

Dimitrievska D.¹, Zdraveska M.¹, Gjorcev A.¹, Todevski D.¹, Janeva E.¹, Vlaski E.²:
Correlation of Total Eosinophils in peripheral blood with bronchoprovocative testing with acetylcholine in patients with bronhchial asthma.

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SUMMARY

The main attributes of bronchial asthma are airway obstruction, spontaneous or treatment induced reversibility, increased bronchial reactivity to exogenous and endogenous stimuli (triggers) and inflammation of the tracheobronchial tree. Since the basic characteristic of asthma is airway hyperreactivity, only proving the existence of airway reactivity can make valid diagnosis. Two methods for airway hyperreactivity detection were used in this study, Eo count in peripheral blood and PC₂₀ to acetylcholine bronchoprovocation. We estimated their corelation.

This study includes 30 patients of the Pulmology and Allergy Clinic, Skopje, with confirmed bronchial asthma. In all of the patients we followed Eo count in peripheral blood and PC₂₀ to acetylcholine bronchoprovocation at the beginning of the study, after 2 and 6 months. The results show statistically significant corelation ($p < 0.05$) between Eo count in peripheral blood and PC₂₀ to acetylcholine.

Our conclusion is that each of them may be used in the diagnosis of asthma, alone or in combination. The total Eo count in peripheral blood can be used as a screening method in the asthma management and as a diagnostic parameter for asthma, especially in cases when bronchoprovocative tests can not be performed.

Key words: bronchial asthma, diagnosis, Eo count, PC₂₀ to acetylcholine.



CORRELATION OF TOTAL EOSINOPHILS IN PERIPHERAL BLOOD WITH BRONCHOPROVOCATIVE TESTING WITH ACETYLCHOLINE IN PATIENTS WITH BRONCHIAL ASTHMA

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INTRODUCTION

Asthma is a chronic inflammatory disorder of the airways in which many cells play a role, in particular mast cells, eosinophils (Eo), and T lymphocytes. In susceptible individuals this inflammation causes recurrent episodes of wheezing, breathlessness, chest tightness, and cough particularly at night and/or early morning. These symptoms are usually associated with widespread but variable airflow limitation that is at least partly reversible either spontaneously or with treatment (1).

Asthma is a disease of the developed world. With the industrial development and urbanisation the number of patients with bronchial asthma is constantly rising. The disease affects approximately 3.2% of the population in Republic of Macedonia (2). In order to estimate the prevalence of bronchial asthma (BA) we have to consider the frequent subdiagnosis of the disease, which is due to non-uniform diagnostic criteria (3;4).

The main feature of bronchial asthma is bronchial hyperreactivity (BHR), which is used for confirmation of the diagnosis of the disease. Bronchoprovocative testing (specific and non-specific) is still widely used as the most accurate method for proving the presence of BHR (5). Still, the diagnostic procedure does not always allow the application of bronchoprovocative tests for discovering the BHR. In some of the patients it is not possible to achieve remission of the disease with normal, or close to normal ventilatory function, which is one of the important conditions for performing the bronchoprovocative testing. In everyday practice, the problem of false negative bronchoprovocative test is also common, especially in the early stage of the disease. Achieving accurate diagnosis in these patients must be done with undirect methods (6).

Inflammation is the direct cause for bronchial hyperreactivity in asthma (7; 8). Many cells and mediators play a role, but eosinophils take the main place in asthmatic inflammation. Increased number and activity of the eosinophils in peripheral blood, bronchial mucosa and bronchoalveolar lavage (BAL) is a valid marker for inflammation and for the bronchial hyperreactivity. The most sensitive is detection of eosinophils in airway mucosa and BAL, but the invasive diagnostic investigation by bronchoscopy is necessary. Although, less sensitive, detection of eosinophils in peripheral blood and estimation of serum ECP and MBP, is possible. This method is not invasive, and it provides valid parameters for the severity of the inflammation (9; 10; 11). Increased total number of peripheral blood's eosinophils is present in 35-39% of patients with newly diagnosed asthma and in 44-76% with chronic asthma. Other studies publish increased number of eosinophils in peripheral blood in 70% of patients with positive bronchoprovocative test. If



the serum level of ECP, MBP and the other markers of eosinophil's activity, we examined together, they can be found positive in 100% of patients with positive bronchoprovocative test (12). Bousquet publishes that eosinophils can be markers of inflammation and a valid parameter for disease severity (13).

AIM OF THE STUDY

The aim of this study is to estimate (show) the correlation of eosinophils in peripheral blood with bronchoprovocative testing with acetylcholine and the possibilities for their application in the diagnosis of bronchial asthma.

MATERIAL AND METHODS

The study includes patients of the Pulmology and Allergy Clinic, Skopje, with confirmed bronchial asthma. The diagnosis was made according to the recommendations of the International consensus for diagnosis and treatment of bronchial asthma (14). We evaluated 30 asthmatics, 21(70%) females and 9 (30%) males, 22 to 61 years old. The average age was 42.20 ± 9.94 years. The following parameters were measured in all of the patients, at the beginning of the study, after 2 and after 6 months of the study:

- Bronchoprovocative test with acetylcholine, by the standard method of the Department of functional diagnosis at the Pulmology and Allergy Clinic. We calculated the PC_{20} for acetylcholine (dose of acetylcholine that provokes a decrease of FEV_1 for more than 20%).

