Original Research

Diagnosing Type 2 Inflammation in COPD: Comparison of Blood and Sputum Eosinophil Assessment in the University of California Los Angeles COPD Phenotyping Study

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Abstract:

Background: COPD phenotyping is an approach for developing tailored therapies. The eosinophilic phenotype is associated with exacerbation risk and response to specific treatments. This study evaluates the relationship between sputum and blood eosinophilia, hypothesizing that sputum eosinophil percentage (SpE%) better reflects disease severity and exacerbation risk than blood eosinophil counts (BEC).

Methods: A single-center, prospective observational cohort enrolled 107 participants aged 40-80 with clinically diagnosed COPD. Participants completed spirometry, a 6-minute walk test, and questionnaires, and blood and sputum samples were provided at baseline and 3 months. BEC and SpE% were measured via routine complete blood counts and flow cytometric analyses (FACS). Eosinophilic phenotype thresholds were defined as BEC≥300 cells/μL and SpE%≥2%, and associations with clinical characteristics and outcomes were investigated.

Results: Adequate sputum specimens were obtained less frequently than blood (60.7% vs 98%). SpE% showed poor repeatability (interclass coefficient 0.36) and poor correlation with FACS (Spearman's ρ=0.008, p=0.58). Conversely, BEC showed higher repeatability (ρ=0.67, p<0.01) and better correlation with FACS (ρ=0.74, p<0.01). More participants were classified as eosinophilic COPD by sputum (33.3%) than by blood (19.6%). BEC values were poorly correlated with SpE% (ρ=0.13, P=0.39), and sputum and blood-based diagnostic criteria showed poor agreement (64.5%, Cohen's κ 0.10). High SpE%, but not high BEC, was associated with lower FEV_1 % predicted.

Conclusions: In stable COPD patients, BEC and SpE% did not correlate well, and blood- and sputum-based diagnostic criteria identified different individuals. Defining eosinophilic COPD requires a better understanding of the biocompartment sampled, testing methods, and cut-off values used.

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Introduction:

Cell biomarkers provide a means of understanding the underlying mechanisms in chronic obstructive pulmonary disease (COPD), potentially predict disease progression, and thereby modify diagnostic and therapeutic approaches (1). A subset of COPD patients has increased absolute blood eosinophil counts (BEC) (2), which has been associated with an increased risk of exacerbations (3) and a favorable clinical response to treatment with inhaled corticosteroids (ICS) (4). These data led to the inclusion of this biomarker as a relevant end-point in clinical trials (5) and a therapeutic algorithm in COPD management (6). Recently, a biologic agent targeting Type 2 inflammation was approved for treating COPD patients with elevated eosinophils (7). Despite this, the nuances of using eosinophils to guide therapy and predict clinical outcomes have remained controversial. Several large cohort studies that have evaluated blood eosinophilia concerning COPD outcomes have revealed discordant results. In an analysis by Hastie et al., (8) blood eosinophils alone were not a reliable biomarker for COPD severity or exacerbation risk, and a high sputum eosinophil percentage (SpE%) was a better biomarker than BEC to identify a patient subgroup with more severe disease, more frequent exacerbations, and increased emphysema on quantitative CT imaging (9).

Analyzing data from the COPD Phenotyping Study (10-13), we sought to evaluate the biomarker profile and clinical characteristics of patients diagnosed with eosinophilic COPD using BEC≥300 cells/μL and SpE%≥2% as criteria and to evaluate differences between these two biomarkers and their clinical implications.

Methods:

The COPD Phenotyping Study was a prospective, observational cohort performed at the University of California, Los Angeles (UCLA) in partnership with Amgen (Thousand Oaks, CA) (10-13). Participants, enrolled from 2016-2019, were ≥40 and ≤80 years of age, had a smoking history ≥10-pack years and a clinical diagnosis of COPD. Clinical stability was defined by being on a stable medication regimen without COPD exacerbations (ECOPD) for ≥3 months before enrollment. Participants were excluded if they had asthma, pulmonary parenchymal disease, or had taken oral corticosteroids within two weeks before enrollment. The study was comprised of three visits. At the baseline Visit 1 (V1) and 3-month follow-up Visit 2 (V2), a detailed medical history was

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obtained, blood and sputum specimens were collected, pulmonary function was assessed by post-bronchodilator spirometry following American Thoracic Society guidelines (14), functional exercise capacity was tested by the six-minute walk test, dyspnea was assessed using the modified Medical Research Council (mMRC) dyspnea scale (15), respiratory symptoms were assessed with the COPD Assessment Test (CAT) (16), and health-related quality of life was assessed with the St. George's Respiratory Questionnaire (SGRQ)(17). All enrolled participants, including GOLD 0 as determined by post-bronchodilator spirometry, were included in the main analysis. One year after the baseline visit, a third visit (V3) consisted of a phone interview to capture symptoms and COPD exacerbation episodes within the year after enrollment in the study. All participants consented to participate in the study, which was approved by the UCLA Office for the Protection of Research Subjects (IRB#14-000748).

Blood and Sputum Sample Collection and Processing

Venous blood sampling and spontaneous sputum sample collection were attempted in all enrolled participants. If spontaneous sputum collection was not feasible, patients with forced expiratory volume in 1 second (FEV_1) ≥1.2 liters on spirometry underwent sputum induction by inhalation of nebulized 3% saline solution after pre-treatment with albuterol. Collected specimens were split and processed within two hours at UCLA or stored on ice and sent to Amgen Thousand Oaks.

Eosinophils were assessed by cell differential counts (CDC) performed at UCLA using manual microscopic assessment for sputum and automated (Sysmex XN-3000) for blood. Supernatants were collected and stored at -80°C and later analyzed at UCLA using Human Luminex® Discovery Assay (Bio-techne, LXSAHM-15). Samples for Fluorescence-Activated Cell Sorting (FACS) were cryopreserved and shipped to Amgen for analysis. Immune cell populations were identified by gating on live, single $CD45^+$ cells. Eosinophils were further characterized within this population by gating on $CD66b^+$ and $CD16^-$/Siglec-8^+ subpopulations.

