

Full blood count parameters for the detection of asthma inflammatory phenotypes

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Summary

Background In asthma, the airway inflammatory phenotype influences clinical characteristics and treatment response. Although induced sputum is the gold standard test for phenotyping asthma, a more accessible method is needed for clinical practice.

Objective To investigate whether white blood cell counts and/or their derived ratios can predict sputum eosinophils or neutrophils in uncontrolled asthma.

Methods This cross-sectional study evaluated 164 treated but uncontrolled asthmatic patients with sputum induction and blood collection. Receiver-operating characteristic (ROC) curves were used to assess the relationship between blood and sputum parameters.

Results There was a significant positive relationship between blood eosinophil parameters and the percentage of sputum eosinophil count. A weak but significant correlation was found between sputum neutrophil percentage and blood neutrophil percentage ($r = 0.219$, $P = 0.005$). ROC curve analysis identified that blood eosinophil percentage count was the best predictor for eosinophilic asthma, with an area under the curve (AUC) of 0.907

($P < 0.001$). The optimum cut-point for blood eosinophil percentage was 2.7%, and this yielded a sensitivity of 92.2% and a specificity of 75.8%. The absolute blood eosinophil count was also highly predictive with an AUC of 0.898 ($P < 0.0001$) at a blood eosinophil cut-off of $0.26 \times 10^9/L$. The blood eosinophil/lymphocyte ratio (ELR) and eosinophil/neutrophil ratio (ENR) were increased in eosinophilic asthma, and the neutrophil/lymphocyte ratio (NLR) was increased in neutrophilic asthma. Neutrophilic asthma could also be detected by blood neutrophil percentages and NLR, but with less accuracy.

Conclusions and Clinical Relevance Blood eosinophil counts and derived ratios (ELR and ENR) can accurately predict eosinophilic asthma in patients with persistent uncontrolled asthma despite treatment. Blood neutrophil parameters are poor surrogates for the proportion of sputum neutrophils. Blood counts may be a useful aid in the monitoring of uncontrolled asthma.

Keywords asthma, blood, eosinophil, neutrophil, sputum

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Introduction

Asthma is a heterogeneous chronic inflammatory condition of the airways. Inflammatory subtypes are recognized based on sputum eosinophil and neutrophil proportions and include eosinophilic asthma, neutrophilic asthma, mixed granulocytic asthma and paucigranulocytic asthma [1]. These different subtypes of inflammation may have different exacerbation risks [2] and different responses to treatment. Although the presence of inflammatory cells in the airways does not diagnose asthma, their measurement is especially useful for the clinical assessment of bronchitis, guiding treatment with corticosteroids [3] and long-term therapy of asthma [4].

Induced sputum cell counts are the gold standard test for defining inflammatory phenotypes. However, access to this test is limited, and there is a need to find simpler methods to assess airway inflammation. Peripheral blood cell counts may be a useful, non-invasive procedure to quantify inflammatory cells to detect the subtypes of inflammation in asthma [5]. This is biologically plausible as eosinophils are produced in the bone marrow and transported by the blood to the airway [6], and blood eosinophilia is recognized as one of the characteristic features of asthma [7, 8]. The epidemiological study on the genetics and environment in asthma (EGEA) found that four blood inflammatory patterns could be defined in the same way as the four subtypes of inflammation in sputum proposed by Simpson *et al.* [1]. These four blood phenotypes showed marked differences in asthma phenotype characteristics [9], suggesting that blood count may be a useful way to detect airway inflammatory phenotype. However, that study did not relate blood counts to sputum cell counts.

Sputum examination has been considered more accurate than measurement of peripheral blood eosinophils for the diagnosis of eosinophilic asthma [7]. However, three recent studies have shown significant, positive associations between blood and sputum eosinophils [10–12] and that the peripheral eosinophil count could predict the eosinophil phenotype in steroid-naïve non-atopic asthma with moderate accuracy [12]. The EXTRA Study also suggested that people with higher baseline eosinophil counts in peripheral blood obtained more benefit from omalizumab therapy than those with lower counts [13]. One study did not find a significant relationship between the peripheral blood and sputum neutrophil counts [11].

