4.25 mg/ml) and C (4.52 mg/ml). The odds ratio for moderate/severe BHR was 5.21 and 5.04 in group B and group C, respectively.

Conclusion Severity of bronchial hyperresponsiveness shows differences according to sensitization to particular allergens. Perennial allergens are more often associated with serious forms of bronchial hyperresponsiveness which also have an impact on the severity and prognosis of bronchial asthma.

Keywords Bronchial asthma · Bronchial hyperresponsiveness · Allergic sensibilization · Pollen allergens · Perennial allergens

Introduction

Allergic sensitization belongs to the most common risk factors contributing to the development of bronchial asthma (BA) in children, adolescents, and adults [1–3]. BA can develop in patients who are sensitized to seasonal (usually pollen) or perennial allergens. Sensitization to seasonal allergens or perennial allergens often occurs either independently or in combinations.

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The association between asthma and sensitization to perennial allergens is well-known [4]. It has been reported that sensitization to indoor perennial allergens and outdoor mold spores increased the risk of bronchial hyperresponsiveness (BHR) and accentuated clinical symptoms in children [5–7]. Similarly, sensitization to house dust mites (HDM) was significantly associated with BA and BHR in adults [8–10]. Other works have confirmed the high risk of BA in patients exposed to the allergens from domestic animals [11–13]. Much attention is devoted to the mold allergens. Species of the genera *Cladosporium* [14] and *Alternaria* are among the most common and the most universally distributed fungi associated with BA [15–17]. On the other hand, pollen allergens are significantly involved in the development of allergic rhinitis [18, 19]. The results of studies concerning the sensitization to pollen allergens and the development of BA are, however, more controversial. Some publications reported that pollen allergens rarely induce BA [20]. Studies from the US, however, confirmed the association between asthma exacerbation and a high concentration of pollen in the air [21, 22].

BHR is a key feature of BA. Mild BHR can also occur in patients with allergic rhinitis without BA; however, the development of BA is supposed in the future. In order to confirm the bronchial hyperresponsiveness, histamine or methacholine provocation tests (BPT) are used with a subsequent determination of PC₂₀ values. Studies which directly compare the severity of BHR, based on the values of PC₂₀, between the groups of patients sensitized to perennial and pollen allergens are relatively rare [23, 24]. The advantage of such a comparison is that the value of PC₂₀ accurately determines the severity of BHR and, consequently, provides information about the severity of the disease [5]. However, in clinical practice, it is not completely clear whether sensitization to pollen or perennial allergens results in the different severity of BHR. Similarly, it is not known which patients (sensitive to pollen or perennial allergens) are at a higher risk of more severe forms of BA.

The aim of our study was to investigate whether differences exist in the severity of BHR between groups of patients who are sensitized to pollen allergens, perennial allergens, and/or a combination of perennial and pollen allergens.

Materials and methods

Patients

The study includes a total of 668 patients hospitalized in the Department of Occupational Medicine and Clinical Toxicology (L. Pasteur University Hospital, Kosice, Slovakia) for the realization of a bronchoprovocation test (BPT) and differential diagnosis of BA. All patients reported clinical symptoms, such as dyspnea, wheezing, and a cough. Of the 668 pa-

tients included in this study, BHR was confirmed by bronchial provocation test in a total of 109 patients. Simultaneously, in these patients, the sensitivity to at least one tested allergen was confirmed. The remaining 559 patients were excluded from the study. Data used in this study were analyzed retrospectively.

Sensitization by allergens

Sensitization was evaluated on the basis of results of skin prick tests, which have been obtained from different allergy outpatient departments. Sensitization to the following groups of allergens was monitored – mixture of tree pollen, mixture of grass pollen, mugwort, ragweed, house dust mites, dog, cat, and molds (*Alternaria* spp. and *Cladosporium* spp.). Based on the results of skin prick tests, the patients were divided into 3 groups – sensitization to pollen allergens (trees, grasses, mugwort, and ragweed) – group A; sensitization to perennial allergens (house dust mites, *Alternaria* spp., *Cladosporium* spp., dog, cat) – group B; sensitization to pollen and perennial allergens in combination – group C.

Bronchial provocation test

Aerosol histamine was generated by a standardized bronchial provocation nebulizer (Provo Jet, Ganshorn GmbH, Germany). The air-compressor created a sufficient amount of respirable fraction of the aerosol (output 1 ml for 1–3 min). The histamine was applied in double dose concentrations (0.1, 0.2, 0.4, 0.8, 1.5, 3.0, 6.0, 12.0 mg/ml) with a total cumulative dose of 172.8 µg.

