European Review for Medical and Pharmacological Sciences 2024; 28: 1060-1065

Is bronchial provocation test positivity associated with blood eosinophil count and cut-off value?

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Abstract. – OBJECTIVE: Asthma is characterized by airway hyperresponsiveness due to chronic inflammation in the airways. One of the main cells involved in airway inflammation is eosinophils. In the current study, a bronchial provocation test (BPT) was performed to demonstrate airway hyperresponsiveness. We investigated the relationship between BPT and blood eosinophil count and the cut-off value of blood eosinophil count.

PATIENTS AND METHODS: In this study, we retrospectively evaluated the data of 246 patients who visited our immunology and allergy clinic, a tertiary reference center, with asthma symptoms between May 2017 and March 2020 and underwent BPT with methacholine for the diagnosis of asthma. The cases were grouped according to the level of BPT positivity and negativity.

RESULTS: Of 246 patients, BPT was positive in 90 (36.6%) and negative in 156 (63.4%). The blood eosinophil measurement of the BPT-positive cases was found to be statistically significantly higher than that of the BPT-negative cases (135 *vs*. 119 cells/μl, respectively, p=0.029). When BPT is grouped according to positivity levels, there was no statistically significant difference in blood eosinophil measurements between subgroups (p=0.174). As a result of the evaluations, the cut-off point obtained for the blood eosinophil count was determined as ≥226 cells/μl. For the blood eosinophil count, for the cut-off value of ≥226 cells/μl, sensitivity was 30.0%, specificity 87.7%, positive predictive value 58.7%, and negative predictive value 68.3%.

CONCLUSIONS: This study shows that BPT positivity is associated with blood eosinophil count. The cut-off value (≥226 cells/μl) determined for blood eosinophil count may be helpful when planning BPT and evaluating the diagnosis of asthma.

Key Words:
Asthma diagnosis, Bronchial provocation test, Eosinophil, Inflammation.

Introduction

Asthma is a chronic inflammatory disease of the airways. It can cause symptoms of varying intensity and frequency, such as chest pressure, coughing, and wheezing. The diagnosis of asthma in symptomatic patients is based on the demonstration of expiratory airflow limitation. Early and late reversibility tests, peak expiratory flow variability, positive exercise tests, and inter-visit spirometry variability can be used to demonstrate expiratory airflow limitation. In the pathogenesis of asthma, there is airway hyperresponsiveness (AHR) that develops due to chronic inflammation. The test used to demonstrate AHR is the bronchial provocation test (BPT)[1]. BPT can be performed using methacholine, histamine, exercise, eucapnic voluntary hyperventilation, and inhaled mannitol[2,3]. In recent years, inflammation in the airways in asthma has been classified as type 2-high and type 2-low. Eosinophils are one of the main cells involved in type 2-high inflammation[4].

There are few studies[5] in the literature showing the relationship between AHR and blood eosinophil count in patients who are steroid-naive and do not receive asthma treatment. Blood eosinophil count is used as a biomarker when determining treatment options and treatment response, especially in severe eosinophilic asthma[6]. In addition, treatment guidance is recommended according to the sputum eosinophil count[1]. However, a blood eosinophil cut-off value related to BPT has not been determined for the diagnosis of asthma.

In this study, we sought to determine the relationship between BPT and the blood eosinophil count and cut-off value in patients with asthma symptoms in a defined population.

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Patients and Methods

We retrospectively reviewed the outpatients who presented with asthma symptoms between May 2017 and March 2020 and underwent non-specific BPT with methacholine to confirm the diagnosis of asthma. Inclusion criteria for the study were as follows: being 18 years and over, having a hemogram taken simultaneously with BPT, being a nonsmoker, and not being previously diagnosed or treated for asthma. American Thoracic Society criteria were used in the application and evaluation of BPT[3]. Demographic data of the patients, BPT results, provocative concentration [methacholine concentration causing 20% decrease in the first second of forced expiration volume (FEV1) level; PC20] values, blood eosinophil counts (cells/µl), and atopy status were recorded. In the evaluation, the patients were divided into four groups according to PC20: Group I, PC20 positive with methacholine concentration of "<1 mg/ml"; Group II, PC20 positive with methacholine concentration between "1 - <4 mg/ml"; Group III, PC20 positive with methacholine concentration between "4 - <16 mg/ml"; Group IV, negative with methacholine concentration of "≥16 mg/ml". In addition, data from the positive groups (Group I+ Group II+ Group III) and the negative group (Group IV) were compared. The study was accomplished according to the guidelines of the Helsinki Declaration and approved by the Clinical Research Ethics Committee of Kartal Dr Lutfi Kırdar City Hospital, Istanbul, Turkey (approval number: 2020/514/181/10-08.07.2020).

