Peripheral blood eosinophils: a surrogate marker for airway eosinophilia in stable COPD

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Introduction: Sputum eosinophilia occurs in approximately one-third of stable chronic obstructive pulmonary disease (COPD) patients and can predict exacerbation risk and response to corticosteroid treatments. Sputum induction, however, requires expertise, may not always be successful, and does not provide point-of-care results. Easily applicable diagnostic markers that can predict sputum eosinophilia in stable COPD patients have the potential to progress COPD management. This study investigated the correlation and predictive relationship between peripheral blood and sputum eosinophilia. It also examined the repeatability of blood eosinophil counts.

Methods: Stable COPD patients (n=141) were classified as eosinophilic or noneosinophilic based on their sputum cell counts (≥3%), and a cross-sectional analysis was conducted comparing their demographics, clinical characteristics, and blood cell counts. Receiver operating characteristic curve analysis was used to assess the predictive ability of blood eosinophils for sputum eosinophilia. Intraclass correlation coefficient was used to examine the repeatability of blood eosinophil counts.

Results: Blood eosinophil counts were significantly higher in patients with sputum eosinophilia (n=45) compared to those without (0.3×10^9/L vs 0.15×10^9/L; P<0.0001). Blood eosinophils correlated with both the percentage (ρ=0.535; P<0.0001) and number of sputum eosinophils (ρ=0.473; P<0.0001). Absolute blood eosinophil count was predictive of sputum eosinophilia (area under the curve =0.76, 95% confidence interval [CI] =0.67–0.84; P<0.0001). At a threshold of ≥0.3×10^9/L (specificity =76%, sensitivity =60%, and positive likelihood ratio =2.5), peripheral blood eosinophil counts enabled identification of the presence or absence of sputum eosinophilia in 71% of the cases. A threshold of ≥0.4×10^9/L had similar classifying ability but better specificity (91.7%) and higher positive likelihood ratio (3.7). In contrast, ≥0.2×10^9/L offered a better sensitivity (91.1%) for ruling out sputum eosinophilia. There was a good agreement between two measurements of blood eosinophil count over a median of 28 days (intra-class correlation coefficient =0.8; 95% CI =0.66–0.88; P<0.0001).

Conclusion: Peripheral blood eosinophil counts can help identify the presence or absence of sputum eosinophilia in stable COPD patients with a reasonable degree of accuracy.

Keywords: sputum eosinophilia, diagnostic accuracy, chronic obstructive pulmonary disease, stability of eosinophil counts

Introduction

Airway eosinophilia, a hallmark feature of asthma, is now a recognized inflammatory pattern in chronic obstructive pulmonary disease (COPD).1–3 Eosinophilic COPD, defined as sputum eosinophils ≥3%, is reported during acute exacerbations in up to 28% of cases,4 and interestingly, in periods of disease stability, it is seen in approximately 34%5 (or 38%)6 of COPD patients. Airway eosinophilia is a reliable predictor...
of responsiveness to inhaled and oral corticosteroid therapies in COPD.6–9

The detection and measurement of airway eosinophilia mostly require the assessment of induced sputum.2 Although sputum induction is considered a direct and reliable method of assessing airway inflammation, it has a number of limitations.10,11 In addition to being unsuitable for point-of-care testing, it requires expertise and may not be always successful (failure rate of up to 30%).10,11 Due to these reasons, the search for minimally invasive and easily applicable diagnostic tools that can predict sputum eosinophilia in COPD and asthma has intensified.4,10,12–15 The use of peripheral blood cell counts as a potential alternative is attracting profound interest owing to its ease of application in clinical practice. The ability of blood eosinophils to predict sputum eosinophilia in patients with asthma has been reported, with promising results.15–18 In COPD, however, very few studies have addressed this, particularly during clinical stability. A recent report in 20 COPD patients and 21 healthy controls has demonstrated the association between bronchial and blood eosinophil counts.19 Studies have also shown the potential ability of blood eosinophils to serve as a marker of response to corticosteroid treatments in exacerbating20 and stable21,22 COPD patients. The clinical characteristics of nonexacerbating COPD patients with persistently elevated levels of blood eosinophils (≥2%) and their longitudinal changes during a follow-up period of 3 years have also been investigated.1 Nevertheless, studies examining the utility of blood eosinophils in detecting sputum eosinophilia in stable COPD are still lacking.

In this study, we hypothesized that peripheral blood eosinophils can serve as a promising surrogate marker for sputum eosinophilia in stable COPD. To test this hypothesis, a cross-sectional analytical study of 141 stable COPD patients was conducted with the aim of investigating the correlation and predictive relationship between peripheral blood and sputum eosinophils. In addition, the stability of peripheral blood eosinophil counts between two measurements over a median period of 28 days was examined.