Exacerbation cohort

As a separate arm of this study, we recruited participants who, in addition to the main cohort inclusion criteria, also had a current severe ECOPD requiring an emergency department visit or hospitalization at UCLA. These COPD patients were enrolled in the study within 24 hours of initial

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admission to the hospital. All participants in this arm of the study were expected to return for a follow-up in-person visit three months after the ECOPD at a self-reported baseline state. Inflammatory biomarkers were compared within this cohort during the exacerbation of COPD and afterward when clinically stable, and values during these episodes were also compared to those in the main steady-state cohort.

Statistical Analysis

To characterize eosinophilic inflammation, we used an absolute BEC≥300 cells/μL as a blood-based and SpE%≥2% of sputum cells as a sputum-based cut-off (18). We compared participant characteristics with and without an eosinophilic phenotype with standard bivariate tests of significance. To determine repeatability of continuous parameters, we used interclass correlation coefficient (ICC)(19) and to assess the agreement of categorical data, we used Cohen's Kappa statistics (κ)(20). Because of non-normal distribution patterns, we evaluated correlation between variables using Spearman's rank correlation coefficient (rho [ρ]). To assess the association of COPD eosinophilic phenotype with clinical outcomes, we used a multivariable-adjusted linear regression model for continuous and a logistic regression model for binary parameters.

Results:

Cohort characteristics

We enrolled 107 participants who completed V1. In-person follow-up visits at 3 months after enrollment were completed by 85% (N=91) of participants, and 66% (N=71) of enrolled participants completed the phone 1-year phone follow-up visit.

As presented in *Table 1*, participants with available BEC (N=107) were 70.0±7.1 (mean±SD) years old and were predominantly white males. Based on post-bronchodilator spirometry, enrolled participants had moderate obstruction (percent-predicted (pp)FEV_1: 62.5±23.7) on average, 10 (9%) were participants who had smoked tobacco at risk of COPD (GOLD 0). In comparison, 51% and 20% of those with COPD were GOLD Group B and E, respectively. Of 91 participants with available CT scans, 89% had emphysema. On review of prescribed medications, only 9% were

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using inhaled corticosteroids (ICS) at baseline, and none reported chronic use of oral corticosteroids.

Collection of specimens

In contrast to the blood specimens collected in all but one participant at V1 and all participants at the 3-month follow-up visit, spontaneous sputum collection yielded an adequate specimen in only 61% of all attempts at V1 and 67% of all attempts (a maximum of two for those who completed both in-person visits). Among those who had completed both visits and had sputum analysis, 14% could not provide sputum specimens at both visits.

Repeatability

The between-visit absolute BEC values correlated well (ρ=0.67, p<0.01) and demonstrated moderate reliability for both absolute BEC (ICC=0.71) and percent blood eosinophils (ICC=0.72). In contrast, sputum eosinophil analysis showed poor correlation (ρ = 0.22, P=0.22) and poor reliability (ICC =0.36) between the two visits.

Classification as an eosinophilic phenotype based on BEC≥300 cells/μL at V1 and V2 showed substantial between-visit agreement (91% agreement, κ=0.64). Evaluating other clinically used BEC thresholds, classification as an eosinophilic phenotype based on BEC≥150 cells/μL showed moderate between-visit agreement (78% agreement, κ =0.57), and the classification as low-eosinophilic COPD, using a threshold of ≤100 cells/μL showed poor agreement between visits (69% agreement, κ =0.3). Classification as an eosinophilic phenotype, defined by ≥2% sputum eosinophils, showed poor agreement between the two visits (agreement 66.7%, κ =0.14).

Methods of specimen analysis

To evaluate the accuracy of the methodology determining eosinophil counts, we compared CDC to the gold standard based on FACS. We analyzed all available sputum specimens from 63 participants. While there was a strong correlation between the two methods for blood specimen analysis (ρ=0.74, p<0.01), the correlation between the sputum analysis methods was poor (ρ=0.008, P=0.58), *Figure 1a-b*

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Correlation Between Specimen Types

BEC at V1 correlated poorly with SpE% (ρ= 0.13, P=0.39). Comparing the maximum values obtained at either visit, BEC_{max} had slightly better, but also poor, correlation with $SpE\%_{max}$ (ρ= 0.16, P=0.21), *Figure 1c*

Blood Eosinophil Testing

Median BEC of 144 (IQR: 86-221) cell/μL and a median percent of 2.3% (IQR: 1.4-3.3) of total white blood-cell counts. As presented in *Figure 2*, when high-BEC stratification is based on ≥300 cells/μL, the prevalence of the high eosinophilic phenotype was 17.9% at V1, or 19.6% if the BEC≥300 cells/μL was captured at either V1 or V2 or 10.1% if this threshold was reached consistently at all available samplings. BEC≥150 cells/μL was more prevalent, with 48.1% of participants at V1 and 57.0% of participants based on any of the two visits. BEC ≤ 100 cells/μL was present in 33% of participants at V1, 45.8% if any of the visits was considered, or 17.6% if consistently BEC ≤ 100 cells/μL.

Sputum Eosinophil testing

The average SpE% was 6.5±20.3 for total sputum cell counts. Using a cut-off of 2% to define eosinophilic COPD at Visit 1, 25% of participants could be classified as having the eosinophilic phenotype, and this number increased to 33.3% if SpE% was ≥2% at any of the two visits at which sputum was sampled (including individuals who had only one and those who had two sputum specimens available for analysis).

Agreement between biocompartment assessments in determining eosinophilic disease

When comparing BEC≥300 cells/μL with SpE%≥2 to determine eosinophilic COPD, only 5 (8%) participants satisfied both criteria, a finding that did not allow this approach to be evaluated for association with clinical outcomes. Blood and sputum-based criteria showed poor agreement (agreement 64.5%, 0.10). Agreement between blood and sputum SpE%≥2 criteria was only fair if once the blood eosinophil criterion was lowered to BEC≥150 cells/μL at all available visits (agreement 67.2%, κ=0.25).

