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Relationship between bronchial hyperreactivity and complete blood count derived inflammatory biomarkers

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ABSTRACT

Aims: Studies specifically linking bronchoprovocation test (BPT) positivity with complete blood count (CBC)-derived biomarkers are limited. We investigated the relationship between CBC-derived biomarkers and BPT positivity in this study.

Methods: We retrospectively evaluated patients who underwent methacholine BPT and simultaneous CBC from May 2017 to March 2020. Non-smoker patients without a prior diagnosis or treatment of asthma were selected. We investigated the relationship between bronchial hyperreactivity and CBC parameters white blood cell (WBC), neutrophil (NEU), lymphocyte (LYM), eosinophil (EOS), platelet counts (PLTs), mean platelet volume (MPV), neutrophil-to-lymphocyte ratio (NLR), eosinophil-to-lymphocyte ratio (ELR), platelet-tolymphocyte ratio (PLR), and the systemic immune-inflammation index (SII).

Results: The study population consisted of 246 adult patients (69.1% female, mean age 40.5±13.1 years) evaluated for asthma-like symptoms. The BPT was negative in 156 (63.4%) patients and positive in 90 patients (36.6%). A relationship was found between EOS count and BPT positivity [135 (interquartile range (IQR) 167) vs 119 (IQR: 99 p=0.04)]. However, no statistically significant relationship was found between BPT positivity and other parameters, WBC, NEU, LYM, PLT, MPV, NLR, ELR, PLR, and the SII.

Conclusions: We identified an association between BPT positivity and EOS count among CBC parameters. However, we found no relationship between BPT positivity and WBC, NEU, LYM, EOS, PLT, MPV, and CBC-derived inflammatory biomarkers such as NLR, ELR, PLR, and SII. In asthmatic patients, a relationship between asthma and CBCderived biomarkers may emerge after inflammation persists for a period of time.

Introduction

Chronic inflammation is the basis of asthma pathogenesis (1). This inflammatory process contributes to bronchial hyperreactivity (BHR), which leads to recurrent symptoms and signs of airway obstruction. One of the diagnostic tools used to demonstrate BHR is the bronchial provocation test (BPT). Non-specific bronchial provocation using methacholine is performed as supporting evidence in the diagnosis of asthma (2). Nevertheless, its routine clinical use remains restricted.

The complete blood count (CBC) represents a basic and universally performed laboratory assessment, available in nearly all clinical settings. Parameters obtained from the CBC can provide insights into systemic inflammation and assist in identifying inflammatory phenotypes of asthma. The link between CBC parameters and diverse inflammatory subtypes of asthma has been assessed in numerous studies (3,4). Moreover, a number of older and recent studies have explored the relevance of hemogram parameters in BHR and asthma (5,6). In recent years, data indicating systemic inflammation in asthma have accumulated increasingly (7). The systemic immune-inflammation index (SII) has been studied as a marker in both inflammatory conditions and malignancies (8-10). However, current evidence exploring the association between SII and BHR remains limited.

In the present research, we sought to explore the relationship between BHR and CBC-derived parameters, including white blood cell (WBC) count, neutrophil (NEU), lymphocyte (LYM), eosinophil (EOS), and platelet (PLT) counts; mean platelet volume (MPV); neutrophil-to-lymphocyte ratio (NLR); eosinophil-to-lymphocyte ratio (ELR); platelet-to-lymphocyte ratio (PLR); and the SII, calculated as (NEUs×PLTs)/LYMs.

Methods

Study design and participants

This study retrospectively enrolled patients presenting to the outpatient department with asthma-like symptoms who underwent non-specific BPT with methacholine for asthma diagnosis between May 2017 and March 2020. Inclusion criteria were age ≥18 years, non-smoker status, no prior diagnosis or treatment for asthma, and availability of CBC results obtained concurrently with the BPT. Exclusion criteria were age <18, smoking, former smoker, diagnosis of asthma, and lack of a CBC simultaneously with BPT. CBC analyses were performed using the Mindray BC-6000 automated hematology analyzer (Mindray Bio-Medical Electronica Co., Ltd., Shenzhen, China). The ethics committee of the University of Health Sciences Türkiye, Sancaktepe Şehit Prof. Dr. İlhan Varank Training and Research Hospital Institution provided formal approval for this research (decision no.: 244, date: 13.12.2023).