**Methods**

**Study design**

A cross-sectional analytical study was conducted involving 141 patients with stable COPD (Figure 1). The data for 71 participants were obtained from our previously published studies.23,24 The remaining 70 participants were recruited from the respiratory ambulatory care clinics at John Hunter Hospital (Newcastle, Australia), the clinical research databases of the Priority Research Centre for Asthma and Respiratory Disease at the University of Newcastle and the Hunter Medical Research Institute (Newcastle, Australia), and through community advertisement. All participants provided written informed consent, and ethics approval was obtained from the Human Ethics Research Committees of the Hunter New England Local Health District (12/12/12/3.06) and the University of Newcastle (H-2013-0010).

**Study participants**

Adults (n=141) with stable COPD and paired blood and sputum cell counts, which were obtained from samples collected during the same visit, were included. COPD diagnosis was confirmed by incompletely reversible airflow limitation (post-bronchodilator forced expiratory volume in 1 second [FEV1] <80% predicted and FEV1/forced vital capacity [FVC] ratio of <0.7). Stable COPD was defined as no increase in bronchodilator use, no use of oral corticosteroids or antibiotics, no unscheduled doctor’s visit, or no hospitalization due to COPD in the past 4 weeks. Participants were assessed for demographic features, lung function, airway and peripheral blood inflammatory cell counts, smoking history, body mass index, preceding year exacerbation history, medical history, dyspnea (modified Medical Research Council [mMRC]),25 comorbidities (Charlson Comorbidity Index),26 and health-related quality of life (St George Respiratory Questionnaire).27 The BODE指数 (body mass index, airflow obstruction, dyspnea, severe exacerbation) index was also calculated.28

**Spirometry**

Airflow limitation was assessed using spirometry (Medgraphics, CPFS/D™ USB Spirometer, BreezeSuite v7.1, MGC
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Diagnosics, Saint Paul, MN, USA) to measure pre- and postbronchodilator FEV₁, FVC, and FEV₁/FVC according to the standards of the American Thoracic Society. The third National Health and Nutrition Examination Survey reference equations were used to calculate percent predicted.

Sputum induction and analysis
Sputum was induced using nebulized 4.5% saline in participants whose FEV₁ was ≥1 L, using our previously described methods. In those with FEV₁ <1 L, 0.9% saline was used. Lower respiratory sputum portions were selected and dispersed using dithiothreitol, and total cell count viability was performed. Cytospins were prepared, stained (May-Grunwald-Giemsa), and a differential cell count was obtained from 400 nonsquamous cells. Sputum samples (May-Grunwald–Giemsa), and a differential cell count was used. Lower respiratory sputum portions were selected as significant when viability was performed. Cytospins were prepared, stained (May-Grunwald–Giemsa), and a differential cell count was obtained from 400 nonsquamous cells. Sputum samples were used to determine the agreement between two measurements, approximately half (53.9%) of the participants were in Quadrant D and 24.8% in Quadrant B. Over half (52.5%) were “frequent exacerbators”, having had two or more exacerbations in the past 12 months. Most (128, 90.8%) of the participants were prescribed maintenance inhaled corticosteroids (ICS) or ICS and long-acting β₂ agonist (LABA) combination therapy (ICS/LABA), and of these, 102 (72.3% of the total population) participants were also taking long-acting muscarinic antagonists (LAMA).

Eosinophilic airway inflammation (sputum eosinophil count ≥3%) was present in 45 (31.9%) participants. Clinical characteristics were similar between those with sputum eosinophilia and those without, except for higher BODE index (mMRC ≥2) (P=0.01) in the latter.

Peripheral blood cell counts/ratios
Both the absolute number and percentage proportion of blood eosinophils were significantly higher in eosinophilic COPD compared with noneosinophilic COPD (0.30×10⁹/L vs 0.15×10⁹/L, P<0.0001 and 3.95% vs 2.07%, P<0.0001, respectively) (Table 2, Figure 2A). Noneosinophilic participants had significantly elevated blood neutrophil counts compared with the eosinophilic group (5.3×10⁹/L vs 4.6×10⁹/L, P=0.02). There was no difference in blood lymphocytes (P=0.84) and total white blood cell counts (P=0.32) between the two groups. Participants with sputum eosinophilia had significantly higher blood eosinophil/neutrophil ratio (ENR) and eosinophil/lymphocyte ratio (ELR), whereas those without had significantly higher neutrophil/lymphocyte ratio (Table 2, Figure 2B–D).