Characteristics of Eosinophilic COPD based on blood eosinophil counts

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Compared to participants with BEC<300 cells/μL, those with BEC ≥300 cells/μL had a higher white blood cell count (WBC) (9.3±6.9 vs. 6.8±2.2 cells/μL, p<0.01) but did not differ in other clinical characteristics including historic ECOPD, *Table 2*. Participants with BEC ≥150 cells/μL at the baseline visit, compared to those with lower BEC, reported a history of asthma more often (35.5% vs. 16.4%, p=0.03), and also had higher total leukocyte counts (7.9±6.7 $x10^3$ cells/μL vs. 6.7±2.5 $x10^3$ cells/μL, p=0.02), *Supplemental Table 1.*

Characteristics of Eosinophilic COPD based on SPE%≥2%

Compared to participants with low sputum eosinophils, those with SpE%≥2 (n=21) had a lower post-bronchodilator percent-predicted FEV_1 (54.0±16.9% vs. 71.7±23.0%, p=0.002) and lower FEV_1/FVC ratio, had a more advanced COPD GOLD spirometric grade and were more symptomatic with a higher mMRC (1.5±1.0 vs. 1.0±1.1, p=0.04) and higher SGRQ score (42.9±22.4 vs. 30.7±24.0, p=0.04). More participants in the SpE≥2% group had severe or very severe (spirometric grades III and IV) COPD compared to those with SpE% <2%(42.9% vs 21.5%, p=0.01). In a multivariable analysis adjusted for age, sex, race, height, smoking status, and a lifetime smoking exposure history, participants with SPE%≥2% had a lower post-bronchodilator percent-predicted FEV_1 (coefficient: -0.23, p=0.04) but did not have more past (1 year before enrollment), or prospective exacerbations over the following year, *Table 3.* In a sub-analysis limited to only participants with sputum eosinophilia and spirometry-proven COPD (GOLD 1-4), we found similar, lower FEV_1 percent-predicted in comparison to those with SpE%<2 (coefficient: -0.07, p<0.01)

Blood Eosinophils and clinical risk stratification

Older participants (age ≥65) had a higher BEC than younger participants (200±160 vs. 152±172 cells/μL, p=0.03), and nominally higher BEC was seen in males (p=0.13), non-Blacks (p=0.09) and former smokers (p=0.25). Those who had ECOPD in the year before enrollment, compared to those who had not, had only a nominally higher BEC (200±128 vs. 190±171 cells/μL, p=0.35).

Participants with sputum eosinophilia (SpE%≥2) had nominally higher BEC (254±203 vs. 167±127, p=0.24) compared to those with lower SpE%. There was no BEC difference between participants receiving vs. those not receiving ICS, regardless of whether or not they had either

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emphysema or chronic bronchitis, nor was there a difference between symptomatic (mMRC ≥2 or CAT ≥10) and non-symptomatic individuals. No difference in BEC was seen when comparing those with high vs normal values of C-reactive protein (CRP) (p=0.87), sedimentation rate (p=0.71), or fibrinogen (p=0.42). There was no difference in BEC between those with or without previous exacerbations (in the year before enrollment). In contrast, those with an exacerbation during the follow-up period had lower BEC at the entry visit (145±120 vs. 211±177, p=0.04).

Participants with a history of asthma had a higher BEC than those without (225±135 vs 179±170 cells/μL, p=0.02). However, neither BEC≥300 cells/μL nor SPE%≥2% was associated with asthma history. In contrast, having low BEC ≤100 cells/μL at V1 was associated with a less prevalent history of asthma (14.8% vs. 39.2%, p<0.02). There was no association between atopy and BEC. Similarly, we found no evidence that the highest SpE% from the two visits correlated with a history of asthma or atopy, nor did those with SpE%≥2 have a history of asthma or atopy more often than those with SpE%<2.

Relationship with ICS use and smoking status

Of the participants who reported using maintenance inhaled medication, 49 (47%) used ICS. Compared to those not using ICS, they had only modestly lower values of BEC_{max} (189±129 vs. 246±199 cells/μL, p=0.26) or $SpE\%_{max}$ (5.5±18.2% vs. 6.5±16.5%, p=0.99).

Compared to participants who formerly smoked, those who currently smoked tobacco had similar $SpE\%_{max}$ (12.9±30.9% vs. 5.6±15.2%, p=0.96) and BEC_{max} (225±191 vs. 224±168 cells/μL, p=0.71).

BEC during Exacerbations

We recruited 13 participants during exacerbations of their COPD. They were 70.8±6.1 years old, were comprised of slightly more females (53.8%), and were predominantly white (76.9%); 57.1% of these participants were currently smoking with a heavy tobacco use history (49.5±39.9 pack-years), and 36.4% had a history of asthma and atopy. Their post-bronchodilator percent-predicted FEV_1 was 57.3±11.6, and 11.1% were on ICS, *Supplemental Table 2*.

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Comparing the biomarker values during an ECOPD to values in the clinically stable state of the same individuals three months later, BEC values were similar: 101±109 cells/μL vs. 102±29.2 cells/μL. In contrast, peripheral blood neutrophils during ECOPD were higher (80.3±12.3% vs. 69.7±7.6%, $p<0.01$) compared to values 3 months later. While the low number of participants in this substudy did not allow for meaningful statistical analysis, inflammatory markers were nominally higher during ECOPD compared to the stable state 3 months later (WBC: 12.4±5.0 $x10^3$ vs. 5.9±2.0 $x10^3$ cells/μL and CRP: 5.8±9.7 vs 4.4±2.2 mg/L). In an unadjusted sensitivity analysis comparing the levels of inflammatory markers during ECOPD in the exacerbation cohort and their levels at steady state of the main COPD cohort, exacerbations were associated with higher levels of WBC, neutrophil percent, CRP, and fibrinogen, *Supplemental Table 3*.

Eosinophil Status and Inflammatory Markers

Multi-analyte Luminex testing found minimal correlation between blood eosinophil status and levels of serum cytokines, particularly IL-4, IL-5, and TNFα. Specifically, when comparing participants with BEC≥300 cells/μL to those with BEC<300 cells/μL, we found no significant correlation with levels of IL-5, IL-4, or IL-13RA1 but noted a mild nominal trend toward higher TNF-alpha concentrations in sera of the higher BEC group (4.2±3.9 pg/mL vs. 3.5±4.6 pg/mL, $p=0.16$). Likewise, when comparing participants with SpE%≥2 to those with fewer eosinophils, there were no differences in IL-5, TNFα, IL-4, or IL-13RA1, *Tables 2 and 3*.