Subject heterogeneity may be responsible for the differing results in these studies. In clinical practice, the detection of asthma airway inflammatory phenotype will be most useful in patients who are uncontrolled on maintenance inhaled corticosteroid therapy, where add-on therapy is being considered. Consequently, the present

study has focused on the use of blood counts in uncontrolled asthma. In addition, we evaluated novel blood cell parameters derived from a full blood count that have been useful in other diseases. Inflammatory cell ratios [14–16], such as the neutrophil/lymphocyte ratio (NLR), have been associated with poor clinical outcomes in several cancers [14], but the relationship between this parameter and the inflammatory phenotype in asthma is unknown. The NLR is considered to reflect a systemic inflammatory response, which can occur in asthma [15]. This study tested the hypothesis that circulating blood cells, and/or their ratios can reflect the airway inflammatory phenotype in patients with poorly controlled asthma.

Materials and methods

Study design and participants

This cross-sectional study recruited eligible patients aged 18–75 years with asthma ($n = 164$). The diagnosis of asthma was established using the American Thoracic Society guidelines based upon all of current episodic respiratory symptoms (past 12 months), doctor's diagnosis and evidence of variable airflow obstruction [17] (airway hyperresponsiveness, bronchodilator response or diurnal variation of peak expiratory flow (PEF)). They also were prescribed maintenance inhaled corticosteroid treatment and remained uncontrolled with an Asthma Control Questionnaire 6 (ACQ 6) score > 0.7 . Participants with an $FEV_1 < 40\%$ predicted, current smokers, ex-smokers who had ceased smoking in the previous year and those with a recent (past 4 weeks) exacerbation or respiratory infection were excluded. Those with significant smoking-related airspace disease (ex-smokers > 10 pack-year history and $DLC0/VA < 70\%$ predicted or smoking history > 10 pack-years and exhaled carbon monoxide > 10 ppm) were also excluded. Participants underwent a clinical assessment, allergy skin test, spirometry with bronchodilator response, sputum induction and blood sampling in turn. All tests were performed on the same day. Measurements were carried out by observers blinded to other results. Subjects gave written informed consent (the ethics approval number is 08/11/19/3.03), and the study received approval from National Health and Medical Research Council (NHMRC project Grant 569246).

Sputum induction

Spirometry (Medgraphics, CPFS/D™ usb Spirometer, BreezeSuite v7.1, Saint Paul, USA) and induced sputum were performed. Sputum was induced by the inhalation of hypertonic saline (4.5%) as described by Gibson *et al.* [18]. Subjects received a standardized mean nebulization time of 13.7 min.

Sputum analysis

Sputum was processed as described [18]. Selected sputum portions were dispersed by dithiothreitol (DTT; Merck millipore, Darmstadt, Germany) and a total cell count performed. Cytospins were prepared and stained with May Grunwald and Giemsa. Cell counts were performed on 400 non-squamous cells. Cell viability and differential cell counts were recorded.

Blood samples

Venous blood samples were collected in ethylenediaminetetraacetic acid (EDTA) anti-coagulated tubes after sputum induction. An automated analyzer (Beckman Coulter LH 780, Miami, FL, USA) determined the differential white blood cell count.

Asthma subtypes defined using sputum eosinophils and neutrophils

Eosinophilic asthma was defined as sputum eosinophils $\geq 3\%$. Neutrophilic asthma was defined as sputum neutrophils $\geq 61\%$. Participants with increased eosinophils and neutrophils were classified as mixed granulocytic asthma. Those with normal levels of both eosinophils and neutrophils were classified as having paucigranulocytic asthma.

Statistical analyses

The results are expressed as mean $\pm$ SD for continuous variables and median with interquartile range when data were not normally distributed. Categorical data were reported using frequencies and percentages. A Kruskal–Wallis test was performed in the different subgroups of subjects with asthma. Spearman's rank correlation coefficient was used to assess the association between blood cell count and sputum cell count. Results were reported as significant when $P < 0.05$. The performance characteristics of the blood count variables were examined by receiver-operating characteristic (ROC) curves to determine the concentrations of eosinophils, neutrophils or monocytes which best defined eosinophilic asthma, neutrophilic asthma or non-eosinophilic asthma based on the sputum cell count. Data analysis was performed using SPSS software, Version 20.0 of the SPSS System for Macintosh.