Correlation between blood and sputum eosinophils
A significant correlation was found between blood eosinophil counts and the proportion (ρ=0.535; P<0.0001) (Figure 3A) and number of sputum eosinophils (ρ=0.473; P<0.0001)
Table 1 Demographics and clinical characteristics of the study population

<table>
<thead>
<tr>
<th>Variable</th>
<th>All participants (n=141)</th>
<th>Eosinophilic* (n=45)</th>
<th>Noneosinophilic* (n=96)</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>69.8±7.7</td>
<td>68.5±8.0</td>
<td>70.3±7.6</td>
<td>0.20</td>
</tr>
<tr>
<td>Sex (male)</td>
<td>89 (63)</td>
<td>30 (67)</td>
<td>59 (61)</td>
<td>0.58</td>
</tr>
<tr>
<td>Postbronchodilator FEV₁ (% predicted)</td>
<td>57.5±17.9</td>
<td>58.3±18.8</td>
<td>57.1±17.6</td>
<td>0.72</td>
</tr>
<tr>
<td>Postbronchodilator FEV₁ (L)</td>
<td>1.3 (1.12, 1.79)</td>
<td>1.56 (1.09, 1.79)</td>
<td>1.48 (1.12, 1.78)</td>
<td>0.48</td>
</tr>
<tr>
<td>Postbronchodilator FEV₁/FVC ratio (%)</td>
<td>56.55 (43.31, 65.57)</td>
<td>55.26 (44.4, 62.65)</td>
<td>57.12 (42.56, 65.92)</td>
<td>0.46</td>
</tr>
<tr>
<td>Reversibility (L)</td>
<td>0.04 (0.006, 0.10)</td>
<td>0.04 (0.008, 0.11)</td>
<td>0.05 (0.008, 0.11)</td>
<td>0.48</td>
</tr>
<tr>
<td>Reversibility (%)</td>
<td>4.46 (6.63, 10.22)</td>
<td>3.9 (0.0, 7.5)</td>
<td>4.67 (0.9, 11.0)</td>
<td>0.48</td>
</tr>
<tr>
<td>CCI score</td>
<td>3.9±1.1</td>
<td>3.8±1.0</td>
<td>3.9±1.1</td>
<td>0.71</td>
</tr>
<tr>
<td>Frequent vs nonfrequent exacerbators*</td>
<td>74 vs 67 (52.5 vs 47.5)</td>
<td>23 vs 22 (51.1 vs 48.9)</td>
<td>51 vs 45 (53.1 vs 46.9)</td>
<td>0.86</td>
</tr>
<tr>
<td>GOLD grade</td>
<td>0.003</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>11 (7.8)</td>
<td>3 (6.7)</td>
<td>8 (8.3)</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>74 (52.4)</td>
<td>24 (53.3)</td>
<td>50 (52.1)</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>45 (31.9)</td>
<td>15 (33.3)</td>
<td>30 (31.3)</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>11 (7.8)</td>
<td>3 (6.7)</td>
<td>8 (8.3)</td>
<td></td>
</tr>
<tr>
<td>GOLD quadrant</td>
<td></td>
<td></td>
<td></td>
<td>0.003</td>
</tr>
<tr>
<td>A</td>
<td>9 (6.4)</td>
<td>5 (11.1)</td>
<td>4 (4.2)</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>35 (24.8)</td>
<td>7 (15.6)</td>
<td>28 (29.2)</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>21 (14.9)</td>
<td>13 (28.9)</td>
<td>8 (8.3)</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>76 (53.9)</td>
<td>20 (44.4)</td>
<td>56 (58.3)</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>30.2 (25.5, 34.7)</td>
<td>30.4 (24.3, 33.3)</td>
<td>30.2 (26.1, 35.9)</td>
<td>0.32</td>
</tr>
<tr>
<td>Ex-smokers</td>
<td>116 (82.3)</td>
<td>36 (80)</td>
<td>80 (83.3)</td>
<td>0.64</td>
</tr>
<tr>
<td>Pack years</td>
<td>37.5 (13.8, 62.5)</td>
<td>35.3 (9.8, 61.3)</td>
<td>30.4 (13.9, 63.4)</td>
<td>0.51</td>
</tr>
<tr>
<td>SGROA</td>
<td>49.7±16.9</td>
<td>45.7±18.0</td>
<td>51.6±16.1</td>
<td>0.052</td>
</tr>
<tr>
<td>BODEx</td>
<td>2 (1.5)</td>
<td>2 (1.3)</td>
<td>3 (2.5)</td>
<td>0.003</td>
</tr>
<tr>
<td>mMRC score ≥2</td>
<td>96 (68.1)</td>
<td>24 (53.3)</td>
<td>72 (75.0)</td>
<td>0.01</td>
</tr>
<tr>
<td>ICS or ICS/LABA combination use</td>
<td>128 (90.8)</td>
<td>41 (91.1)</td>
<td>87 (96.0)</td>
<td>0.60</td>
</tr>
<tr>
<td>ICS dose (µg/d)</td>
<td>500 (250, 500)</td>
<td>500 (250, 500)</td>
<td>500 (250, 500)</td>
<td>0.74</td>
</tr>
<tr>
<td>LAMA use</td>
<td>110 (78.0)</td>
<td>35 (77.8)</td>
<td>75 (78.1)</td>
<td>0.56</td>
</tr>
<tr>
<td>Prior history of asthma</td>
<td>69 (48.9)</td>
<td>23 (51.1)</td>
<td>46 (47.9)</td>
<td>0.86</td>
</tr>
</tbody>
</table>