Discussion:

Eosinophil measurement has become a recommended routine in the COPD assessment (21). The relationships between eosinophilia, exacerbations, and responsiveness to inhaled corticosteroids have been well established (22). However, it seems there is more to be understood about the role of eosinophils in the airway inflammation of chronic obstructive pulmonary disease (23). We conducted a single-center, observational study that aimed to better understand the role of biomarkers in clinical phenotyping of participants with current and former tobacco smoking with or at risk of COPD. In this analysis, we investigated the nuances of eosinophil assessment in routine care and explored the clinical repercussions of different methods of diagnosing the eosinophilic COPD phenotype. We showed significant heterogeneity in the identification of

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eosinophilic COPD stemming from how one defines and measures eosinophilia, including whether to use blood or sputum, the assay technique, cutoff thresholds, and whether one requires repeated samplings for assessment of consistency of the measured values.

Several findings from our analysis merit discussion. The prevalence of eosinophilic COPD as a clinical manifestation of Type 2 inflammation (24) ranged in our study from 17.9 to 57.0%, depending on the method used for assessing the eosinophilic phenotype. This finding suggests that the technical differences in its assessment can significantly impact variability in the diagnostic criteria for the eosinophilic COPD phenotype (20-40%)(25). On the other hand, the low-eosinophilic population, defined by BEC≤100 cells/μL, was more prevalent in our analysis than the eosinophilic phenotype. It has been shown that BEC≤100cells/μL carry risks of worsened clinical outcomes (26), underscoring the importance of additional research focused on our understanding of non-Type 2 immune responses in COPD and precision medicine approaches in these patients.

While the percentage of blood eosinophils can also be used (7, 27-29), various cut-off values of absolute BEC have been used to diagnose the eosinophilic phenotype in COPD (21, 30). BEC analysis is readily available and repeatable, with automated CDC assessment being adequate, but its association with clinical outcomes was limited in our study, supporting previously published data (8). Compared to blood eosinophilia, sputum eosinophilia has been reported to be a better marker of the eosinophilic COPD phenotype (8, 9) and has been associated with a history of exacerbations, lower FEV_1, lower exercise capacity, greater symptoms, and poorer quality of life in COPD individuals (2, 3, 31, 32). In the SPIROMICS cohort, high concentrations of sputum eosinophils were a better biomarker than high concentrations of blood eosinophils for identifying a patient subgroup with more severe disease, more frequent exacerbations, and increased emphysema (8). Recognizing the limitation of our smaller cohort, our results aligned with these findings, suggesting that SpE%≥2 has greater sensitivity to identify a clinically sicker population of patients compared to blood analysis. Unfortunately, however, sputum analysis in our cohort showed relatively poor repeatability, with many participants demonstrating discordance in SpE% between two visits three months apart. Moreover, sputum eosinophils are not readily accessible, and a large portion of individuals with COPD are incapable of producing adequate sputum. In our

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study, approximately a third of individuals could not provide an adequate spontaneous sputum specimen after one (39.3%) or up-to-two sampling attempts (32.7%) despite adequate hydration.

Repeating laboratory testing twice over three months in a steady state of disease but using the same threshold for eosinophilia for sputum and blood led to an increased prevalence of eosinophilic COPD by up to 9% over the initial prevalence based on the baseline assessment, *Figure 2*. Understanding how to interpret variable lab results is of significant relevance as shown in the ECLIPSE study, in which 49% of study participants had only intermittent elevations of SpE%, and the stability of eosinophil counts was significantly lower in patients with COPD than in controls (23, 33). Despite acknowledging the suboptimal repeatability of BEC, especially when higher BEC thresholds are used, current guidelines do not leave clear recommendations on how many blood results should be considered when determining the presence of eosinophilic inflammation in COPD (6).

The lab methodology of specimen processing was relevant in our study. In contrast to flow cytometry, which can be considered to be the gold standard for assessment of eosinophils in sputum (34), routine CDC tends to underestimate percentages of these cells in the sputum. This may not necessarily be inherent to the sputum bio-compartment but could be a consequence of the presence of cellular debris and dead cells, as well as the opinion of the interpreter. While this finding should not suggest that more complex and expensive flow cytometry should be routinely used, it calls attention to the fact that many factors can influence sputum white blood cell analysis.

This analysis has some limitations. We chose to use cut-off values rather than to consider eosinophils as a continuous variable since categorical data with defined cut-points allows for a better prediction of clinical outcomes with knowledge of sensitivity and specificity. (2). This approach was also chosen from the practical standpoint, acknowledging the wide use of cut-off values to determine recommended therapeutic approaches (6). Our cohort is relatively small and was not powered to determine significant associations with major clinical outcomes over an extended period. However, our use of a second in-person visit three months after enrollment and a one-year follow-up over the phone provided us with the opportunity to assess the stability of eosinophilic biomarkers despite the inadequacy of our study design for evaluating long-term

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longitudinal outcomes. Some strengths include a well-described longitudinally followed cohort of individuals with or at risk of COPD. All individuals were evaluated twice over a short period offering a unique chance to assess repeatability of multiple measures. We extended the sputum analysis beyond routine cell count to compare the latter with flow cytometric analysis and evaluated both cross-sectional and longitudinal clinical outcomes. While not the only study longitudinally comparing blood and sputum, our analysis adds to the current knowledge about the methods used to determine eosinophilic COPD.

Conclusions:

Both blood and sputum eosinophil counts offer helpful information in identifying Type-2 eosinophilic inflammation. Nevertheless, our data demonstrate that relatively small differences in the methodology of diagnosing the eosinophilic COPD phenotype may have significant repercussions not only on the designation of a COPD phenotype as "eosinophilic," but also on guiding therapeutic decisions. From the practical standpoint, further research focused on standardizing the clinical interpretation of eosinophilia is needed.

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Author Contributions:

WBL, RBG, DPT, CBC, HP, and IZB contributed to the conception and design of the study. SAI, RW, JB, TB assisted with the acquisition of data. WBL, RW, RGB, and IZB provided statistical analysis support. WBL, RW, IZB were the primary composers of the manuscript. All authors contributed to the data interpretation as well as to the editing and final approval of the manuscript.