Significant for the objectification of bronchial hyperresponsiveness is a decline in the value of the one-second forced vital capacity (FEV₁) of 20% of its base value. The concentration of histamine which resulted in decreased FEV₁ value of more than 20% (PC₂₀) was monitored in each patient; BHR was present if a decrease in FEV₁ by 20% of its base value occurred with a concentration of histamine of ≤12 mg/ml. The severity of BHR was determined by the value of PC₂₀ – mild >4 mg/ml of histamine or moderate/severe <4 mg/ml of histamine.

Statistical analysis

Statistical analysis was performed using the software DELL-STATISTICA version 13.0. The individual variables are characterized by the number of values, the mean value, an estimate of the arithmetic average, and by a selected standard deviation. To compare the differences in total IgE antibodies, eosinophils leukocytes, and PC₂₀ between the groups, an analysis of variance (ANOVA) was used. Obtained data did not show a normal distribution; thus, the Kruskal–Wallis test was used. For the comparison of patients with mild BHR and moderate/severe BHR, the χ^2 test was

Table 1 Characteristics of the patients

Group		A (pollen)	B (perennial)	C (mix)
Number of patients (<i>n</i>)		31	36	42
Sex	Men (<i>n</i>)	10	8	19
	Women (<i>n</i>)	21	28	23
Average age (years)		35.53 (±10.57)	41.54 (±11.21)	36.24 (±11.44)
Number of eosinophils (10 ⁹ /l)		0.29 (±0.22)	0.26 (±0.21)	0.42 (±0.59)
Total IgE (IU/ml)		271.82 (±282.16)	275.25 (±474.74)	449.59 (±517.27)
Average PC ₂₀ (mg/ml)		7.46 (±3.66)	4.25 (±3.00)	4.52 (±3.57)

used. In group A, we tested the hypothesis of equality of the mean values of parameters PC₂₀ by an unpaired T-test in patients who had BPT performed during the pollen season (March–September) and outside the pollen season (October–February). The differences at a level of $p < 0.05$ were considered significant.

Results

The average age of 109 tested patients included in the analysis was 38 years (range of 16–64 years). Upon evaluations of sensitization, most patients were assigned to group C (hypersensitivity to both pollen and perennial allergens). The highest average values for serum eosinophils and total IgE were found in group C. But no statistically significant difference in these values was found between all groups (Table 1). The most frequent allergen in the group A was grass pollen. HDM was the most important allergen in the group B; other allergens in this group were relatively rare. In the group C, similarly to group A, most patients were sensitized by grass pollen. Similarly, sensitization to HDM was quite common, but not so frequent as in group B. Sensitization to *Alternaria* spp. was more frequent in group C in comparison to group B (Table 2).

The highest average value of PC₂₀ was in group A; in groups B and C, the average values of PC₂₀ did not differ. The values of PC₂₀ in the individual groups are present in Fig. 1. A statistically significant difference was confirmed between groups A, B, and C in the PC₂₀ parameter at a level of significance $p < 0.01$, while the

difference appeared between the groups A and B at a level of $p < 0.01$ and the groups A and C at a level of $p = 0.02$.

Similarly, comparing the severity of BHR between the groups, in group A the patients with mild BHR prevailed significantly, in contrast to groups B and C, where the patients with moderate/severe BHR predominated. The odds ratio for moderate/severe BHR was 5.21 and 5.04 in group B and group C, respectively (Fig. 2).

Comparing the values of PC₂₀, no statistically significant difference was found between the patients in group A, in which the test was carried out during the pollen season (March–September; average value of PC₂₀ = 8.06 mg/ml) and outside the pollen season (October–February; average value of PC₂₀ = 6.90 mg/ml).

Discussion

The study shows significant differences in the severity of BHR based on sensitization to different groups of allergens. Perennial allergen sensitization was associated with a lower threshold of responsiveness to histamine (PC₂₀) in comparison with pollen allergen sensitization. Also, a group of patients sensitized to the combination of pollen and perennial allergens had a lower value of PC₂₀ compared to those sensitized to pollen allergens only. Our findings are consistent with some earlier studies which were conducted using nonspecific BPT. Verdiani and Prieto analyzed the nonasthmatics patients with allergic rhinitis. They confirmed a higher prevalence of a higher degree of BHR in patients with perennial rhinitis compared to those with seasonal rhinitis. However, their study did not include patients with asthma [24, 25]. Craig et al., on a large sample of mild to moderate asthmatic patients, compared the group of patients sensitized to perennial allergens, and to the combination of perennial and seasonal allergens, with a group of patients sensitized only to seasonal allergens. This study reported the lower PC₂₀ values and higher levels of total IgE and exhaled nitric oxide in the group of patients with a perennial allergen sensitization. However, patients with isolated seasonal allergen sensitization represented only 2% of the total population [23].