Notes: *Participants with sputum eosinophil count ≥3%; †participants with sputum eosinophil count <3%; ‡Frequent exacerbators: ≥2 exacerbations in the previous year, and nonfrequent exacerbators: <2 exacerbations in the previous year; †ICS dose calculated as beclomethasone equivalents where 1 µg of beclomethasone = 1 µg budesonide = 0.5 µg fluticasone; ‡P-value for the comparison of eosinophilic vs noneosinophilic COPD. Data expressed as mean ± SD, median (interquartile range) or as number (%).

Abbreviations: FEV₁, forced expiratory volume in 1 second; FVC, forced vital capacity; CCI, Charlson comorbidity index; GOLD, Global Initiative for Chronic Obstructive Lung Disease; BMI, body mass index; SGROA, St George Respiratory Questionnaire; BODEx, body mass index, airflow obstruction, dyspnea, severe exacerbation; mMRC, modified Medical Research Council; ICS, inhaled corticosteroids; LABA, long-acting β₂ agonist; LAMA, long-acting muscarinic antagonists; SD, standard deviation.

(Figure 3B). Similarly, percentage sputum eosinophils correlated reasonably well with both blood ELR (p=0.488; P<0.0001) and blood ENR (p=0.592; P<0.0001). No significant association was observed between percentage sputum neutrophils and blood neutrophil/lymphocyte ratio (p=0.0287; P=0.7355).

Table 2 Blood cell count parameters and blood cell ratios

<table>
<thead>
<tr>
<th>Blood cell counts/ratios</th>
<th>All participants (n=141)</th>
<th>Eosinophilic* (n=45)</th>
<th>Noneosinophilic* (n=96)</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood eosinophils (×10⁹/L)</td>
<td>0.20 (0.10, 0.30)</td>
<td>0.30 (0.20, 0.40)</td>
<td>0.15 (0.10, 0.20)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Blood eosinophils (%)</td>
<td>2.70 (1.56, 3.92)</td>
<td>3.95 (2.94, 5.08)</td>
<td>2.07 (1.35, 3.25)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Blood neutrophils (×10⁹/L)</td>
<td>5.08±1.67</td>
<td>4.60±1.53</td>
<td>5.30±1.70</td>
<td>0.02</td>
</tr>
<tr>
<td>White blood cells (×10⁹/L)</td>
<td>7.62±1.95</td>
<td>7.38±1.77</td>
<td>7.73±2.03</td>
<td>0.32</td>
</tr>
<tr>
<td>Blood lymphocytes (×10⁹/L)</td>
<td>1.78±0.65</td>
<td>1.76±0.58</td>
<td>1.78±0.68</td>
<td>0.84</td>
</tr>
<tr>
<td>Blood NLR</td>
<td>2.84 (2.15, 3.85)</td>
<td>2.59 (2.07, 3.23)</td>
<td>3.00 (2.24, 4.00)</td>
<td>0.04</td>
</tr>
<tr>
<td>Blood ELR</td>
<td>0.12 (0.07, 0.18)</td>
<td>0.15 (0.13, 0.24)</td>
<td>0.09 (0.06, 0.16)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Blood ENR</td>
<td>0.04 (0.02, 0.06)</td>
<td>0.07 (0.04, 0.08)</td>
<td>0.03 (0.02, 0.05)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Notes: *Participants with sputum eosinophil count ≥3%; †participants with sputum eosinophil count <3%; ‡P-value for the comparison of eosinophilic vs noneosinophilic COPD. Data expressed as mean ± SD, median (interquartile range) or as number (%).

Abbreviations: NLR, neutrophil/lymphocyte ratio; ELR, eosinophil/lymphocyte ratio; ENR, eosinophil/neutrophil ratio; SD, standard deviation.

