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# Specific bronchial hyperreactivity and hypersensitivity in patients with allergic asthma

## Specifična bronhijalna hiperreaktivnost i kožna preosetljivost kod bolesnika sa alergijskom astmom

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**Abstract**

**Background/Aim**. Bronchial asthma is a disease that is characterized by the variability of the clinical picture, physical and functional status and the existence of bronchial hypersensitivity and hyperreactivity with varying degrees. Bronchial responsiveness and sensitivity are tested in patients with clinically suspected existence of asthma and normal spirometry test. The aim of the study was to analyze the patients with atopic asthma and study test results of skin sensitization to inhaled allergens, nonspecific bronchial hyperreactivity and specific hyperreactivity estimated by bronchial provocation tests with inhalant allergens. **Methods**. The prospective study at the Pulmonology Clinic of the Military Medical Academy in Belgrade Serbia, during 2014, included 70 male subjects aged 18–30 years, who had perennial asthma symptoms. All subjects were nonsmokers, with normal spirometry findings, with normal radiological chest findings and with no symptoms of respiratory infection over the past two months. All respondents were tested with skin prick tests with inhalant allergens and nonspecific bronchial provocation test with histamine. On the basis of histamine test, subjects were divided into two groups: the group I, in which there was a slight degree of hypersensitivity [provocation concentration of histamine causing a 20% fall in forced expiratory volume – $PC_{20}$ = 6.09 ± 1.1 mg/mL], and the group II with negative histamine test ($PC_{20}$ = 14.58 ± 6.34 mg/mL). Specific bronchial provocation test was performed in all patients, and the selection of the allergens was carried out based on the results of testing of skin hypersensitivity. **Results.** Results of skin sensitization show the highest incidence of mites *Dermatophagoides pterronissinus* (83.3% group I and 85.0% group II) followed by grass pollen (53.3% group I and 52.0% group II), and house dust (33.3% group I and 50.0% group II). There were no statistically significant differences in allergens between groups ($p > 0.05$). In both groups, spirometry findings were within normal values [forced vital capacity – FVC and forced expiratory volume 1 – FEV1 > 80% predictive value], but statistically significant difference was found in FEV1 between groups ($p < 0.05$). Specific bronchial provocation tests with solutions of inhaled allergens in both groups caused a significant decline in FEV1 (≥ 20%) in all patients individually. No statistically significant differences were found neither between groups, nor between individual allergens (average decline in FEV1: Group I 32.9 ± 2.4% and group II 31.5 ± 2.2%). **Conclusion**. There is no relationship between the degree of specific and non-specific bronchial hyperreactivity in patients with allergic asthma.

**Key words:**
**asthma; bronchial spasm; hypersensitivity; allergens; skin tests; histamine; spirometry.**

**Apstrakt**

**Uvod/Cilj**. Bronhijalna astma je bolest koja se odlikuje varijabilnošću kliničke slike, fizičkog i funkcijskog statusa i postojanjem bronhijalne hipersenzitivnosti i hiperreaktivnosti različitog stepena. Bronhijalna reaktivnost i senzitivnost ispituje se kod bolesnika sa kliničkom sumnjom na postojanje astme i normalnim spirometrijskim testom. Cilj rada je bio da se kod bolesnika sa atopijskom astmom analiziraju rezultati testova kožne preosetljivosti na inhalacione alergene, nespecifične bronhijalne hiperreaktivnosti i specifične hiperreaktivnosti procenjene bronhoprovokacijskim testovima sa inhalacionim alergenima. **Metode**. Prospektivnom studijom u Klinici za pulmologiju Vojnomedicinske akademije tokom 2014. godine, obuhvaćeno je 70 ispitanika muškog pola starosti od 18 do 30 godina, koji su imali višegodišnje simptome astme. Svi su bili nepušači, urednog spirometrijskog i radiološkog nalaza na snimku grudnog koša, bez simptoma i znakova respiratornih infekcija tokom prethodna dva meseca. Svim ispitanicima rađeni su testovi kožne preosetljivosti sa inhalacionim alergenima i nespecifični bronhoprovokacijski test sa histaminom. Na osnovu