Statistical Analysis

Statistical analyses were performed using SPSS version 25.0 (IBM Corp., Armonk, NY, USA). Descriptive statistical methods (mean, standard deviation, median, percentage, minimum, maximum) were used when evaluating study data. The conformity of the quantitative data to the normal distribution was tested with the Shapiro-Wilk test and graphical examinations. The Mann-Whitney U test was used for comparisons between two groups of quantitative variables that did not show normal distribution. The Kruskal-Wallis test was used for comparisons between groups of more than two non-normally distributed quantitative variables. ANOVA test was used for the comparison of normally distributed quantitative variables between more than two groups. Pearson's Chi-square test was used to compare qualitative data. Diagnostic screening tests (sensitivity, specificity, positive predictive value, negative predictive value) and receiver operating curve (ROC) analysis were used to determine the predictive value for the parameters. Statistical significance was accepted as $p<0.05$.

Results

Among the 455 screened patients, data from 246 patients who met the inclusion criteria, of whom 170 (69.1%) were females and 76 (30.9%) males, were evaluated. There was no statistical difference between the study groups in terms of gender, age distribution, or presence of atopy (Table I).

The blood eosinophil count of the BPT-positive group was found to be statistically significantly higher than the BPT-negative group (135 *vs.* 119 cells/µl, respectively, $p=0.029$). BPT results were grouped according to PC20 levels; no statistically significant difference was found between subgroups in blood eosinophil of the groups ($p=0.174$) (Table II).

As a result of the evaluations, the cut-off point obtained for the blood eosinophil count was determined as ≥226 cells/µl. For the blood eosinophil count cut-off value of ≥226 cells/µl, sensitivity was 30.0%, specificity 87.7%, positive predictive value 58.7%, and negative predictive value 68.3%. The area under the ROC obtained for the blood eosinophil count was determined as 58.3% with a standard error of 3.8% (Table III, Figure 1).

Discussion

In our study, the blood eosinophil count was statistically significantly higher in the BPT-positive group ($p=0.029$). However, there was no significant difference between the groups in terms of blood eosinophil counts according to BPT positivity levels. As another important result in our study, we found the blood eosinophil count cut-off point for BPT positivity as ≥226 cells/µl (sensitivity 30.0%, specificity 87.7%, positive predictive value 58.7%, negative predictive value 68.3%).

In a study[5] of asymptomatic individuals, when blood eosinophil counts were compared between BPT-positive and BPT-negative groups [0.46±0.21 *vs.* 0.21±0.01 K/µl (mean ± SD)], a statistically significant difference was found, which is similar to our study ($p<0.002$). The patients in our study had symptoms suggestive of asthma. There was no relationship between BPT

Table I. Evaluation of the groups in terms of number of patients, gender, age, and presence of atopy.

		BPT Positive n (%)			BPT Negative n (%)		
		Group I	Group II	Group III	Group IV	Total, n (%)	*p*-value
Gender	Female	9 (3.6)	18 (7.3)	40 (16.3)	103 (41.9)	170 (69.1)	0.42†
	Male	4 (1.6)	8 (3.3)	11 (4.5)	53 (21.5)	76 (30.9)	
Atopy	Yes	6 (2.4)	14 (5.7)	17 (6.9)	58 (23.6)	95 (38.6)	0.30†
presence	No	7 (2.9)	12 (4.9)	34 (13.8)	98 (39.8)	151 (61.4)	
Age, year ± SD (median)		41.4±16.4 (39.0)	40.4±16.0 (36.0)	39.8±14.0 (39.0)	40.7±12.1 (40.0)	40.5±13.1 (39.0)	0.97‡
Total, n (%)		13 (5.3)	26 (10.6)	51 (20.7)	156 (63.4)	246 (100)	

†Chi-square test, ‡ANOVA test; BPT: Bronchial Provocation Test, SD: Standard Deviation.

Table II. Relationship between blood eosinophil counts and BPT results.