Receptor operating characteristic (ROC) curve analysis

Absolute blood eosinophil count was predictive of sputum eosinophilia with AUC of 0.76 (95% confidence interval [CI] =0.67–0.84; P<0.0001) (Figure 4). Percentage blood eosinophils, blood ELR, and blood ENR were also predictive of
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Sputum eosinophilia with AUCs of 0.80 (95% CI = 0.73–0.86), 0.74 (95% CI = 0.65–0.83), and 0.81 (95% CI = 0.73–0.89), respectively. The sensitivities and specificities of different cutoff points of absolute blood eosinophil counts were evaluated together with their ability to correctly classify patients (Table 3). A summary of sensitivity and specificity for percentage blood eosinophils and blood ENR of different cutoff points is provided in the Supplementary material (Tables S1 and S2). The absolute blood eosinophil count threshold that balanced sensitivity and specificity on the ROC curve was found to be $0.3 \times 10^9$/L (300/μL), with a sensitivity of 60%, specificity of 76%, and a positive likelihood ratio of 2.5.

Figure 2 Scatter dot plot comparing.

Notes: (A) Blood eosinophil count, (B) eosinophil/neutrophil ratio, (C) eosinophil/lymphocyte ratio, and (D) neutrophil/lymphocyte ratio between eosinophilic ($\geq$3% sputum eosinophils) and noneosinophilic COPD (<3% sputum eosinophils). Graphs represent individual data points and a median as a bar (red).

Abbreviations: ENR, eosinophil/neutrophil ratio; ELR, eosinophil/lymphocyte ratio; NLR, neutrophil/lymphocyte ratio.

Figure 3 Scatter plots for correlations between sputum and blood eosinophil counts.

Notes: (A) Correlation between percentage sputum eosinophils and absolute blood eosinophil count ($\times 10^9$/L). Vertical dotted line represents upper limit of normal for percentage sputum eosinophils. (B) Correlation between absolute sputum eosinophil counts ($\times 10^4$/mL) and absolute blood eosinophil count ($\times 10^9$/L).
At this cutoff point, blood eosinophil counts enabled the correct identification of the presence or absence of sputum eosinophilia in 71 cases out of 100. A higher cutoff point of $\geq 0.4 \times 10^9$/L (400/$\mu$L) gave a greater specificity (91.7%), a higher positive likelihood ratio (3.7), and an essentially similar classifying ability. In contrast, a higher degree of sensitivity (91.1%) was achieved at a peripheral blood eosinophil cutoff point of $0.2 \times 10^9$/L (200/$\mu$L).

Based on the peripheral blood eosinophil threshold of $\geq 0.3 \times 10^9$/L, 76% of the noneosinophilic participants (73 out of 96) would be correctly characterized as not having sputum eosinophilia (true negatives) while the remaining 24% as false positives. On the other hand, 60% of the eosinophilic participants (27 out of 45) would be accurately identified as having sputum eosinophilia (true positives) while the remaining 40% as false negatives. Two-by-two contingency tables for the blood eosinophil cutoff points of $0.2 \times 10^9$/L and $0.4 \times 10^9$/L are provided in the Supplementary material (Tables S3 and S4).

Clinical characteristics of participants classified by blood eosinophil counts

Blood eosinophilia (blood eosinophil count $\geq 0.4 \times 10^9$/L) was present in 22 (15.6%) participants (Table 4). Patients with blood eosinophilia had a higher postbronchodilator FEV$_1$% predicted and a lower BODE index score compared to those without ($< 0.4 \times 10^9$/L). All other clinical parameters were similar between the two groups. There were no differences in blood eosinophil counts between males and females ($0.2 [0.1, 0.3] \times 10^9$/L and 0.2 [0.1, 0.3] $\times 10^9$/L; $P=0.9087$), ex-smokers and never smokers ($0.2 [0.1, 0.3] \times 10^9$/L and 0.2 [0.1, 0.3] $\times 10^9$/L; $P=0.8848$), frequent and nonfrequent exacerbators ($0.2 [0.1, 0.3] \times 10^9$/L and 0.2 [0.1, 0.3] $\times 10^9$/L; $P=0.96$), or between patients taking ICS/LABA and those who did not ($0.2 [0.1, 0.3] \times 10^9$/L and 0.1 [0.1, 0.2] $\times 10^9$/L; $P=0.221$).

Stability study

This study also assessed the stability of peripheral blood eosinophil counts in those participants who had repeated measurements of blood cell counts obtained from blood samples collected at two visits spaced a median of 28 (22.5, 35.5) days apart (n=46). There was a good agreement between the two measurements, with an intraclass correlation coefficient of 0.8 (95% CI =0.66–0.88; $P<0.0001$). The bias of measurement was negligible (0.002±0.13 [×10^9]/L), with equal scatter around the bias line, indicating no systematic measurement bias (Figure 5).