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histaminskog testa ispitanici su podeljeni u dve grupe: I grupu, kod koje postoji lakši stepen hipersenzitivnosti [provokaciona koncentracija histamina koja uzrokuje 20% smanjenja forsiranog ekspiratornog volumena u jednoj sekundi ($PC_{20}$) = 6,09 ± 1,1 mg/mL] i II grupu sa negativnim histaminskim testom ($PC_{20}$ = 14,58 ± 6,34 mg/mL). Kod svih ispitanika izvršeno je specifično bronhoprovokacijsko testiranje, a izbor alergena vršen je na osnovu rezultata testiranja kožne preosetljivosti. **Rezultati.** Rezultati kožne preosetljivosti pokazuju najveću učestalost grinje *Dermatophagoides pterronissinus* (83,3% u grupi I i 85% u grupi II), zatim polena trave (53,3% u grupi I i 52% u grupi II) i kućne prašine (33,3% u grupi I i 50% u grupi II), bez statistički značajne razlike u zastupljenosti alergena između ovih grupa ($p > 0,05$). U obe grupe spirometrijski nalaz bio je u granicama referentnih vrednosti [forsirani vitalni kapacitet (FVC) i forsirani ekspiratorni volumen u jednoj sekundi (FEV21) > 80% prediktivne vrednosti], ali je postojala statistički značajna razlika u vrednosti FEV1 između grupa ($p < 0,05$). Specifičnim bronhoprovokcijskim testovima sa rastvorima inhalacionih alergena kod obe grupe ispitanika izazvano je značajano smanjenje FEV1 (≥ 20%) kod svih ispitanika. Nije bilo statistički značajne razlike u plućnoj funkciji među grupama, a ni u preosetljivosti na pojedine alergene (prosečano smanjenje FEV1: I grupa – 32,9 ± 2,4% i II grupa – 31,5 ± 2,2%). **Zaključak.** Nije utvrđena direktna povezanost između stepena specifične i nespecifične bronhijalne hiperreaktivnosti kod bolesnika sa alergijskom astmom.

**Ključne reči:**
**astma; bronhusi, spazam; hipersenzibilnost; alergeni; koža, testovi; histamin; spirometrija.**

## Introduction

Bronchial asthma is a chronic inflammatory disease of the airways that is clinically characterized by attacks of shortness of breath, especially in the titration phase, followed by wheezing, cough and sputum tough secretions [1, 2].

The cause of asthma is unknown but it is thought that there is a tendency to develop the disease, which is transmitted as an autosomal dominant inheritance; also, numerous external and internal factors are determined (eg. atopy) that can trigger immune reaction in the airways. A specific type of chronic inflammation of the bronchial mucosa with dominant engaging lymphocytes (CD4+, Th2), eosinophils and metahromal cells in the airway mucosa forms the basis for bronchial hyperreactivity, expression and chronicity of the disease. In addition to edema, epithelial damage and increased mucus production, inflammation leads to irreversible morphological changes such as subepithelial fibrosis and hypertrophy of smooth muscle, resulting in the so-called airways remodeling [1–4].

The diagnosis of asthma is based on a history of problems, physical and pathological findings of the lung, pulmonary function tests (spirometry, which confirms the limitation of airflow, which usually registers obstructive disorders of ventilation – reducing the value of forced expiratory volume in one second (FEV1) and relations with the vital capacity (VC) Tiffeneau index – FEV1/VC × 100), skin tests to inhalant allergens and basic indicators of immune status and inflammation in the bronchial tree [eosinophilic leukocytes in peripheral blood, immunoglobulin E in serum and eosinophils in sputum and nitric oxide (NO) in exhaled air). When the spirometry test is normal, and clinical picture indicates asthma, broncho-provocation testing is performed (non-specific bronchial provocation test – histamine, methacholine and others, or specific bronchial provocation test with inhalant allergens, which is less common) [4–12].

The aim of the study was to analyze the correlation between non-specific bronchial hyperreactivity and specific bronchial hyperreactivity in patients with allergic asthma, estimated by bronchial provocation test with inhaled allergens, as well as to assess their relationship.

## Methods

The prospective study at the Pulmonology Clinic in the tertiary health care university hospital, the Military Medical Academy, Belgrade, Serbia, during 2014, included 70 male subjects aged 18 to 30 years, who had had for many years asthma symptoms (shortness of breath, difficulty breathing, wheezing, fatigue, night choking and dry or productive cough). All were non-smokers.