Results

We evaluated 164 subjects with uncontrolled asthma who underwent sputum induction and blood sample collection (Table 1). The median age of subjects was 59.8 years. Sixty-one (37%) were male, and 42% were

Table 1. Demographic characteristics of the participants

N	164
Sex, M/F (%M)	61/103 (37%)
Age, years	59.80 (51.20–67.54)
Prior smoking, Y/N (%ex)	66/93 (42%)
Pack-years, years	11.3 (2.50–28.0)
BMI, kg/m ²	30.72 $\pm$ 6.79
Atopy, n (%)	111 (71%)
ACQ-6	1.67 (1.17–2.33)
FEV ₁ % predicted	73.8 $\pm$ 20.4
FEV ₁ /FVC, %	68.9 $\pm$ 10.5
PD ₁₅ , mL, median (Q1, Q3)	5.37 (2.55, 8.97)
Change FEV ₁ post short-acting beta agonist, % median (Q1, Q3)	17.0 (13.3, 23.0)
ICS therapy	
ICS dose (μ g)	1200 (800–2000)
Low dose, n (%)	24 (14.1%)
Moderate dose	57 (33.5%)
High dose	83 (48.8%)
Oral steroids, n (%)	6 (3.5%)

BMI, body mass index; FEV₁, forced expiratory volume in 1 s; FVC, forced vital capacity; ACQ, Asthma Control Questionnaire; ICS, inhaled corticosteroids.

Data are presented as mean $\pm$ SD or median (range).

Low dose: ≤ 500 μ g/day beclomethasone; moderate dose: 500–1000 μ g/day beclomethasone; high dose: >1000 μ g/day beclomethasone.

ex-smokers with an average 11.3 pack-year smoking history. The mean (SD) predicted FEV₁ was 73.8% (20.4%). Of the 157 patients who underwent allergy skin testing, 111 (71%) had atopy. All subjects took inhaled corticosteroid, 98.5% (266/270) were taking LABA, and 3.7% (6/164) used maintenance oral corticosteroids. Evidence of variable airflow obstruction was observed in 160 participants, 100 of whom demonstrated a significant response to bronchodilator, a further 30 had airways hyperresponsiveness, and 30 demonstrated peak flow or FEV₁ variability.

An adequate sputum sample (viability $>40\%$ and squamous count $<50\%$) was obtained in 138 (84.1%) participants. The statistical analysis was initially performed using only these 138 subjects; however, as this gave similar results when all 164 subjects' blood test results were used, we chose the bigger number of subjects to report. Total cell and viability data were not available in 11 subjects due to the small volume of lower airway plugs available, and the percentage of cells on direct smear was used to assign inflammatory phenotype for these participants. Subjects were classified as having eosinophilic asthma ($n = 71$, 43.3%), neutrophilic asthma ($n = 25$, 15.2%), mixed granulocytic asthma ($n = 14$, 8.5%) and paucigranulocytic asthma ($n = 54$, 32.9%). Clinical features among the four inflammatory subtypes were similar except for higher lung function in the paucigranulocytic phenotype (data not shown). The median

of ACQ-6 was 1.67; there were no significant differences between inflammatory phenotypes ($P > 0.05$).

Blood cell profiles

The total white blood cell counts were similar across phenotypes. Eosinophilic and mixed granulocytic asthma both showed an increase in the number and proportion of circulating eosinophils ($P < 0.001$, Table 2). Subjects with NA had a higher percentage of blood neutrophils compared with all other phenotypes, but the absolute neutrophil count was not different. This change in blood neutrophil percentage was explained by a reduction in the number and proportion of eosinophils compared with eosinophilic phenotypes and a reduction in the number and proportion of lymphocytes compared with eosinophilic and paucigranulocytic asthma. Monocytes were similar across groups.

Blood inflammatory cell ratios

The blood neutrophil/lymphocyte ratio (NLR) and platelet/lymphocyte ratio (PLR) were significantly increased in neutrophilic asthma compared with paucigranulocytic asthma (Table 2, Fig. 1). In contrast to the sputum results, the blood neutrophil/monocyte ratio (NMR) was not significantly different between phenotypes. The blood eosinophil/lymphocyte ratio (ELR), eosinophil/neutrophil ratio (ENR) and eosinophil/monocyte ratio (EMR) were significantly higher in eosinophilic and mixed granulocytic asthma compared with neutrophilic asthma (Fig. 1). The blood monocyte/lymphocyte ratio (MLR) was not different between groups.