Discussion

This study, which assessed the ability of peripheral blood eosinophils for detecting sputum eosinophilia in stable COPD, had three main findings. First, peripheral blood eosinophil count was shown to distinguish patients with sputum eosinophilia from those without, thereby indicating its potential use as a diagnostic biomarker for eosinophilic COPD. Second,
Table 4 Demographics and clinical characteristics of participants with and without blood eosinophilia

<table>
<thead>
<tr>
<th>Variable</th>
<th>Participants with blood eosinophilia (≥0.4×10^9/L)</th>
<th>Participants without blood eosinophilia (&lt;0.4×10^9/L)</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>22 (15.6)</td>
<td>119 (84.4)</td>
<td>NA</td>
</tr>
<tr>
<td>Age (years)</td>
<td>70.1±7.5</td>
<td>69.7±7.8</td>
<td>0.84</td>
</tr>
<tr>
<td>Sex (male)</td>
<td>13 (59.1)</td>
<td>76 (63.9)</td>
<td>0.81</td>
</tr>
<tr>
<td>Postbronchodilator FEV₁ (% predicted)</td>
<td>65.1±19.3</td>
<td>56.1±17.4</td>
<td>0.03</td>
</tr>
<tr>
<td>Postbronchodilator FEV₁ (L)</td>
<td>1.56 (1.2, 1.88)</td>
<td>1.48 (1.12, 1.79)</td>
<td>0.31</td>
</tr>
<tr>
<td>Postbronchodilator FEV₁/FVC ratio (%)</td>
<td>58.82±14.83</td>
<td>54.6±14.79</td>
<td>0.23</td>
</tr>
<tr>
<td>Reversibility (L)</td>
<td>0.025 (0, 0.12)</td>
<td>0.045 (0.01, 0.1)</td>
<td>0.51</td>
</tr>
<tr>
<td>Reversibility (%)</td>
<td>2.5 (0, 12.03)</td>
<td>4.55 (0.95, 9.66)</td>
<td>0.51</td>
</tr>
<tr>
<td>CCI score</td>
<td>4 (3.5)</td>
<td>4 (3.4)</td>
<td>0.14</td>
</tr>
<tr>
<td>Frequent vs nonfrequent exacerbators*</td>
<td>13 vs 9 (59.1, 40.9)</td>
<td>61 vs 58 (51.2, 48.8)</td>
<td>0.64</td>
</tr>
<tr>
<td>Number of severe exacerbation</td>
<td>0 (0, 1)</td>
<td>0 (0, 1)</td>
<td>0.60</td>
</tr>
<tr>
<td>GOLD grade</td>
<td></td>
<td></td>
<td>0.23</td>
</tr>
<tr>
<td>I</td>
<td>4 (18.2)</td>
<td>7 (5.9)</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>12 (54.5)</td>
<td>62 (52.1)</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>5 (22.7)</td>
<td>40 (33.6)</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>1 (4.5)</td>
<td>10 (8.4)</td>
<td></td>
</tr>
<tr>
<td>GOLD quadrant</td>
<td></td>
<td></td>
<td>0.16</td>
</tr>
<tr>
<td>A</td>
<td>0 (0)</td>
<td>9 (7.6)</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>3 (13.6)</td>
<td>32 (26.9)</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>6 (27.3)</td>
<td>15 (12.6)</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>13 (59.1)</td>
<td>63 (52.9)</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>31.1 (26.7, 34.5)</td>
<td>30.2 (25.5, 34.9)</td>
<td>0.91</td>
</tr>
<tr>
<td>Ex-smokers</td>
<td>19 (86.4)</td>
<td>97 (81.5)</td>
<td>0.77</td>
</tr>
<tr>
<td>Pack years</td>
<td>22.3 (9.42)</td>
<td>38.8 (15, 65)</td>
<td>0.06</td>
</tr>
<tr>
<td>SGRQ</td>
<td>44.2 (32.8, 66.6)</td>
<td>50.2 (38, 61.1)</td>
<td>0.89</td>
</tr>
<tr>
<td>BODEx</td>
<td>2 (1.3)</td>
<td>3 (1.5)</td>
<td>0.047</td>
</tr>
<tr>
<td>mMRC score ≥ 2</td>
<td>12 (54.5)</td>
<td>84 (59.6)</td>
<td>0.14</td>
</tr>
<tr>
<td>ICS or ICS/LABA combination use</td>
<td>22 (100)</td>
<td>106 (89.1)</td>
<td>0.22</td>
</tr>
<tr>
<td>ICS dose* (μg/d)</td>
<td>500 (400, 500)</td>
<td>500 (250, 500)</td>
<td>0.16</td>
</tr>
<tr>
<td>LAMA use</td>
<td>16 (72.7)</td>
<td>94 (79)</td>
<td>0.52</td>
</tr>
<tr>
<td>Prior history of asthma</td>
<td>12 (55)</td>
<td>57 (48)</td>
<td>0.65</td>
</tr>
</tbody>
</table>

Notes: *Frequent exacerbators: ≥2 exacerbations in the previous year, and nonfrequent exacerbators: <2 exacerbations in the previous year; †ICs dose calculated as beclomethasone equivalents where 1 μg of beclomethasone = 1 μg budesonide = 0.5 μg fluticasone. Data expressed as mean ± standard deviation, median (interquartile range) or as number (%); *P-value for the comparison of participants with and without blood eosinophilia.