All the patients were tested by hypersensitivity skin tests to inhalant allergens (Torlak, Institute of Virology, Vaccines and Sera, Belgrade, Serbia) and nonspecific bronchial provocation test with histamine (Fluka Histamine dichydrochloride, Sigma-Aldrich, Germany). Patch testing was done, a standardized, prick method with dual control (saline solution and histamine). Allergens and dual controls were injected intradermally to produce a small bleb, and the outcome measure was an increase in the size of the wheal after 20 minutes. Allergens needed to be diluted (100–1000 fold) from the concentrations used for skin prick testing. There were skills required to inject correctly and interpret the result.

Spirometry finding in all patients was within normal ranges (SpiroPro, Erich Jaeger GMBH). Chest Radiological findings in all patients were normal and there were no symptoms of respiratory infection over the past 2 months. Nonspecific bronchial hypersensitivity and hiperreactivity were tested with histamine solutions with the help of the device for inhalation (Inhalog 2, Drägerwerk AG Lübeck). The tests were performed by cumulative technique until reaching the threshold of sensitivity ($PC_{20}$) – provocation concentration of inhaled histamine that led to a drop in FEV1 by 20% compared to baseline. The value of $PC_{20}$ was calculated by algorithmic transformation of the measurement results. The histamine test was estimated as negative if $PC_{20}$ was not reached, even with the concentration of histamine higher than 8 mg/mL of histamine. The value of $PC_{20}$ in the range of 4–7.9 mg/mL, ment slight degree of nonspecific bronchial hypersensitivity [6–12].

On the basis of histamine test results subjects were divided into two groups: the group I, in which there was a slight degree of hypersensitivity ($PC_{20}$ = 4–7.9 mg/mL), and the group II with negative histamine test ($PC_{20}$ = 8 mg/mL).

Specific bronchial provocation tests were performed in all patients, 24 hours after histamine test. The allergens were selected on the basis of the results of skin testing (Torlak, Institute of Virology, Vaccines and Sera, Serbia). The initial concentration of the solution of allergen was 1,000 units of total nitrogen (TNU). The test was considered positive when it led to a drop in FEV1 of 20%, and more activity compared to the initial value. The reactivity was evaluated on the dose of allergen that had a major response of the bronchi, and on the basis of decrease in FEV1 [6, 9–14]. Due to possible late asthmatic reactions, the subjects were being observed for 7 hours after the test.

All attribute variables were presented in the form of the frequency of certain categories, and statistical significance between the individual categories was tested by $\chi^2$ test. Continuous variables were presented as arithmetic means and standard deviations ($\bar{x} \pm SD$). Continuous variables were compared using Student's *t*-test or Mann-Whitney *U*-test. The normality of the data was assessed by using Kolmogorov-Smirnov test. All the analyses were estimated at $p < 0.05$ level of the statistical significance.

## Results

Average age, duration of the disease and parameters of pulmonary ventilation in study participents are presented in Table 1. There were statistically significant difference of these parameters between the group with mild degree of hypersensitivity and the group with negative histamine test. The analysis of the average age and disease duration in our patients established that patients in the group II were older in comparison with those in the group I, while the duration of illness was significantly shorter in the group II in comparison with the group I.

In both groups, spirometry findings were within normal values [forced vital capacity – FVC and FEV1 > 80% of predictive value), but there was a statistically significant difference in FEV1 between groups ($p < 0.05$) and FEV1/ FVC index ($p < 0.05$).

The results of skin sensitization to inhaled allergens are shown in Table 2. There were no significant differences in allergens between groups ($p > 0.05$). The most common allergens were mites *Dermatophagoides pterronissinus*, grass pollen and house dust.

The degree of nonspecific bronchial sensitivity, determined by histamine test is shown in Table 3. Statistical analysis showed that there was a significant difference in the percentage fall in FEV1 between the groups I and II ($p < 0.05$). Nonspecific bronchial provocation test with histamine was negative for the whole group II (a significant decline in FEV1 was not caused in any of the patients), while in the group I it was minor (average fall FEV1: 1.44 ± 0.34 L or 27.2 ± 5.7%).