Data collection

Data collected included demographic characteristics, BPT results, provocative concentration 20 (PC20) values, atopy status, and CBC parameters (WBC, NEU, LYM, EOS, PLT, MPV, NLR, ELR, PLR, and SII). The study population was divided into four categories based on PC20 values: Group 1: PC20 <1 mg/mL. Group 2: PC20 1–<4 mg/mL. Group 3: PC20 4–<16 mg/mL. Group 4: Negative BPT result (PC20 ≥16 mg/mL). Additionally, comparisons were made between patients with a positive BPT (Groups 1-3) and those with a negative result (Group 4).

Bronchial provocation test

One approach to assess airway reactivity is the application of methacholine challenge testing. Methacholine bronchial challenge tests were performed and interpreted following the recommendations of the American Thoracic Society. Five methacholine chloride concentrations (0.0625, 0.25, 1, 4, and 16 mg/mL) were prepared in sterile vials using normal saline as diluent, placed in a labeled holder, and stored at 2-8 °C until use. The solutions were administered in quadrupling concentrations (every other dilution) according to the standard bronchial challenge protocol (11). Baseline spirometry was performed, and the target forced expiratory volume in one second (FEV_1) was calculated, which indicates a 20% fall in FEV_1 [baseline (or diluent) FEV_1 x 0.8].

Outcomes

The primary outcome is to evaluate the relationship between BPT and CBC-derived inflammatory markers. The secondary outcome is to evaluate the difference between groups, according to the degree of BPT positivity.

Statistical Analysis

All statistical analyses were carried out with IBM SPSS Statistics for Windows, version 25 (IBM Corp., Armonk, NY, USA). Data were summarized using descriptive measures including mean, standard deviation, median, interquartile range (IQR), number and percentages. The Shapiro-Wilk test and graphical methods were applied to assess the normality of distribution for quantitative variables. When the variables did not follow a normal distribution, two-group comparisons were performed using the Mann-Whitney U test, whereas comparisons among more than two groups were carried out with the Kruskal-Wallis test. One-Way Analysis of Variance was applied for normally distributed quantitative variables involving more than two groups. Categorical variables were examined using the Pearson chi-square test, with statistical significance defined as $p<0.05$.

Results

This investigation involved 246 adult subjects as the study population. Participants (69.1% female, mean age 40.5±13.1 years) were evaluated for asthma-like symptoms. BPT positivity was identified in 36.6% of the cases. In the BPT-negative group, the patients' mean age was 40.7±12.1 years, whereas in the BPT-positive group it was 40.2±14.8 years.

No statistically significant differences were observed between the BPT-positive and BPT-negative groups with respect to age, sex, atopy, or body mass index. Median total immunoglobulin E (IgE) levels were significantly elevated in the BPT-positive group (52.7 IU/mL) compared with the BPT-negative group (25.0 IU/mL) (p=0.027). However, no significant association was found between the extent of BPT positivity and total IgE levels (p=0.13) (Table 1).

No statistically significant relationship was observed between BPT positivity and CBC-derived parameters such as WBC count, NEU count, LYM count, PLT count, MPV, NLR, ELR, PLR, or SII. Peripheral blood EOS counts were significantly greater in the BPT-positive group (median 135 cells/μL; IQR 167) than in the BPT-negative group (median 119 cells/μL; IQR 99) (p=0.040). A comprehensive summary of the results is provided in Table 2. When evaluating the four PC20-based groups (Group I, Group II, Group III, and Group IV), no statistically significant differences were observed in parameters such as WBC, NEU, LYM, or PLT counts; MPV; NLR; ELR; PLR; SII; or EOS counts among the subgroups.

Discussion

This investigation revealed that peripheral EOS levels were markedly higher among patients with positive BPT results, suggesting a potential link between EOS inflammation and BHR. Although leukocyte counts appeared elevated in BPT-positive individuals, this difference did not reach statistical significance. These findings highlight EOS count as the most sensitive CBC parameter in distinguishing patients with BPT positivity.

Annesi et al. (5) examined the association between WBC count and BHR using methacholine in a population-based sample of 324 men. They demonstrated that elevated WBC counts (≥8000 cells/mm³) were associated with BPT positivity; however, the study included smokers and male patients only (5). According to another report, impaired lung function was associated with increased WBC counts in a negative direction (12). In contrast, our study included both male and female non-smokers and found no statistically significant relationship between WBC count and either BPT positivity or degree of BHR. The discrepancy may stem from differences in study populations regarding smoking status.

In our study, LYM counts did not show a statistically significant association with BPT positivity or the severity of BHR, aligning with findings from a previous study that also reported no such relationship (13). However, some studies have indicated that LYM percentages may be significantly reduced in patients with asthma (14).