Our work shows a higher importance of perennial allergens for the development of severe forms of BHR

Table 2 Sensitization to individual allergens (numbers of patients in percentage)

Allergen	Group		
	A (pollen)	B (perennial)	C (mix)
Tree pollen	48.39	–	59.52
Mixture of grass pollen	74.20	–	71.43
Mugwort	64.52	–	57.14
Ragweed	41.94	–	35.71
House dust mites	–	91.67	64.29
Dog	–	13.89	28.57
Cat	–	11.11	19.05
<i>Alternaria</i>	–	22.22	50.00
<i>Cladosporium</i>	–	22.22	38.01

Fig. 1 Comparison of values of PC₂₀ in the individual groups obtained by application of Tukey–Kramer test

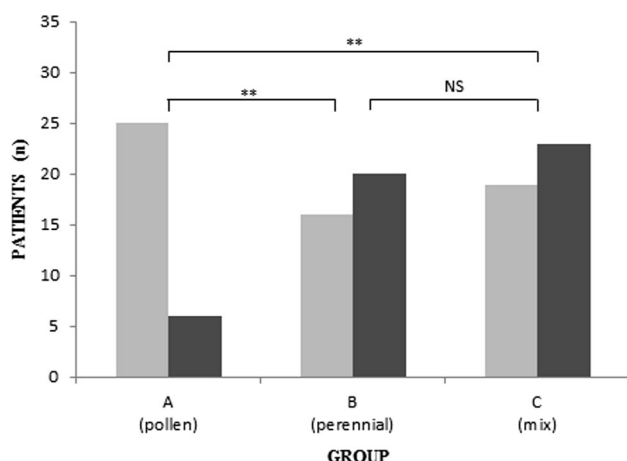
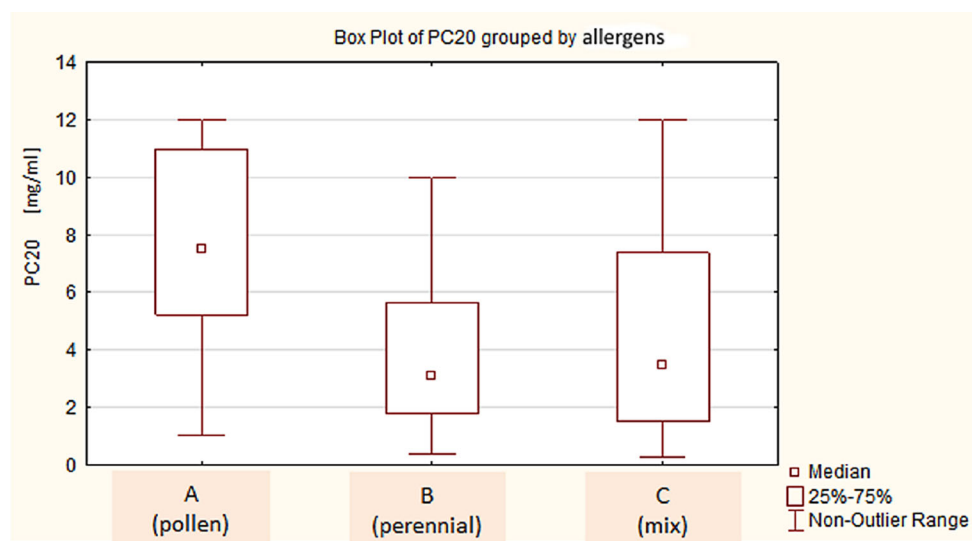


Fig. 2 Comparison mild and moderate/severe bronchial hyperresponsiveness (BHR) in individual groups (gray columns mild BHR, black columns moderate/severe BHR, ** $p < 0.01$, NS not significant)

compared to pollen allergens. Moderate and severe BHR occurred more frequently in groups B and C, i.e., where patients were sensitized to perennial allergens against group A – patients with isolated pollen sensitization. The difference in PC₂₀ values was not recorded between groups B and C. Therefore, in the patients sensitized by the combination of perennial and pollen allergens, the severity of the BHR is mostly caused by the perennial allergens.