Specific bronchial provocation tests with solutions of inhaled allergens in both groups caused a significant decline in FEV1 (≥ 20%) in all patients individually (the group I: 32.9 ± 2.4%; the group II: 31.5 ± 2.2%; $p = 0.0151$). The results are shown in Table 4.

## Discussion

Asthma is a major burden for governments, healthcare providers and patients [15]. The annual costs of the European economy of healthcare and lost productivity due to asthma are estimated as €33,9 billion [16]. This is a disease that is characterized by the variability of the clinical picture, physical and functional status which is caused by chronic inflammation with the presence, also variable, bronchial hypersensitivity and hyperreactivity.

Bronchial hyperresponsiveness and hypersensitivity were tested in patients with clinical suspicion of the existence of asthma, in whom the disorder in pulmonary ventilation was not registered by spirometry test. Positive bronchial provocation test can confirm the diagnosis of bronchial asthma. Standardized method of bronchial challenge with

**Table 1**

**Characteristics of study participants**

| Parameters | Group I (n = 30) | Group II (n = 40) | *p* |
|---|---|---|---|
| | $\bar{x} \pm SD$ | $\bar{x} \pm SD$ | |
| Average age (years) | 19.9 ± 1.3 | 20.8 ± 1.6 | 0.0116 |
| Disease duration (months) | 11.1 ± 1.9 | 8.2 ± 2.2 | < 0.0001 |
| FVC, (L)* | 5.24 ± 0.64 (95.0)* | 5.36 ± 0.71 (96.8)* | 0.4616 |
| FEV1, (L)* | 4.36 ± 0.53 (88.2)* | 4.67 ± 0.71 (101.8)* | 0.0402 |
| FEV1/ FVC | 83.7 ± 6.4† (98.3)* | 86.7 ± 5.73† (104.5)* | 0.0470 |

**Group I – patients with high degree of hypersensitivity to histamine; group II – patients with negative histamine test; FVC – forced vital capacity; FEV1 – forced expiratory volume in one second; †Tiffeneau index – the ratio of FEV1/FVC, expressed as a percentage; *percentage of values obtained in relation to the standard values for this population of patients; $\bar{x}$ – arithmetic mean; SD – standard deviation.**

**Table 2**

**Skin tests with inhalant allergens**

| Allergens | Group I (n = 30) | Group II (n = 40) | *p* |
|---|---|---|---|
| *Dermatophagoides pterronissinus* | 25 (83.3) | 34 (85.0) | |
| Grass pollen | 16 (53.3) | 21 (52.0) | |
| House dust | 10 (33.3) | 20 (50.0) | |
| Weed pollen | 6 (20.0) | 11 (27.5) | 0.9542 |
| Feathers | 5 (16.7) | 5 (12.5) | |
| Tree pollen | 5 (16.7) | 6 (15.0) | |
| Linen | 1 (3.3) | 2 (5.0) | |

**Group I – patients with high degree of hypersensitivity to histamine; Group II – patients with negative histamine test.**

**Table 3**

**Nonspecific bronchial hyperreactivity test results in the study participants**

| Parameter | Group I | | | Group II | | | *p* |
|---|---|---|---|---|---|---|---|
| | Value | n | % | Value | n | % | |
| Reactivity degree | Minor (4 mg/mL ≤ $PC_{20}$ ≥ 7.9 mg/mL) | 30 | 100 | Insignificant ($PC_{20}$ ≥ 8 mg/mL) | 40 | 100 | - |
| Average fall of FEV1 (L) $\bar{x} \pm SD$ | 1.44 ± 0.34 | 30 | 27.2 ± 5.7 | 0.68 ± 0.26 | 32 | 12.48 ± 4.09 | < 0.0001 |
| $PC_{20}$, $\bar{x} \pm SD$ | 6.09 ± 1.1 | 30 | 100 | 14.58 ± 6.34 | 32 | 75.0 | < 0.0001 |

**Group I – patients with high degree of hypersensitivity to histamine; Group II – patients with negative histamine test; $PC_{20}$ – provocation concentration of histamine, which leads to a fall in forced expiratory volume in one second (FEV1) of 20%; $\bar{x}$ – arithmetic mean; SD – standard deviation.**