Associations between blood and sputum cell profiles

For the whole group, there was a significant positive relationship between blood eosinophil parameters and the percentage of sputum eosinophils. These blood eosinophil parameters included not only the eosinophil percentage and absolute blood eosinophil count ($r = 0.691$, $P < 0.001$; $r = 0.683$, $P < 0.001$; respectively), but also ratios like ELR, ENR and EMR ($r = 0.654$, $P < 0.001$; $r = 0.670$, $P < 0.001$; $r = 0.669$, $P < 0.001$; respectively). We also found weaker but significant correlations between sputum neutrophil percentage and blood neutrophil percentage ($r = 0.219$, $P = 0.005$), blood lymphocytes ($r = -0.171$, $P = 0.029$), blood NLR ($r = 0.178$, $P = 0.023$), blood ENR ($r = -0.205$, $P = 0.008$) and blood PLR ($r = 0.158$, $P = 0.043$). A similar relationship was found between the proportion of sputum macrophages and blood EMR ($r = -0.222$, $P = 0.004$).

There was no significant relationship between sputum lymphocytes and blood parameters.

Table 2. Blood count parameters according to sputum inflammatory phenotype

	All	Neutrophilic asthma	Eosinophilic asthma	Mixed granulocytic asthma	Paucigranulocytic asthma	P-value
White blood cell ($\times 10^9/L$)	7.45 $\pm$ 1.83	7.21 $\pm$ 1.76	7.50 $\pm$ 1.74	7.53 $\pm$ 1.72	7.47 $\pm$ 2.03	0.963
Haemoglobin, g/L	140.73 $\pm$ 12.81	137.16 $\pm$ 14.57	142.69 $\pm$ 12.13	141.07 $\pm$ 15.30	139.72 $\pm$ 12.02	0.461
Platelets ($\times 10^9/L$)	246.48 $\pm$ 71.32	248.36 $\pm$ 49.96	244.63 $\pm$ 64.54	218.43 $\pm$ 106.06	255.30 $\pm$ 77.19	0.758
Neutrophils ($\times 10^9/L$)	4.44 $\pm$ 1.43	4.75 $\pm$ 1.33	4.38 $\pm$ 1.39	4.18 $\pm$ 1.15	4.43 $\pm$ 1.59	0.430
Eosinophils ($\times 10^9/L$)	0.24 (0.12–0.40)	0.10 (0.10–0.25) [§]	0.40 (0.27–0.54) [*]	0.34 (0.30–0.48) [*]	0.14 (0.10–0.20)	0.000
Lymphocytes ($\times 10^9/L$)	2.11 $\pm$ 0.71	1.75 $\pm$ 0.63 [*]	2.06 $\pm$ 0.51	2.22 $\pm$ 0.67	2.32 $\pm$ 0.88	0.010
Monocytes ($\times 10^9/L$)	0.54 $\pm$ 0.19	0.52 $\pm$ 0.17	0.54 $\pm$ 0.18	0.57 $\pm$ 0.25	0.55 $\pm$ 0.21	0.878
Eosinophils, %	3.2 (1.8–5.6)	1.7 (1.4–3.1) [§]	5.3 (3.7–7.8) [*]	5.5 (3.9–6.3) [*]	1.9 (1.3–2.5)	0.000
Blood NLR	2.17 (1.57–2.76)	2.69 (1.95–3.63) [§]	2.17 (1.63–2.55)	1.96 (1.30–2.56)	1.72 (1.38–2.81)	0.006
Blood ELR	0.11 (0.07–0.20)	0.08 (0.06–0.12) [§]	0.20 (0.11–0.30) [*]	0.16 (0.11–0.25) [*]	0.07 (0.04–0.09)	0.000
Blood ENR	0.06 (0.03–0.11)	0.03 (0.02–0.05) [§]	0.09 (0.06–0.15) [*]	0.09 (0.08–0.13) [*]	0.03 (0.02–0.05)	0.000
Blood EMR	0.43 (0.25–0.79)	0.25 (0.20–0.41) [§]	0.77 (0.50–1.20) [*]	0.65 (0.42–1.44) [*]	0.25 (0.18–0.37)	0.000
Blood NMR	8.27 (6.54–10.48)	9.00 (7.32–11.88)	8.10 (6.67–9.90)	7.04 (5.17–11.81)	8.29 (6.13–11.02)	0.313
Blood MLR	0.25 (0.19–0.33)	0.30 (0.23–0.40)	0.25 (0.19–0.32)	0.26 (0.18–0.33)	0.23 (0.17–0.34)	0.099
Blood PLR	123.43 (94.61–155.61)	133.75 (119.59–193.29) [*]	123.70 (95.10–155.56)	114.65 (66.88–147.92)	108.31 (87.78–145.93)	0.015

NLR, neutrophil/lymphocyte ratio; ELR, eosinophil/lymphocyte ratio; ENR, eosinophil/neutrophil ratio; EMR, eosinophil/monocyte ratio; MLR, macrophage/lymphocyte ratio; PLR, platelet/lymphocyte ratio.