Abbreviations: FEV₁, forced expiratory volume in 1 second; FVC, forced vital capacity; CCI, Charlson Comorbidity Index; GOLD, Global Initiative for Chronic Obstructive Lung Disease; BMI, body mass index; SGRQ, St George Respiratory Questionnaire; BODEx, body mass index, airflow obstruction, dyspnea, severe exacerbation; mMRC, modified Medical Research Council; ICS, inhaled corticosteroids; LABA, long-acting β₂ agonist; LAMA, long-acting muscarinic antagonists; NA, not applicable.

Figure 5 Bland–Altman plot showing the difference between the absolute blood eosinophil counts of two measurements against the mean of the absolute blood eosinophil counts of the two measurements.

Notes: The blue line represents the mean of differences (bias line). Horizontal dotted lines represent the 95% limits of agreement (mean difference ±1.96 SD).

Abbreviation: SD, standard deviation.

we have shown that blood eosinophil counts and their ratios (ELR and ENR) are elevated in eosinophilic COPD and correlate reasonably well with sputum eosinophilia. Third, blood eosinophil counts between two measurements over a median period of 28 days were found to be stable.

The diagnostic performance of peripheral blood eosinophils in identifying eosinophilic airway inflammation in mild, moderate, and severe asthma has been previously investigated.13–18,34 Studies examining the potential utility of blood eosinophils in COPD, particularly during stable conditions, are however few.3,19–21 One study in COPD has shown that peripheral percentage blood eosinophil count (>2%) can serve as a sensitive biomarker to determine sputum eosinophilia (>3%) during exacerbations (AUC 0.85 [95% CI =0.78–0.93], sensitivity =90%, specificity =60%).4 This AUC result is similar to our present AUC result in stable COPD.
As noted by Korevaar et al,
optimal cutoff points for
diagnostic biomarkers of airway eosinophilia selected by
balancing sensitivity and specificity on a ROC curve may
not be clinically applicable, given that their sensitivity and/or
specificity is often suboptimal compared to that of reference
standard tests, such as bronchoalveolar lavage and sputum
induction. From the clinical point of view, the choice of a
cutoff point is also partly determined by the clinical question. In
view of this, we have evaluated the sensitivity and specificity
of blood eosinophil counts at different cutoff points (Table 3).
According to our data, in patients with stable COPD, peripheral
blood eosinophil counts can help correctly identify the
presence or absence of sputum eosinophilia in 71 cases
out of 100 at cutoff points of \( \geq 0.3 \times 10^9/L \) or \( \geq 0.4 \times 10^9/L \).
Nevertheless, the higher cutoff point had a higher positive
predictive value (PPV) (number of true positives/[number of
true positive + number of false positives]) and a much better
specificity to rule in sputum eosinophilia. This implies that
patients with blood eosinophil counts above the threshold of
\( 0.4 \times 10^9/L \) would most likely have COPD with eosinophilic
airway inflammation. Akin to the suggestion of Fowler et al.,
such patients may not necessarily need to undergo induction
of sputum for the assessment of airway eosinophilia. Patients
with blood eosinophil count of \( < 0.4 \times 10^9/L \) may however
need further assessment because the proportion of false nega-
tives at the cutoff point of \( 0.4 \times 10^9/L \) is high (~69%). As a
matter of interest, a similar blood eosinophil cutoff point of
\( \geq 0.45 \times 10^9/L \) has been reported to help correctly identify
sputum eosinophilia in patients with severe asthma with a
specificity of 97%, sensitivity of 49.3%, and PPV of 89.2.
Another recent study has also reported a blood eosinophil
cutoff point of \( \geq 0.41 \times 10^9/L \) (specificity of 95%, sensitivity of
36%, and PPV of 79) for detecting sputum eosinophilia in a
population of asthmatic patients of different phenotypes.

For a clinician who wants to rule out sputum eosinophilia,
on the other hand, a peripheral blood eosinophil cutoff point
with a high sensitivity would be of interest. Based on our
results, patients are unlikely to have sputum eosinophilia if
their blood eosinophil count is below \( 0.2 \times 10^9/L \) because the
sensitivity at the cutoff point of \( 0.2 \times 10^9/L \) is 91.1%.