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Declaration of Interests:

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Table 1: Demographics and Baseline Clinical Characteristics of the COPD Phenotyping Cohort

Baseline:	
Age, years, (mean±SD)	70±7.1
Female, N (%)	40 (39.3%)
Race (Black)	28 (26.2%)
BMI (mean±SD)	27.2±5.4
Smoking history, pack-years (mean±SD)	47.5±30.2
Active smoking, N (%)	25 (23.4%)
Asthma history, N (%)	27 (25.2%)
Atopy, N (%)	32 (30.5%)
FEV1 (L) (mean±SD)	3.3±1.4
FEV1, % (mean±SD)	62.5±23.7
FVC, % (mean±SD)	92.4±40.7
FEV1/FVC, % (mean±SD)	51.5±15.4
6MWD, m (mean±SD)	382±132
GOLD stage (1, 2, 3, 4) N (%)	
Stage 0	10 (9.3%)
Stage 1	19 (17.8%)
Stage 2	42 (39.2%)
Stage 3	28 (26.2%)
Stage 4	8 (7.5%)
GOLD group (CAT - A, B, E) N (%)	
Group A	28 (29.2%)
Group B	49 (51.0)
Group E	19 (19.8%)
Exacerbation history in previous year, % positive	0.2±0.4
Emphysema, N (%)	91 (89.2%)
Chronic bronchitis, N (%)	49 (44.9%)
On inhaled steroid, N (%)	47 (49.0%)
mMRC, score (mean±SD)	2.2±1.1
CAT, score (mean±SD)	14.3±8.6
SGRQ, score (mean±SD)	34.3±22.5
WBC, cells/uL x10^3 (mean±SD)	7.3±3.7
Ne/Ly ratio (mean±SD)	191±163
ESR, mm/hr (mean±SD)	22.7±18.6
CRP, mg/L (mean±SD)	0.8±1.4
Fibrinogen, mg/dL (mean±SD)	351±88

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Sputum Eos, % (mean±SD)	6.5±20.3

Baseline Characteristics. Data are presented as mean±SD or No. (%). BMI: Body mass index; FEV1: Forced expiratory volume in 1 second; FVC: Forced vital capacity; 6MWD = 6-minute walk distance; GOLD: Global Initiative for Chronic Obstructive Lung Disease; CAT: COPD Assessment Test; mMRC: modified Medical Research Council; SGRQ: St. George's Respiratory Questionnaire; WBC: White blood cell; Ne/ly: Neutrophil to lymphocyte ratio; ESR: Erythrocyte sedimentation rate; CRP: C-reactive protein

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Table 2: Characteristics of Eosinophilic COPD based on blood eosinophil counts ≥300cells

Baseline:	BEC≥300 cells/µL (n = 21)	BEC<300 cells/µL (n = 86)	p Value
Age, years (mean±SD)	69.7±8.2	70.1±6.9	0.97
Female, N (%)	5 (23.8%)	37 (43.0%)	0.11
Race (Black)	3 (14.3%)	25 (29.1%)	0.16
BMI (mean±SD)	27.4±5.3	27.1±5.3	0.87
Smoking history, pack-years (mean±SD)	46.3±29.7	47.8±30.5	0.79
Active smoking, N (%)	4 (19.1%)	21 (24.4%)	0.60
Asthma history, N (%)	8 (38.1%)	19 (29.1%)	0.13
Atopy, N (%)	6 (30%)	26 (30.6%)	0.96
FEV1 (L) (mean±SD)	1.8.±0.6	1.6±0.7	0.20
FEV1, % (mean±SD)	64.2±19.4	62.1±24.6	0.61
FVC, % (mean±SD)	89.9 ±19.9	93±44.4	0.97
FEV1/FVC, % (mean±SD)	54.1±16.1	50.8±15.3	0.31
6MWD, m (mean±SD)	404.3±90.6	376.7±139.2	0.45
GOLD stage (1, 2, 3, 4) N (%)			0.72
Stage 0	2 (9.5%)	8 (9.3%)	
Stage 1	3 (14.3%)	16 (18.6%)	
Stage 2	11 (52.4%)	31 (36.1%)	
Stage 3	4 (19.0%)	24 (27.9%)	
Stage 4	1 (4.8%)	7 (8.1)	
GOLD group (CAT - A, B, E) N (%)			0.95
Group A	5 (26.3%)	23 (29.9%)	
Group B	10 (52.6%)	39 (50.7%)	
Group E	4 (21.1%)	15 (19.4%)	
Exacerbation history in previous year, % positive	4 (19.1%)	15 (17.7%)	0.88
Emphysema, N (%)	15 (79.0%)	76 (91.6%)	0.11
Chronic bronchitis, N (%)	10 (47.6%)	38 (44.2%)	0.78
On inhaled steroid, N (%)	6 (33.3%)	41 (52.6%)	0.14
mMRC, score (mean±SD)	1.5±1.2	1.2±1	0.30
CAT, score (mean±SD)	14.5±7.9	14.2±8.8	0.71
SGRQ, score (mean±SD)	34.6±22	34.2±22.8	0.78
WBC, cells/uL x10^3 (mean±SD)	9.3±6.9	6.8±2.2	<0.01*
Ne/Ly ratio (mean±SD)	3.5±2.5	3.4±2.7	0.93
ESR, mm/hr (mean±SD)	20.5±12.8	23.3±19.8	0.91
CRP, mg/L (mean±SD)	1.1±1.7	0.7±1.3	0.15
Fibrinogen, mg/dL (mean±SD)	356.6±111.6	350.1±81.5	0.86
Sputum Eos, % (mean±SD)	8.6±17.2	5.9±21.2	0.74
Sputum Ne, % (mean±SD)	41.5±37.2	43.7±37.1	0.68
Serum Interleukin-5, pg/mL , (mean±SD)	4.9.±4.2	3.8±2.9	0.73
Serum Interleukin-4, pg/mL , (mean±SD)	31.1±36.9	45.4±70.6	0.44
Serum IFN-gamma, pg/mL , (mean±SD)	294.3±767.9	112.5±293.7	0.90
Serum TNF-alpha, pg/mL , (mean±SD)	4.2±3.9	3.5±4.6	0.16
Serum Intereukin-6, pg/mL , (mean±SD)	2.5±2.7	4.8±9.9	0.81
Serum Interleukin-33, pg/mL , (mean±SD)	150.1±339.1	82.0±182.9	0.51
Serum Interleukin-10, pg/mL , (mean±SD)	4.4±5.7	2.8±2.0	0.71
Serum Interleukin-13RA1, pg/mL , (mean±SD)	5370±1508	4987±1447	0.43