Data are presented as mean $\pm$ SD or median (range).

^{*} $P < 0.008$ according to Bonferroni principle, paucigranulocytic asthma is used as the comparator.

[§] $P < 0.008$ according to Bonferroni principle, mixed granulocytic asthma is used as the comparator.

[#] $P < 0.008$ according to Bonferroni principle, eosinophilic asthma is used as the comparator.

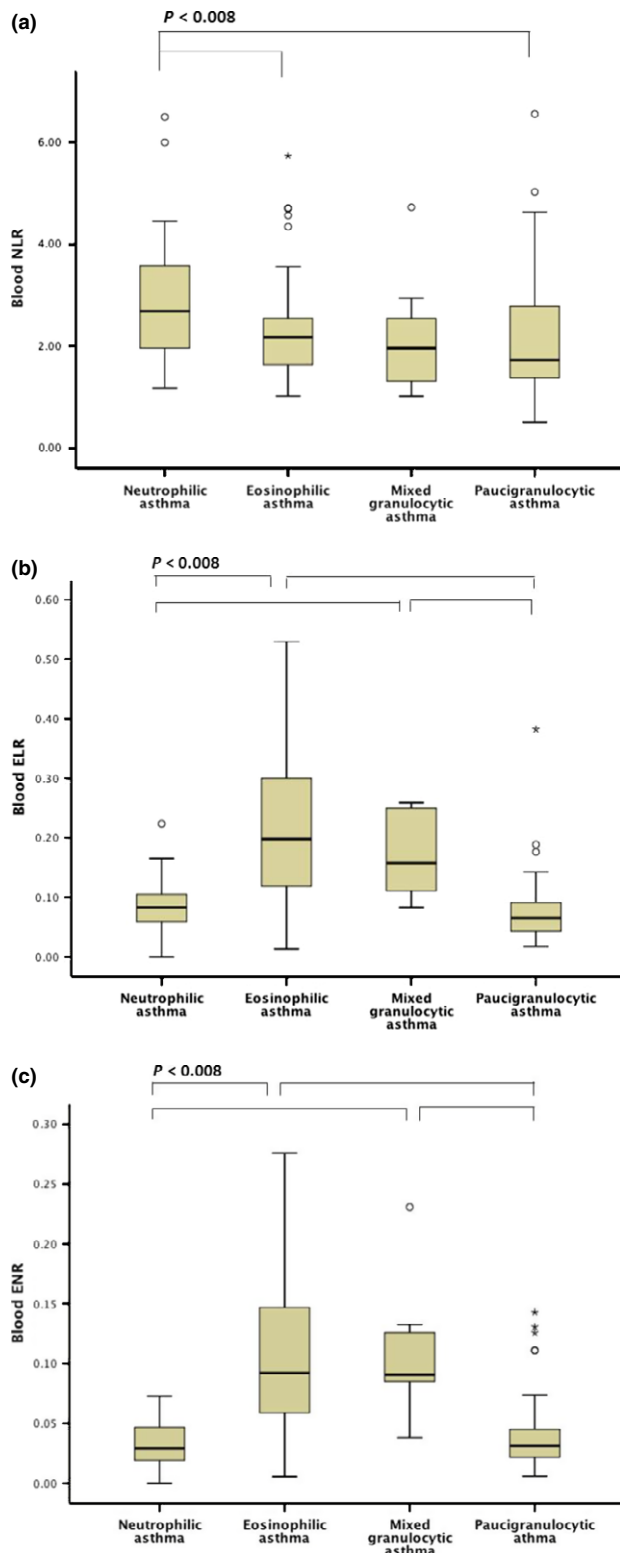


Fig. 1. Blood ratios according to the sputum inflammatory phenotypes. The box plot shows the median and interquartile values of ratios. (a) Blood Neutrophil/Lymphocyte ratio (NLR) in four inflammatory phenotypes; (b) blood ELR in four inflammatory phenotypes; (c) blood ENR in four inflammatory phenotypes. "o" denotes extreme value; "*" denotes outliers.

Determination of alternative measures to sputum cell profiles for detecting eosinophilic asthma and neutrophilic asthma

The ROC curve analysis identified blood eosinophil percentage as the best predictor for eosinophilic phenotypes, with an area under the curve (AUC) of 0.907 ($P = 0.000$). The optimum cut-point for blood eosinophil percentage was 2.7%, and this yielded a sensitivity of 92.2%, a specificity of 75.8%, 75.5% positive predictive value and 92.3% negative predictive value. The absolute blood eosinophil count was also highly predictive with an area under the curve of 0.898 ($P < 0.0001$) at a blood eosinophil cut-off of $0.26 \times 10^9/L$.