Table 1. Comparison of demographic characteristics, BMI, and total IgE levels between BPT-positive and BPT-negative groups

		BPT positive			BPT negative	Total	p-value
		Group I	Group II	Group III	Group IV		
		13 (5.3)	26 (10.6)	51 (20.7)			
		90 (36.6)			156 (63.4)	246 (100)	
Gender	Female, n (%)	9 (3.6)	18 (7.3)	40 (16.3)	103 (41.9)	170 (69.1)	0.42[a]
		67 (27.2)					
	Male, n (%)	4 (1.6)	8 (3.3)	11 (4.5)	53 (21.5)	76 (30.9)	
		23 (9.4)					
Atopy presence	Yes, n (%)	6 (2.4)	14 (5.7)	17 (6.9)	58 (23.6)	95 (38.6)	0.30[a]
		37 (41.1)					
	No, n (%)	7 (2.8)	12 (4.9)	34 (13.8)	98 (39.9)	151 (61.4)	
		53 (21.5)					
Age, years, mean ± SD		41.4±16.4	40.4±16.0	39.8±14.0	40.7±12.1	40.5±13.1	0.97[b]
		40.2±14.8					0.80[c]
Body mass index, median, (IQR)		31.0 (9.5)	25.45 (9.8)	26.8 (7.8)	27.6 (7.25)	27.2 (7.55)	0.25[d]
		26.6 (8.02)					0.75[e]
Total IgE (IU/mL), median, (IQR)		51.3 (65.9)	134.6 (190.59)	36.7 (74.15)	25.0 (60.98)	30.9 (72.14)	0.13[d]
		52.7 (152.01)					0.027[e]

[a]Pearson chi-square test, [b]One-Way ANOVA test, [c]Independent sample t test, [d]Kruskal-Wallis, eMann-Whitney U test
BMI: Body mass index, IgE: Immunoglobulin E, BPT: Bronchoprovocation test, SD: Standard deviation, IQR: Interquartile range

Table 2. Comparison of CBC parameters and CBC-derived inflammatory biomarker findings of BPT-positive and BPT-negative groups

	PC20-16 (2 Groups)	Mean	SD	Median	IQR	p-value
Leukocyte (x10^9/L)	Negative	7.36	2.32	6.95	2.70	0.454[a]
	Positive	7.41	1.89	7.10	2.33	
Neutrophil (x10^9/L)	Negative	4.54	2.04	4.20	2.10	0.749[a]
	Positive	4.43	1.51	4.20	1.13	
Lymphocyte (x10^9/L)	Negative	2.12	0.57	2.10	0.80	0.068[a]
	Positive	2.24	0.53	2.30	0.83	
Eosinophil (x10^9/L)	Negative	142	113	119	99	**0.040**[a]
	Positive	185	157	135	167	
Thrombocyte (x10^9/L)	Negative	264.40	67.72	254.00	84.00	0.617[b]
	Positive	259.93	67.10	250.50	86.50	
MPV (fL)	Negative	8.93	1.03	8.80	1.30	0.784[a]
	Positive	9.03	1.27	8.85	1.23	
NLR	Negative	2.29	1.49	2.02	1.04	0.326[a]
	Positive	2.04	0.77	1.92	0.84	
ELR	Negative	0.07	0.06	0.05	0.04	0.118[a]
	Positive	0.09	0.09	0.06	0.09	
PLR	Negative	133.74	53.44	123.03	51.69	0.121[a]
	Positive	120.72	36.32	115.52	49.5	
SII	Negative	634.03	630.47	514.56	297.25	0.349[a]
	Positive	533.43	254.28	493.40	266.98	

[a]Mann-Whitney U test, bIndependent sample t test
CBC: Complete blood count, BPT: Bronchoprovocation test, MPV: Mean platelet volume, NLR: Neutrophil-to-lymphocyte ratio, ELR: Eosinophil-to-lymphocyte ratio, PLR: Platelet-to-lymphocyte ratio, SII: Systemic immune-inflammation index, PC20: Provocative concentration 20, SD: Standard deviation, IQR: Interquartile range

The link between peripheral EOS counts and BHR has already been documented in earlier studies. In one study, a peripheral EOS cut-off of ≥226 cells/μL was associated with modest sensitivity but high specificity in predicting BHR (15). Consistent with this, our study also revealed a significant elevation in EOS counts among patients with BPT positivity, supporting the role of EOSs as a relevant marker in the assessment of BHR.