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Follow up period:			
Exacerbations during 1 year follow up, % present, n (%)	5 (23.8%)	22 (26.2%)	0.82
Number of ECOPD in follow up, N (%)	0.2±0.4	1±2.1	0.25
Change in SGRQ	(-6.4)±13.8	1.2±13.3	0.13
Change in CAT	(-2.3)±4.5	(-0.1)±6.6	0.22

Baseline Characteristics When Stratified by BEC≥300 cells/uL. Data are presented as mean±SD or No. (%). BMI: Body mass index; FEV1: Forced expiratory volume in 1 second; FVC: Forced vital capacity; 6MWD = 6-minute walk distance; GOLD: Global Initiative for Chronic Obstructive Lung Disease; CAT: COPD Assessment Test; mrc: Medical Research Council; SGRQ: St. George's Respiratory Questionnaire; WBC: White blood cell; Ne: Neutrophil; Ly: Lymphocyte; ESR: Erythrocyte sedimentation rate; CRP: C-reactive protein; Eos: Eosinophil; IFN: Interferon; TNF: Tumor necrosis factor; ECOPD: Exacerbation of COPD

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Table 3: Characteristics of Eosinophilic COPD based on sputum eosinophil percentage ≥2%

Baseline:	SpE%≥2% (n = 21)	SpE%<2% (n = 42)	p Value
Age, years (mean±SD)	69.3±9.2	71.3±5.7	0.77
Female, N (%)	7 (33.3%)	17 (40.5%)	0.58
Race (Black)	3 (14.3%)	15 (35.7%)	0.08
BMI (mean±SD)	28.1±4.9	27.6±5.0	0.89
Smoking history, pack-years (mean±SD)	43.4±22.9	50.9±30.7	0.41
Active smoking, N (%)	5 (23.8%)	9 (21.45)	0.83
Asthma history, N (%)	1 (4.8%)	8 (19.1%)	0.13
Atopy, N (%)	5 (23.8%)	13 (31.0%)	0.55
FEV1 (L) (mean±SD)	1.5±0.5	1.8±0.6	0.05*
FEV1, % (mean±SD)	54.0±16.9	71.7±23.0	<0.01*
FVC, % (mean±SD)	101.1±82.9	3.4±0.9	0.12
FEV1/FVC, % (mean±SD)	47.1±15.6	54.9±13.3	0.04*
6MWD, m (mean±SD)	379±163	399±126	0.42
GOLD stage (1, 2, 3, 4) N (%)			0.01*
Stage 0	1 (4.8%)	4 (9.5%)	
Stage 1	0 (0.0%)	16 (38.1%)	
Stage 2	11 (52.4%)	13 (31.0%)	
Stage 3	8 (38.1%)	7 (16.7%)	
Stage 4	1 (4.8%)	2 (4.8)	
GOLD group (CAT - A, B, E) N (%)			0.23
Group A	4 (20.0%)	16 (42.1%)	
Group B	11 (55.0%)	16 (42.1%)	
Group E	5 (25.0%)	6 (15.8%)	
Exacerbation history in previous year, % positive	0.2±0.4	0.1±0.4	0.44
Emphysema, N (%)	19 (95.0%)	36 (87.8%)	0.38
Chronic bronchitis, N (%)	14 (66.7%)	18 (42.9%)	0.08
On inhaled steroid, N (%)	8 (44.4%)	16 (40.0%)	0.75
mMRC, score (mean±SD)	1.5±1.0	1.0±1.1	0.04*
CAT, score (mean±SD)	16.8±7.8	13.1±9.4	0.07
SGRQ, score (mean±SD)	42.9±22.4	30.7±24.0	0.04*
WBC, cells/uL x10^3 (mean±SD)	7.3±2.8	7.2±4.5	0.40
Ne/Ly ratio (mean±SD)	3.6±2.2	3.5±3.6	0.33
ESR, mm/hr (mean±SD)	23.0±16.7	24.6±20.8	0.89
CRP, mg/L (mean±SD)	0.7±0.9	0.9±0.6	0.73
Fibrinogen, mg/dL (mean±SD)	342.1±85.8	350.9±82.9	0.91
Sputum Eos, % (mean±SD)	15.7±29.9	0.1±0.3	n/a
Sputum Ne, % (mean±SD)	64.4±5.4	63.2±12.5	0.51
Serum Interleukin-5, pg/mL , (mean±SD)	5.0±4.2	3.9±2.5	0.71
Serum Interleukin-4, pg/mL , (mean±SD)	19.4±19.2	35.8±45.8	0.10
Serum IFN-gamma, pg/mL , (mean±SD)	59.1±52.4	169.3±446.6	0.89
Serum TNF-alpha, pg/mL , (mean±SD)	2.4±1.9	3.1±3.9	0.86
Serum Intereukin-6, pg/mL , (mean±SD)	1.9±0.8	5.8±12.5	0.72
Serum Interleukin-33, pg/mL , (mean±SD)	41.0±33.5	115.3±264.3	0.91
Serum Interleukin-10, pg/mL , (mean±SD)	1.7±1.4	3.4±2.7	0.19

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Serum Interleukin-13RA1, pg/mL , (mean±SD)	4228±489	2714±944	0.26
Follow up period:			
Exacerbations during 1 year follow up, % present, n (%)	9 (42.9%)	7 (17.5%)	0.03*
Number of ECOPD in follow up, N (%)	0.4±0.7	0.6±0.8	0.91
Change in SGRQ	(-5.0)±14.1	0.3±13.1	0.57
Change in CAT	(-3.0)±5.2	1.1±7.0	0.05*