There was no difference between the blood eosinophil percentage and the absolute blood eosinophil count in the detection of eosinophilic asthma ($P > 0.05$). Blood eosinophil ratios (ELR, ENR, EMR) were also as efficient as blood eosinophil count to detect sputum eosinophilia (AUC = 0.892, $P < 0.001$; AUC = 0.891, $P < 0.001$; AUC = 0.898, $P < 0.001$; respectively; Table 3, Fig. 2).

Neutrophilic asthma could also be detected by blood parameters, but with less accuracy. Blood neutrophil percentage yielded a 61.5% sensitivity and 68.8% specificity for neutrophilic asthma (Table 3, Fig. 3). Blood NLR with a cut-off point of 1.74 gives a sensitivity of 76.9% and a specificity of 41.6% (AUC = 0.612, $P = 0.035$) for neutrophilic asthma. Although the absolute value of blood lymphocytes and blood ENR and PLR was correlated with the sputum neutrophil percentage, a ROC curve of these parameters did not show useful values (AUC = 0.385, $P = 0.031$; AUC = 0.406, $P = 0.076$; AUC = 0.587, $P = 0.103$; respectively; Table 3, Fig. 3).

Discussion

In adults with uncontrolled asthma on inhaled corticosteroids, blood cell counts and derived parameters offer value in the assessment of inflammatory phenotypes. Blood eosinophil counts and derived ratios can accurately predict eosinophilic asthma. Blood neutrophil percentage and blood NLR are increased in neutrophilic asthma and are also weakly predictive, but not enough to be clinically useful. These results suggest that blood counts can be useful in assessing asthma eosinophil asthma and may have a role in selecting add-on therapy. We also identified some additional findings, namely reduced blood lymphocytes and increased NLR in neutrophilic asthma, which may reflect systemic inflammation.

There is a need to include simple and accessible biomarkers in the management of asthma; however, there is disagreement in the literature as to the value of blood cell counts. Our results resolve these issues by showing that blood eosinophils (whether absolute eosinophil

Table 3. Summary ROC curve analyses of blood parameters for predicting inflammatory phenotype

	Area under the curve (AUC)	P-value	95% Confidence interval		Cut-off point	Sensitivity, %	Specificity,%	PPV, %	NPV, %
			Lower boundary	Upper boundary					
Predicting eosinophilic asthma (sputum eosinophil count of ≥3%)									
Eosinophils (× 10 ⁹ /L)	0.898	0.000	0.851	0.945	0.26	83.1	82.8	81.0	84.7
Eosinophils,%	0.907	0.000	0.862	0.953	2.70	92.2	75.8	75.5	92.3
Blood ELR	0.892	0.000	0.843	0.940	0.10	89.6	74.4	75.8	88.9
Blood ENR	0.891	0.000	0.840	0.941	0.05	89.6	77.0	77.5	89.3
Blood EMR	0.898	0.000	0.853	0.943	0.26	98.7	49.4	63.3	97.7
Predicting neutrophilic asthma (sputum neutrophil count ≥61%)									
Neutrophils, %	0.623	0.020	0.519	0.728	61.52	61.5	63.2	38.1	81.7
Lymphocytes (× 10 ⁹ /L)	0.385	0.031	0.277	0.493	2.54	65.9	48.0	23.8	85.2
Blood NLR	0.612	0.035	0.508	0.715	1.74	76.9	41.6	29.1	85.3
Blood ENR	0.406	0.076	0.303	0.508					
Blood PLR	0.587	0.103	0.483	0.690					

ELR, eosinophil/lymphocyte ratio; ENR, eosinophil/neutrophil ratio; EMR, eosinophil/macrophage ratio; NLR, neutrophil/lymphocyte ratio; PLR, platelet/lymphocyte ratio; PPV positive predictive value; NPV negative predictive value.