In their study, Bedolla-Barajas et al. (16) assessed peripheral leukocyte distributions among asthmatic and non-asthmatic patients, reporting no notable differences in either WBC or LYM counts and percentages. Their study included patients with established asthma diagnoses, while our cohort consisted of individuals undergoing BPT as part of the diagnostic work-up for asthma. Another report indicated that, once confounding factors were considered, significant associations with asthma prevalence remained only for WBC and NEU counts (17).

Evidence from various studies supports the notion that PLTs may participate in allergic inflammation as well as in asthma (18). MPV has been studied as a possible marker of systemic inflammation and PLT activation in asthma (19). In our study, no relationship was found between BPT positivity and PLT count or MPV. In contrast, other studies demonstrated a relationship between MPV and asthma, reporting lower MPV values in patients with asthma (6).

A study evaluating CBC-based inflammatory markers reported that NLR, PLR, and SII were linked to higher mortality from respiratory and other causes in adults with asthma (17). In contrast, our findings did not demonstrate any significant association between BPT positivity and CBC-derived indices, including NLR, ELR, PLR, and SII.

NLR has been associated with chronic inflammatory conditions (20). Elevated NLR levels have been reported in children with asthma and allergic asthma (21). However, in our study involving adult patients, no relationship was observed between BPT positivity and NLR. The literature shows inconsistency on this subject, with some studies reporting no association between NLR and asthma (16). In another study, NLR was investigated as an objective biomarker to assess exacerbation severity in asthma and the need for hospitalization in children (22). However, findings remain inconclusive.

ELR has been found to be elevated in asthma and has been correlated with the response to omalizumab in severe asthma (16,23). Additionally, ELR levels have been shown to be higher

in both allergic and non-allergic rhinitis compared to healthy controls (24). Our findings showed that ELR values did not differ significantly between patients with positive and negative BPT results.

Our findings are in line with earlier reports indicating no meaningful differences in PLR between subjects diagnosed with asthma and those without disease (16). In contrast, Tahseen et al. (25) reported significantly elevated PLR values in asthmatic patients compared with healthy controls.

SII has demonstrated prognostic value in various diseases (26,27), and its correlation with asthma has been reported (17). Nevertheless, no significant association was identified between BPT positivity and SII in our study.

Asthma-related inflammation is a chronic process, and various phenotypes and endotypes of asthma have been identified (28). Among the inflammatory phenotypes, the paucigranulocytic type is the most common, followed by the EOS type (4). An overlap between different inflammatory phenotypes may occur. These phenotypic variations may explain the absence of associations between BPT results and CBC-based inflammatory markers in the initial diagnostic evaluation in our study.

There are a number of limitations to this study. It was a single-center, retrospective study. Nevertheless, it provides valuable clinical insights due to its sample size and specific patient population. Moreover, it was conducted in a tertiary care hospital, which serves as the largest referral center in the region. Data collection was performed electronically through the hospital's online database, minimizing the risk of incorrect or missing data. Second, the study included only patients with asthma-like symptoms who underwent non-specific BPT with methacholine and had available CBC results. Therefore, the findings may not be generalizable to the broader population. However, the results may aid clinicians in managing patients with BHR or those presenting with asthma symptoms during follow-up.

Conclusion

Among CBC parameters, EOS count demonstrated a significant association with BPT positivity, highlighting its potential role as an accessible and clinically useful biomarker in patients with asthma-like symptoms. In contrast, our analysis did not demonstrate significant associations between BPT positivity and other variables, including WBC, neutrophil and lymphocyte counts, EOS percentage, platelet number, MPV, or CBC-based inflammatory indices such as NLR, ELR, PLR, and SII. Given the practical limitations of performing bronchial provocation testing in routine clinical settings, the identification of elevated eosinophil levels, readily obtained from a simple CBC, may serve as a valuable adjunct in both the diagnostic evaluation and longitudinal management of this patient population. Considering that asthma represents a long-standing inflammatory disorder characterized by dynamic clinical features, these associations may become even more pronounced over time.

Ethics

Ethics Committee Approval: The ethics committee of the University of Health Sciences Türkiye, Sancaktepe Şehit Prof. Dr. İlhan Varank Training and Research Hospital institution provided formal approval for this research (approval no.: 244, date: 13.12.2023).

Informed Consent: Retrospective study.

Footnotes

Authorship Contributions

Surgical and Medical Practices: M.K., Concept: M.K., E.T., F.D., Design: M.K., E.T., F.D., Data Collection or Processing: M.K., E.T., F.D., Analysis or Interpretation: M.K., E.T., F.D., Literature Search: M.K., E.T., F.D., Writing: M.K., E.T., F.D.

Conflict of Interest: The authors declared no conflict of interest.

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