Data are presented as mean±SD or No. (%). BMI: Body mass index; FEV1: Forced expiratory volume in 1 second; FVC: Forced vital capacity; 6MWD = 6-minute walk distance; GOLD: Global Initiative for Chronic Obstructive Lung Disease; CAT: COPD Assessment Test; mMRC: modified Medical Research Council; SGRQ: St. George's Respiratory Questionnaire; WBC: White blood cell; Ne: Neutrophil; Ly: Lymphocyte; ESR: Erythrocyte sedimentation rate; CRP: C-reactive protein; Eos: Eosinophil; IFN: Interferon; TNF: Tumor necrosis factor; ECOPD: Exacerbation of COPD

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Figure 1: a) Correlation of sputum eosinophil percentage measured by routine CDC with microscopy and FACS, b) Correlation of blood eosinophil counts measured by routine automated CDC and FACS, and c) Correlation of blood eosinophil counts with sputum eosinophil percentage

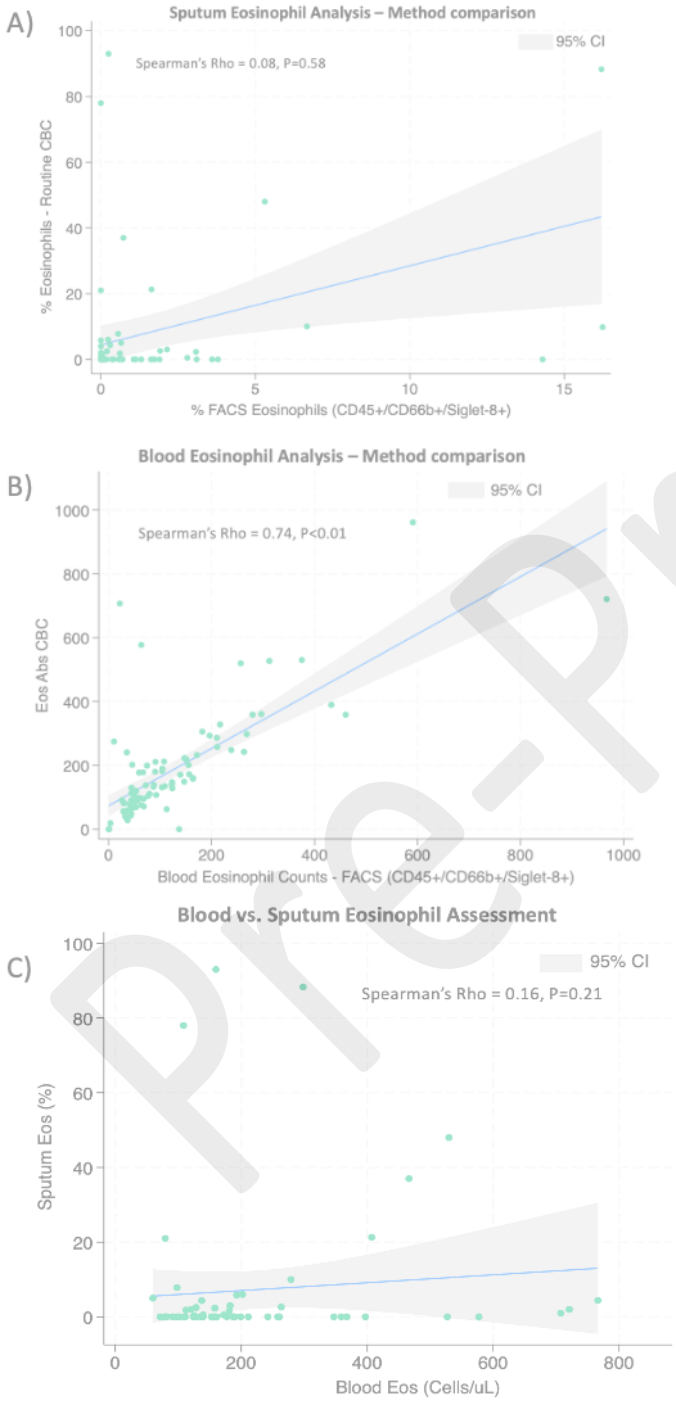

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Figure 2: Eosinophilic disease prevalence in COPD phenotyping cohort using different biocompartments (blood and sputum) and different cut-off threshold values to diagnose eosinophilic COPD

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Online Supplement

Supplemental Table 1: Characteristics of Eosinophilic COPD based on blood eosinophil counts ≥150cells