- Total number of eosinophils in peripheral blood by the standard method of the Institute for clinical biochemistry.

Standard statistical methods (average, standard deviation and correlation) were used in the calculation of the data.

RESULTS

In 22(73.30%) of patients we found an increased number of total eosinophils in peripheral blood. At the beginning of the study, the eosinophils number was 90 - $480 \times 10^6/l$ (averages $276 \pm 60.90 \times 10^6/l$). After 2 months the number of eosinophils was 120 - $420 \times 10^6/l$ ($254 \pm 51.20 \times 10^6/l$), compared to 80- $380 \times 10^6/l$ ($219.67 \pm 47.51 \times 10^6/l$) at the sixth month of the study.

In all of the patients we found positive bronchoprovocative acetylcholine test. PC_{20} to acetylcholine was in the range from 1000 to 5000 γ (average of 4033 ± 873.12) at the beginning of the study, 2000 - 5000 (4166.67 ± 806.45) after two months and from 4000 to 5500 ($4933.33 \pm 361, 29$) at the end of the sixth month of the study.



Table 1 and Diagram 1 present the values of the eosinophil number in peripheral blood and the PC₂₀ of acetylcholine.

	Eo	PC ₂₀	R
0 period	276	4033	-0.027
2 months	254	4166.67	-0.01
6 months	219.67	4933.33	-0.02

Table 1
Eo- total number of eosinophils in peripheral blood; PC₂₀ - PC₂₀ to acetilcholine;
R - quote of correlation

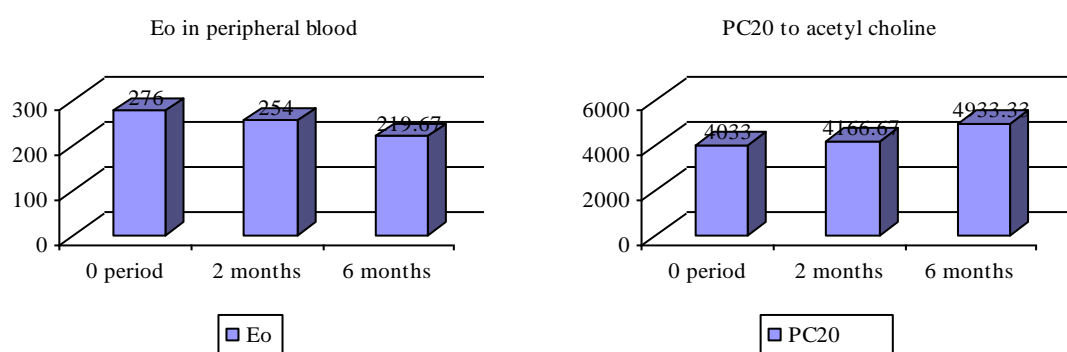


Diagram 1

The statistical analysis of the data showed a statistically significant ($p < 0.05$) negative correlation between the total number of eosinophils in peripheral blood and PC₂₀ of acetyl choline.

DISCUSSION

Not one of the diagnostic tests, currently used, is 100% sensitive for establishing the diagnosis of asthma, especially not in the early stage of the disease (11). The sensitivity increases with the combination of two positive parameters (15;16). The most sensitive method for proving the bronchial hyperreactivity is the bronchoprovocative tests. They have been applied for over 20 years in the routine clinical practice (5). Positive bronchoprovocative test means almost certain asthma in most of the cases. Still positive provocative tests have also been found in the healthy population (5; 15; 17; 18). At the beginning of the disease, the airway provocation frequently shows negative results.

Another parameter which can detect the airway hyperresponsiveness, although indirectly, is the number of eosinophils in airway mucosa, BAL and peripheral blood. For detection of eosinophils in bronchial mucosa and BAL bronchoscopy is necessary. Estimation of eosinophils in peripheral blood is a simple and noninvasive method and there



is positive correlation between eosinophils in peripheral blood and eosinophils in BAL (12).

High quote of correlation (up to $R=0.70$) between the number and activity of eosinophils and the bronchial hyperreactivity was shown by many authors (9; 11; 12; 19).

Our study showed statistically significant correlation between the total number of eosinophils in peripheral blood and PC20 to acetylcholine at all three measurement periods. The quote of correlation was $R=0.27$ for the first measurement, $R=0.10$ at 2 months, and $R=0.20$ at the end of the study (after 6 months). The quote of correlation calculated in our results is comparable to the results shown in other studies (11; 12; 13). The practical value of the positive correlation between the two diagnostic methods lies in the possibility to use them separately or in combination for the diagnosis of asthma. We emphasize that it is not an imperative to carry out a bronchoprovocative test to establish the diagnosis of asthma in a patient with an indicative history of the disease, especially if all of the criteria for performing the test are not, or can not be met. Detection and following the number and activity of eosinophils with detailed history of the disease, characteristic clinical presentation and other markers of inflammation can point to a diagnosis of bronchial asthma.

CONCLUSION

The results of our study show a statistically significant correlation between the total number of eosinophils in the peripheral blood and PC20 to acetylcholine. The number and activity of eosinophils can be used as a valid marker in asthma management and as a diagnostic parameter for asthma, especially in cases when bronchoprovocative tests can not be performed.

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