It is interesting to note that in our study, there was discord-
cance between sputum and blood eosinophils in approxi-
mately 40% of the patients with eosinophilic COPD at a
peripheral blood eosinophil cutoff point of \( 0.3 \times 10^9/L \). This
observation is similar to that of a recent study in patients
with uncontrolled asthma where one-third of the participants
exhibited discordance between blood and sputum eosino-
phils.\(^{35}\) In order to gain a better insight into our true-positive
(27 patients) and false-negative (18 patients) cohorts, their
demographic features, clinical characteristics, and sputum
cell counts were compared. The results nevertheless revealed
no significant difference between the two groups (Table S5).
However, it should be highlighted that this analysis may have
been underpowered due to small sample size.

Plausible causes for the discordance between sputum and
blood eosinophils may include the imbalance between the
production and subsequent clearance of eosinophils
by airway macrophages\(^{36}\) and variations in the process of
recruitment of eosinophils into the airways.\(^{35}\) The fact that
only a proportion of the eosinophilic COPD patients had
blood eosinophilia may suggest the involvement of more than
one distinct biological mechanisms underlying eosinophilic
airway inflammation within this COPD phenotype.\(^{37}\) As in
the case of asthma, Th2 cytokines could be responsible for
inflammation in eosinophilic COPD.\(^{37}\) Nevertheless, eosino-
philic airway inflammation in the absence of elevated levels
of Th2 has also been reported in some COPD patients.\(^{38}\) One
potential mechanism for the non-Th2 eosinophilic inflamma-
tion in COPD could be the epithelial-innate lymphoid cell
type 2 (ILC2) pathway, which has been suggested to play
a similar role in severe nonallergic asthma.\(^{39}\) Obviously,
further investigation is warranted to understand the mecha-
nism underlying the different endotypes of the eosinophilic
COPD phenotype.

Our data demonstrated that blood eosinophils and their
ratios (ELR and ENR) were elevated in patients with sputum
eosinophilia compared with those without. Absolute blood
eosinophil counts correlated reasonably well with both the
absolute sputum counts and percentage sputum eosinophils,
which is in agreement with the findings of Wagener et al,\(^{18}\)
Zhang et al,\(^{16}\) and Fowler et al\(^{17}\) in asthma, but not with those
of Hastic et al\(^{14}\) and Amorim et al.\(^{40}\) In accordance with a
previous finding in asthma,\(^{16}\) in our study, both blood ENR
and ELR also correlated with percentage sputum eosinophils
and were predictive of eosinophilic COPD with AUCs of
0.81 and 0.74, respectively. Recently, Khatri et al\(^{41}\) have
suggested that ratios of blood cell types, such as ELR and
ENR, may minimize variations associated with measurement,
sample processing, and therapies and yield a more accurate
diagnostic performance over actual blood cell counts. This
certainly will be a topical issue for future studies in COPD.

According to Price et al,\(^{42}\) blood eosinophilia (defined
as \( \geq 0.5 \times 10^9/L \)) occurs in 10% of stable COPD patients and
is associated with higher rate of exacerbations, particularly in
nonsmokers receiving maintenance therapy. Elevated levels of
blood eosinophils in COPD patients have also been associated
with increased risk of mortality from exacerbations. Nevertheless, our analysis indicated no significant difference in blood eosinophil counts between frequent and nonfrequent exacerbators. Similarly, there was no difference in the number of severe exacerbations in the past 12 months between patients with blood eosinophilia ($\geq 0.4 \times 10^9/L$) and those without ($<0.4 \times 10^9/L$). It is worth noting here that up to 90% of our study population was using either ICS/LABA alone or in combination with LAMA, which are known to reduce the risk of exacerbation. All things considered, the aforementioned discrepancies may possibly be due to differences in the characteristics of study populations.

In our study, the stability of peripheral blood eosinophil counts between two measurements over a median period of 28 days was found to be acceptable. This implies that a single measurement of blood eosinophil count may provide indicative information about eosinophilic airway inflammation status in stable COPD. The stability of peripheral blood eosinophil counts in repeated measurements in COPD population has been reported in other studies as well.\textsuperscript{20,21}

### Conclusion

In this study, we found a predictive relationship between blood and sputum eosinophils in stable COPD. Peripheral blood eosinophils were stable between two measurements, suggesting that a single blood eosinophil count may potentially serve as a reliable marker for eosinophilic COPD. A potential limitation of this study may be the fact that we did not have bronchoalveolar lavage or endobronchial biopsy samples for the assessment of airway eosinophilia.

The clinical relevance of our work lies in the fact that a simple blood test may allow the clinician to positively diagnose eosinophilic COPD. In relation to this, the question of when to use ICS in COPD is of high clinical importance. Biomarkers that allow for the identification of patients who are most likely to respond to ICS, and consequently minimize harm arising from inappropriate treatment, have the potential to significantly progress COPD management. In this regard, it will be interesting and a worthwhile endeavor to examine ICS response at different blood eosinophil thresholds in future prospective studies.

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### References