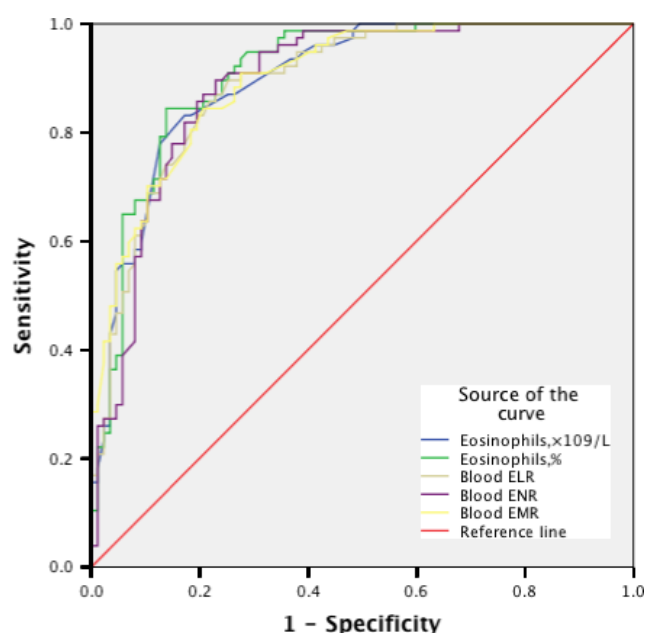


Fig. 2. ROC curve of blood eosinophil parameters and sputum eosinophil count $\geq 3\%$.

counts or percentages) were an excellent predictor of eosinophilic asthma in the clinically important subgroup of patients who remain uncontrolled while on maintenance asthma therapy. There are several studies that also show a positive relationship between eosinophils in blood and sputum eosinophils [19–22]. This is biologically plausible because eosinophils are bone marrow-derived cells that target the airway under the influence of inflammatory cytokines such as IL-5 and eotaxin. Few studies have used ROC to assess the ability of blood eosinophils to detect airway eosinophilic

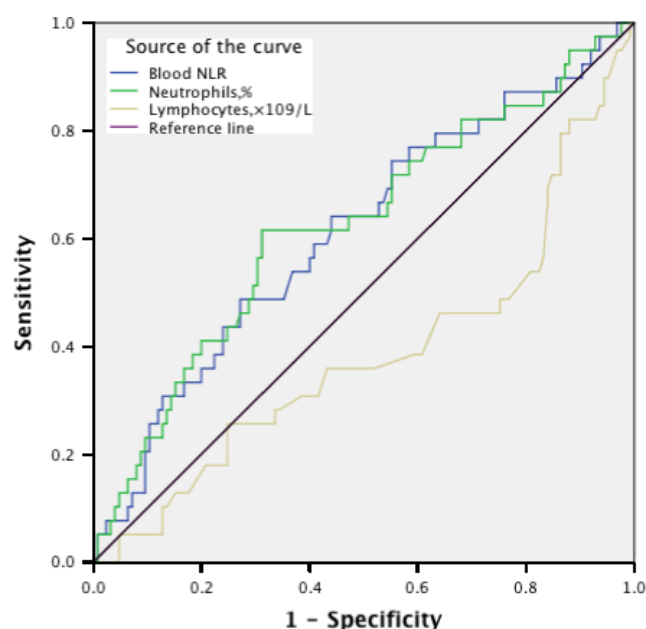


Fig. 3. ROC curves of blood Neutrophil/Lymphocyte Ratio (NLR) and neutrophils that best identified a sputum neutrophil count $\geq 61\%$.

phenotypes [11, 12, 23]. However, two recent studies questioned the predictability of the blood eosinophil count for airway eosinophilia [24, 25]. One study did not use sputum to evaluate the airway eosinophilia but biopsy specimens and did not control for oral corticosteroid use. Another study found a non-significant trend between peripheral blood and sputum eosinophil counts ($r = 0.34$, $P = 0.067$), but the sample size was only 31 patients and so may have been underpowered [25].

Our study is the first study to address the relationship between blood and sputum eosinophils in a large group

of patients with uncontrolled asthma who were using inhaled corticosteroids. This is a clinically important group, where careful monitoring and add-on therapy are considered necessary. Guidelines recommend that patients with uncontrolled asthma should be seen at least every 3 months and that treatment should be adjusted based on monitoring [26]. The method of monitoring should be simple, assessable and low in price. Blood eosinophil count may be a good choice, and our study supports this and has shown higher predictive values than other studies [11, 12, 23]. This may have been because of targeting a subject group who present with persistent symptoms despite inhaled corticosteroid therapy. Although the fact that peripheral blood eosinophils are a good biomarker of sputum eosinophils is not novel, our study found that it was particularly useful in uncontrolled asthma for detecting sputum eosinophilia than in other subgroups of asthma [12, 23]. A peripheral blood eosinophil count has been found to be a very good surrogate of sputum eosinophilic inflammation in COPD [27], and the cut offs are similar. Perhaps peripheral blood eosinophil identifies an eosinophilic bronchitis that will be responsive to ICS regardless of the actual disease label. Because of their accuracy and convenience, blood eosinophil counts can be used in the clinic for detecting airway eosinophilia in uncontrolled asthma.