Perennial allergens interact with an airway epithelia in a different way, in comparison with the pollen allergens. Typical perennial allergens, which are important for inducing asthma in every age, are house dust mites (HDM). The clinical relevance depends on the degree of sensitization and specific HDM allergen involved. The pediatric population of patients with asthma showed higher levels of allergen-specific IgE antibodies to HDM allergens, especially rDer p2, rDer p5, and nDer p1, than nonasthmatic sensitized patients [26]. A positive association between the level of

IgE antibodies and respiratory symptoms was demonstrated in adults [27]. HDM allergens, similarly to mold and pet allergens, are small ($<10\mu\text{m}$) and penetrate deeper into the lower respiratory tract. Very significant is mainly their proteolytic activity that disrupts connections between the epithelial cells. Moreover, HDM allergens activate the costimulatory receptors such as PAR₂ and TLR and, thus, increase the Th2 response [28]. *Alternaria* spp. as a strong asthagen activate PAR₂ (protease-activated receptor) in the respiratory epithelium. *Alternaria*-specific serine protease activity causes release of IL-33, which consequently triggers allergic inflammatory reaction [29]. For the onset of symptoms, the exposure to these allergens is important. In Scandinavia, where the climate is cold and dry, the occurrence of the HDM and mold is low; therefore, these allergens are only sporadically responsible for the onset of allergic diseases. The strongest effects to the lower respiratory tract were attributed to domestic animal allergens. An increased incidence of asthma and wheezing was associated with a positive skin test to the cat, dog, or horse [2]. Warm et al. has shown that asthma was associated with sensitization to animal-related allergens regardless of age. Allergic rhinitis was also associated with sensitization to animals and to pollen allergens, regardless of age [3].

A less significant effect of pollen allergens in the lower airways was also demonstrated in our study. It is generally explained by the size of pollen and the length of exposure during the year. Pollen particles are relatively large compared to perennial allergens and are deposited in the nose with a minor penetration into the lower respiratory tract [30]. On the other hand, it is clear that during flowering, the pollen is one of the most common causes of allergic asthma. Very important factor is also the concentration in the surrounding environment. Studies from different regions of the US have shown a relationship between asthma exacerbations and high concentra-

tion of pollen of trees, grasses, and weeds [21, 22, 31]. Outside the peak season of pollen production, asthma exacerbations were significantly less common [22]. It is not clear by which mechanism pollen can induce BA. It is considered that allergic inflammation in the lower airways is probably not due to the whole pollen granules, but their particles. These are larger than 10 μm , and the predilection site of their localization is the upper airways. In this case, especially important are the starch granules containing pollen antigens, which are smaller than 5 μm and are able to penetrate to the lower airways. Badorrek et al. applied two chamber challenges (a mixture of pollen plus starch granules or only starch granules) in patients with allergic rhinitis and mild intermittent asthma. Nasal symptoms were more significant in the test with the pollen grains compared to the test with only starch granules, which are not deposited in the nose. After both tests, increased levels of exhaled nitric oxide (eNO), confirming that inflammation of the lower respiratory tract is caused by the starch granules, have been observed. However, the levels of FEV₁ did not show a significant decrease in both tests. The presence of pollen grains or starch granules does not alter bronchi in the meaning of bronchoconstriction [32]. This might be another explanation of the lower impact of pollen on BHR compared to perennial allergens. Verdiani et al. explains that prolonged inhalation of allergens induces repeated allergic reactions, which can increase BHR. Patients are exposed to pollen allergens only during the pollen season, while those with perennial allergies are exposed to allergens all year round [25].

In our work, the bronchial provocation tests were performed throughout the year; therefore, the impact of the pollen season on the study remains questionable. High concentration of allergens in the environment can increase BHR. In the Italian study, the lowest value of PC₂₀ was found during the spring and autumn. During these seasons, there is the highest concentration of pollen and dust mites in this region [33]. However in our study, when comparing PC₂₀, no significant differences were found between patients with hypersensitivity to pollen allergens, in which BPT was performed during the “pollen season” (from March to September, when the concentration of pollen in Slovakia is the highest) and outside the “pollen season”. Therefore, we assume that the pollen season did not significantly influence the results of the study.

In conclusion, the study demonstrates that the severity of bronchial hyperresponsiveness shows differences according to sensitization to individual allergens. Perennial allergens are more often associated with serious forms of bronchial hyperresponsiveness which also have an impact on the severity and prognosis of bronchial asthma.

Conflict of interest S. Perečinský, M. Varga, J. Petrovičová, O. Ragač, K. Perečinská, A. Jančová, L. Murínová, T. Bačinský, and Ľ. Legáth declare that they have no competing interests.

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