Baseline:	BEC≥150 cells/uL (n = 51)	BEC<150 cells/uL (n = 55)	p Value
Age, years (mean±SD)	70.3±7	69.8	0.78
Female, N (%)	15 (29.4%)	26 (47.3%)	0.06
Race (Black)	12 (23.5%)	15 (27.3%)	0.66
BMI (mean±SD)	27.5±5.6	26.9	0.61
Smoking history, pack-years (mean±SD)	50.2±29.5	45.7	0.58
Active smoking, N (%)	11 (21.6%)	14 (25.5%)	0.64
Asthma history, N (%)	18 (35.3%)	9 (16.4%)	0.03*
Atopy, N (%)	16 (32%)	16 (29.6%)	0.79
FEV1 (L) (mean±SD)	1.7±0.7	1.7±0.7	0.58
FEV1, % (mean±SD)	60.8±22.7	64.6±24.5	0.46
FVC, % (mean±SD)	95.4±54.9	90±21	0.98
FEV1/FVC, % (mean±SD)	50.4±17.3	52.6±13.6	0.67
6MWD, m (mean±SD)	372.2±124.9	393.2±137.2	0.58
GOLD stage (1, 2, 3, 4) N (%)			0.61
Stage 0	5 (9.8%)	5 (9.1%)	
Stage 1	7 (13.7%)	12 (21.8%)	
Stage 2	20 (39.2%)	22 (40%)	
Stage 3	16 (31.4%)	11 (20%)	
Stage 4	3 (5.9%)	5 (9.1%)	
GOLD group (CAT - A, B, E) N (%)			0.16
Group A	9 (20%)	19 (38.0%)	
Group B	26 (57.8%)	22 (44.0%)	
Group E	10 (22.2%)	9 (18.0%)	
Exacerbation history in previous year, % positive	0.2±0.4	0.2±0.4	0.70
Emphysema, N (%)	43 (89.6%	47 (88.7%)	0.88
Chronic bronchitis, N (%)	27 (52.9%)	21 (38.2%)	0.13
On inhaled steroid, N (%)	22 (51.2%)	24 (46.2%)	0.63
mMRC, score (mean±SD)	1.3±1.1	1.1±1.0	0.28
CAT, score (mean±SD)	15.4±8.1	13.1±9.1	0.08
SGRQ, score (mean±SD)	37.1±22.3	31.3±22.4	0.11
WBC, cells/uL x10^3 (mean±SD)	7.9±6.7	6.7±2.5	0.02*
Ne/Ly ratio (mean±SD)	3.3±2.0	3.5±3.2	0.96
ESR, mm/hr (mean±SD)	21.6±16.5	23.8±20.5	0.73
CRP, mg/L (mean±SD)	0.8±1.3	0.8±1.5	0.36
Fibrinogen, mg/dL (mean±SD)	343.8±92.6	356.9±83.2	0.48
Sputum Eos, % (mean±SD)	11.6±27.3	0.9±2.0	0.25
Sputum Ne, % (mean±SD)	62.5±9.5	64.6±11.1	0.45
Serum Interleukin-5, pg/mL , (mean±SD)	4.5±3.4	3.6±3.0	0.31
Serum Interleukin-4, pg/mL , (mean±SD)	32.9±37.3	51.3±82.7	0.49
Serum IFN-gamma, pg/mL , (mean±SD)	176.9±479.0	120.7±372.4	0.42
Serum TNF-alpha, pg/mL , (mean±SD)	3.7±3.0	3.6±5.5	0.06
Serum Intereukin-6, pg/mL , (mean±SD)	3.3±6.7	5.4±10.8	0.33

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Serum Interleukin-33, pg/mL , (mean±SD)	103±214	88.9±237.4	0.39
Serum Interleukin-10, pg/mL , (mean±SD)	3.4±3.8	2.7±2.3	0.59
Serum Interleukin-13RA1, pg/mL , (mean±SD)	5138.4±1608.8	4963.9±1223.6	0.66
Follow up period:			
Exacerbations during 1 year follow up, % present, n (%)	13 (25.5%)	13 (24.5%)	0.91
Number of ECOPD in follow up, N (%)	0.2±0.5	0.2±0.4	0.71
Change in SGRQ	(-2.8)±14.7	1.8±12.5	0.34
Change in CAT	(-2.0)±6.2	0.8±6.1	0.04*

Data are presented as mean±SD orNo. (%). BMI: Body mass index; FEV1: Forced expiratory volume in 1 second; FVC: Forced vital capacity; 6MWD = 6-minute walk distance; GOLD: Global Initiative for Chronic Obstructive Lung Disease; CAT: COPD Assessment Test; mMRC: Medical Research Council; SGRQ: St. George's Respiratory Questionnaire; WBC: White blood cell; Ne: Neutrophil; Ly: Lymphocyte; ESR: Erythrocyte sedimentation rate; CRP: C-reactive protein; Eos: Eosinophil; IFN: Interferon; TNF: Tumor necrosis factor; ECOPD: Exacerbation of COPD

Supplemental Table 2: Demographics and clinical characteristics of exacerbation cohort

Exacerbators Cohort (n=13)	
Age, years (mean±SD)	70.8±6.1
Male, N (%)	6 (46.2%)
Race (Black)	2 (15.4%)
BMI (mean±SD)	25.2±4.1
Smoking history, pack-years (mean±SD)	49.5±39.9
Active smoking, N (%)	7 (57.1%)
Asthma history, N (%)	5 (36.6%)
Atopy, N (%)	5 (36.4%)
Chronic bronchitis, N (%)	3 (60%)
FEV1, % (mean±SD)	57.3±11.6
FVC, % (mean±SD)	81.3±13.7
FEV1/FVC, % (mean±SD)	51.7±9.1
CAT, score (mean±SD)	18.8±11.0
SGRQ, score (mean±SD)	41.4±27.6
Sputum Eos, % (mean±SD)	7.2±10.3
Sputum Ne, % (mean±SD)	21.4±31.1

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Data are presented as mean±SD or No. (%). BMI: Body mass index; FEV1: Forced expiratory volume in 1 second; FVC: Forced vital capacity; 6MWD = 6-minute walk distance; GOLD: Global Initiative for Chronic Obstructive Lung Disease; CAT: COPD Assessment Test; mMRC: modified Medical Research Council; SGRQ: St. George's Respiratory Questionnaire; Ne: Neutrophil; Eos: Eosinophil

Supplemental Table 3: Blood biomarkers a) during and b) after COPD exacerbation. Comparison with c) the main cohort with stable COPD

Variable	a) During Exacerbation (n=13)	b) After Exacerbation (n=3)	Difference pre and post (P value)	c) Stable Cohort (N = 106)	Difference *(p value)
WBC, cells/uL x10^3 (mean±SD)	12.4±5.0	5.9±2.0	n/a	7.3±3.7	<0.01
Neutrophil, % (mean±SD)	80.3±12.3	69.7±7.6	n/a	63.6±10.3	<0.01
Ne/Ly ratio (mean±SD)	13.4±14.6	4.5±2.4	n/a	3.4±2.7	<0.01
BEC, cells/uL, (mean±SD)	101±109	102±29.2	n/a	196.6±170.5	0.02
ESR, mm/hr (mean±SD)	33.8±34.2	37±32.4	n/a	22.7±18.6	0.30
Fibrinogen, mg/dL (mean±SD)	449.2±148.3	575.3±135.2	n/a	350.6±87.6	<0.01
CRP, mg/L (mean±SD)	5.8±9.7	4.4±2.2	n/a	0.8±1.4	<0.01

Data are presented as mean±SD. WBC: White Blood Cells; NLR: Neutrophil to Lymphocyte Ratio; BEC: Blood Eosinophil Count; ESR: Erythrocyte Sedimentation Rate; CRP: C-reactive Protein

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