		Blood eosinophil count, cell/µl		
		Median (min-max)	Mean ± SD	*p*-value
BPT	Negative (n=156)	119 (0-670)	142±113	**0.029**†
	Positive (n=90)	135 (20-952)	185±157	
BPT subgroups by PC20 level	Group I (n=13)	137 (75-620)	196±154	0.174‡
	Group II (n=26)	144 (25-730)	199±173	
	Group III (n=51)	127 (20-952)	166±164	
	Group IV (n=156)	119 (0-670)	142±113	

†Mann-Whitney U test, ‡Kruskal-Wallis test; BPT: Bronchial Provocation Test, SD: Standard Deviation, min-max: minimum-maximum.

Table III. Diagnostic screening tests for blood eosinophil count according to BPT positivity and ROC results.

	Cut-off	Area Under Curve (95% Confidence Interval)	Sensitivity (%)	Specificity (%)	Positive Predictive Value (%)	Negative Predictive Value (%)	*p*-value
Blood eosinophil count, cell/µl	**226**	**0.583** (0.509-0.658)	30.0	87.7	58.7	68.3	**0.029**

BPT: bronchial provocation test, ROC: receiver operating curve.

positivity levels and blood eosinophil counts in either the mentioned study or our study.

In a meta-analysis[7], the relationship between sputum eosinophilia in induced sputum and peripheral blood eosinophil count was examined. Twelve studies involving 1,967 adult patients with asthma or suspected asthma were evaluated for the relationship between sputum eosinophilia and blood eosinophilia. In these studies, blood eosinophil counts were in the 220-320 cells/µl range, sensitivity was 0.71 (CI: 0.65 - 0.76), and specificity was 0.77 (0.70 - 0.83) when the cut-off value for sputum eosinophilia was ≥3%. In the meta-analysis, in six studies with sputum eosinophilia ≥2% as a reference, 1,180 adult patients were evaluated, and blood eosinophil counts were reported to be in the range of 210-415 cells/µl, sensitivity 0.66 (CI: 0.56 - 0.75), and specificity 0.83 (CI: 0.66 - 0.94). In this meta-analysis[7], when sputum eosinophilia ≥3% is taken as a reference, it was concluded that the peripheral blood eosinophil count may be moderately diagnostic in demonstrating eosinophilic inflammation in asthma. Most of the patients in these studies were using inhaled steroids and were receiving asthma treatment. The studies were conducted to determine the asthma endotype. The patients in our study had never been treated for asthma and were steroid-naive. We can say that the cut-off value of ≥226 cells/µl for the blood eosinophil count we

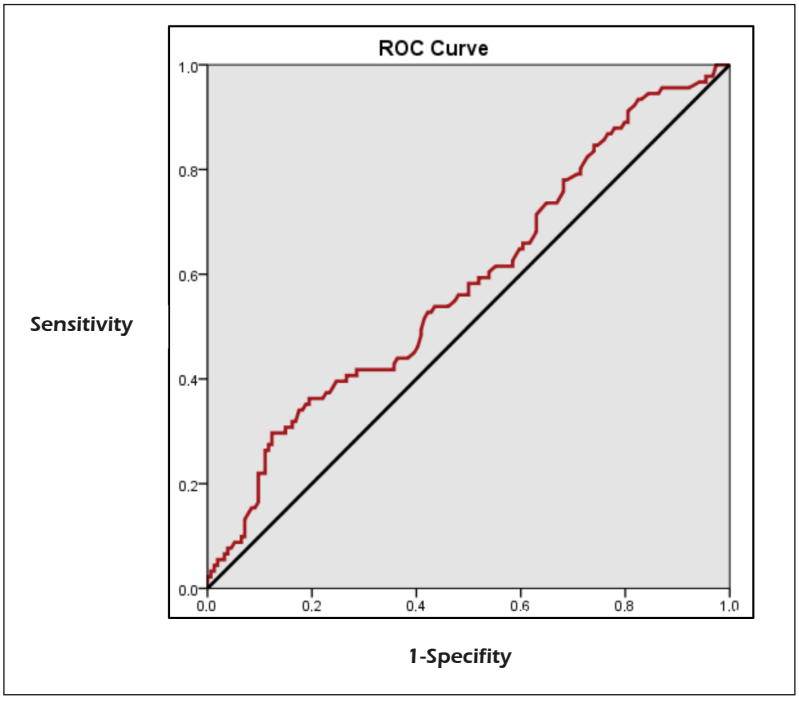

Figure 1. Receiver operating curve (ROC) for blood eosinophil count by the presence of bronchial hyperreactivity.

found is within the range of values in this meta-analysis. However, the fact that our patients are steroid-naive makes our study different.