**Table 4**

**Specific bronchial provocation test results in the study participants**

| Parameters | Group I | | Group II | | *p* |
|---|---|---|---|---|---|
| | n (%) | ↓ FEV1 (%) | n (%) | ↓ FEV1 (%) | |
| Allergen | | | | | |
| *Dermatophagoides pterronissinus* | 24 (80.0) | 33.6 | 28 (70.0) | 30.8 | |
| Grass pollen | 4 (13.3) | 28.5 | 10 (25.0) | 34.2 | 0.7858 |
| House dust | 2 (6.6) | 33.0 | 2 (5.0) | 28.0 | |
| Total | 30 (100) | 32.9 ± 2.4* | 40 | 31.5 ± 2.2* | 0.0151 |

**Group I – patients with high degree of hypersensitivity to histamine; Group II – patients with negative histamine test; FEV1 – forced expiratory volume in one second; * arthitmetic mean ($\bar{x}$) ± standard deviation (SD).**

histamine determined the degree of hypersensitivity in all patients, and this served as a criterion for distribution of patients per groups: moderate and slight degree of hypersensitivity and negative histamine test [6, 8–14, 17]. In this study the analyzed groups were with the slight degree of hypersensitivity and negative histamine test.

The test results of skin sensitization to inhaled allergens showed no difference in the prevalence of allergens tested by groups of respondents. The intensity of skin reaction was used for the selection of allergens which will be used for specific bronchial provocation testing. In our study, the most common allergens were mites *Dermatophagoides pterronissinus*, grass pollen and house dust. In other studies, skin prick test with standard extracts including house dust mites, animal dander, molds, pollens etc. were also performed on patients according to the herbal geography of the local area. The common aeroallergens were house dust mites (88.5%), molds (82.9%), animal dander (79.5%), weeds (77.6%), trees (75.5%) and grass pollen (71.5%) [18]. Bazarbachi et al. [19] found to identify sensitized patients on the eleven allergens, Dermatophagoides pteronyssinus, *Dermatophagoides farinae*, *Blomia*, *Parietaria, grass*, *Salicaceae, oak*, *Oleaceae*, dog, cat, and cockroaches.

Cockroft et al. [20, 21] and Boulet et al. [22] studied 25 patients with asthma and examined the relationship between specific bronchial hypersensitivity, skin hypersensitivity and nonspecific bronchial hypersensitivity to histamine. They concluded that there was a positive correlation between specific hypersensitivity to inhaled allergens and nonspecific hypersensitivity to histamine.

In this study, the specific bronchial provocation test with solutions of different concentrations of allergens caused a significant response in all 70 patients. The analysis of FEV1 decline achieved during the test did not find significant differences between the first and the second group. By analyzing the distribution of the concentration of allergens, the existence of significant differences between the groups with different levels of nonspecific bronchial hypersensitivity was not established.

Examining the effect of inhalation of repeated low doses of allergens in bronchial asthmatics with atopy in 1998, Sulakvelidze et al. [23] reported that despite a lack of significant response to bronchial allergen (FEV1 decline to 5%) in induced sputum after inhalation of allergens, a significant increase in eosinophil number was recorded, as well as the increase in the level of eosinophilic cationic protein (ECP) and an increase in levels of cytokines (interleukins IL-5).

In addition to the studies that presented the results of experiments, in 1996 Djukanovic et al. [24] published the results of the study in which they carried out cytological and histological analysis of inflammatory response development in the bronchus before and after natural exposure of allergic patients with bronchial asthma to grass pollen. They confirmed, as well as some other authors, that exposure to an allergen leads to the induction of inflammation in bronchus with the engagement of T lymphocytes, mast cells and eosinophilic leukocytes. In natural conditions, seasonal allergen exposure of sensitized persons will lead to enhanced secretion of proinflammatory cytokine IL-4 and the mobilization and activation of T lymphocytes and eosinophils [25].

## Conclusion

Presented prospective study did not demonstrate existance of a compulsory direct relationship between the degree of specific and non-specific bronchial hyperreactivity in patients with allergic asthma.

Testing specific bronchial hyperreactivity, as a simulation of natural processes, should be performed in selected patients with suspected allergic asthma.

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