In another study[8] evaluating BPT positivity and blood eosinophil counts, the sensitivity was found to be 80.0%, specificity 61.0%, positive predictive value 52.5%, and negative predictive value 85.7% when the cut-off value was 150 cells/µl. The value we found was a higher blood eosinophil count with 226 cells/µl. However, compared to the number of patients evaluated in our study (246), only 51 patients were evaluated in the mentioned study[8].

Allergic asthma due to aero-allergen sensitivity is the most common asthma phenotype[9]. Previous studies[10,11] have shown that the late-phase asthmatic response that develops with allergen exposure is associated with blood eosinophil counts. However, in our study, no relationship was found between BPT positivity and the presence of atopy. The reason for this may be that asthma is a heterogeneous disease and may occur with different pathogenetic mechanisms[3]. In addition, nonspecific bronchial hyperresponsiveness (BHR) was evaluated with methacholine in our study. We think that the results may be different if BPT is performed with the responsible aeroallergen in atopic patients with asthma symptoms.

At the same time, eosinophils are likely to contribute to asthma exacerbations[12,13]. Studies[14] support that elevated blood eosinophil count is associated with increased severe asthma attacks. Although we found high blood eosinophil counts in BPT-positive patients, we did not find a statistical difference between the groups according to BPT positivity levels (Table II). This suggested that there may not be a relationship like asthma exacerbations between BPT positivity level and blood eosinophil counts.

In our study, one-time blood eosinophil counts measured on the same day as BPT were used. However, in a study[15] in which patients with eosinophilic severe asthma were treated, it was shown that the blood eosinophil counts in the placebo group could increase over time and exceed 400 cells/µl. Studies[16] have also shown that the blood eosinophil count decreases after meals and exercise, and the diurnal rhythm of the eosinophil count peaks at midnight and drops to its lowest level in the middle of the day. In a cohort study[17], blood eosinophil counts, and variability were found to be significantly higher in asthmatic patients than in the normal population. The use of a single blood eosinophil measurement is one of the limitations of our study due to this variability. However, we think that simultaneous monitoring of blood eosinophil counts with BPT increases the value of our study.

In newly published studies[18,19], blood eosinophil subtypes in asthmatics were different from those of chronic obstructive pulmonary

disease patients, smokers, and healthy people. Eosinophils were studied in two subtypes: resident eosinophils (rEos; Siglec-8+ CD62L+ IL-3Rlo) and inflammatory eosinophils (iEos; Siglec-8+ CD62Lo IL-3Rhi). It has been found that the rate of iEos in blood eosinophils is higher in asthmatics than in other groups. In another study20, the iEos rate was found to be statistically higher in patients with mild eosinophilic asthma than in those with severe non-eosinophilic asthma. This suggests that the distribution of subtypes rather than the number of blood eosinophils may be more determinant in asthma and bronchial hyperreactivity.

Conclusions

BPT is a relatively difficult, inaccessible, and time-consuming test. A hemogram is an easily accessible, inexpensive, and simple test that shows blood eosinophils. Therefore, when selecting patients for BPT among patients with asthma symptoms, patients with a blood eosinophil count above 226 cells/μl can be given priority. In addition, it can be considered that a blood eosinophil count of ≥226 cells/μl may be a guide to support the clinical diagnosis of asthma in patients with contraindications for BPT. For the diagnosis of asthma, studies with blood eosinophil counts, as well as eosinophil subtypes, may be more helpful in the future. In order to use a cut-off point of blood eosinophil for bronchial provocation test, more studies should be performed in different populations.

Conflict of Interest
The authors declare that they have no conflict of interest.

Ethics Approval
The study was accomplished according to the guidelines of the Helsinki Declaration and verified by the Clinical Research Ethics Committee of Kartal Dr Lutfi Kırdar City Hospital, Istanbul, Turkey (approval number: 2020/514/181/10-08.07.2020).

Informed Consent
Written informed consent was obtained from all participants.

Funding
The authors report there is no funding associated with the work featured in this article.

Authors' Contributions
All the authors have made substantial contributions to the conception and design of the study, data acquisition, data analysis and interpretation, drafting of the article or critically revising it for important intellectual content, and final approval of the version to be submitted.

Data Availability
The data that support the findings of this study are available on request from the corresponding author.

Acknowledgments
Authors would like to thank Protranslate for the English editing.

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