Derived blood eosinophil parameters, such as the ENR, EMR and ELR, also predicted eosinophilic asthma. This is the first study to report these parameters in asthma. They can be used to divide asthmatic patients into two groups: eosinophilic asthma and non-eosinophilic asthma, similar to sputum and blood eosinophil counts. In our study, we confirmed that these novel parameters derived from blood had the same trend as blood eosinophil count in differentiating asthma phenotypes and that they also had considerable predictive powers for sputum eosinophils. It seems that these new cellular ratios may be complementary to blood eosinophils for detecting sputum eosinophilia; however, they may not offer specific advantages over cellular differential counts. Our data show that blood eosinophils can be used as a surrogate for the detection of eosinophilic airway inflammation in asthmatics who are uncontrolled on maintenance ICS therapy. This extends previous reports considerably by showing the utility of blood counts in a large group of well-characterized adults with poorly controlled asthma taking inhaled corticosteroids and long-acting beta agonist. As this is a clinically important population where there is a need to review therapy, our results could be expected to aid in treatment decisions. We have also examined the utility of other white blood cell populations and their ratios and show that blood eosinophils are a superior predictor of the presence of eosinophilic bronchitis.

Although the percentage of blood neutrophils was related to the percentage sputum neutrophil, the strength of the association was low and a ROC curve analysis showed poor accuracy. This result is in accordance with previous studies [11, 28]. Many factors probably affect sputum neutrophils. In a cross-sectional study, Brooks et al. [29] found an association between age and sputum neutrophils in both asthmatic and non-asthmatic subjects. Hastie et al. [28] reported that combined age, absolute blood neutrophil counts and asthma duration were significantly associated with percentage sputum neutrophils. In addition, infection [30] and higher doses of inhaled corticosteroids may increase airway neutrophils [31].

Our study, for the first time, has demonstrated that patients with neutrophilic asthma had higher NLR and PLR than paucigranulocytic asthma. The NLR is a parameter which combines neutrophils as a marker of innate inflammation and lymphocytes as a regulator of allergic inflammation [32, 33]. Blood NLR is reported to detect the overall inflammatory and stress status of the body [34]. A high blood NLR is associated with poor clinical prognosis in many chronic diseases such as cardiac disease [32], malignancy [16] and chronic kidney disease [35]. Moreover, patients with a higher blood PLR also had adverse outcomes in cancers [36], cardiovascular diseases [37] and renal diseases [38]. To our knowledge, this is the first report of NLR and PLR in asthmatic patients. The reason why NLR and PLR were increased in neutrophilic asthma remains unclear, but it may reflect systemic inflammation that is associated with neutrophilic airway inflammation [39]. Alternatively, it may be a reflection of a low blood lymphocyte count. We observed that blood lymphocytes in neutrophilic asthma were decreased and had a weak negative association with sputum neutrophils. This is a novel finding and may be a marker of the stress response for adverse physiologic conditions, such as systemic inflammation.

Limitations and Future research

As blood neutrophils had a weak correlation with sputum neutrophils, further study should use age-corrected values to evaluate this relationship [29]. Whether NLR and PLR are directly related to systemic inflammation in neutrophilic asthma needs to be investigated in the future using biomarkers of systemic inflammation. Many studies have demonstrated poor outcomes in patients with a high NLR and/or PLR in chronic inflammatory diseases [16, 32, 40, 41], and a prospective study is needed to evaluate the incidence of adverse events in asthma with different levels of NLR and PLR. Further investigation of the ENR's usefulness for differentiating phenotypes of asthma would also benefit from bigger sample sizes.

Conclusion

This study shows that blood eosinophil counts, and their derived ratios the ELR, ENR and EMR, can accurately detect eosinophilic asthma in patients with uncontrolled asthma. It suggests that the peripheral eosinophil count is a useful tool for monitoring sputum eosinophil percentages in uncontrolled asthma. However, blood neutrophil counts and NLR are poorly related to sputum neutrophil percentages and have less utility. This study shows the promise of a simple and accessible blood test, when used in the appropriate clinical setting.

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Author's contributions

Conceived and designed the experiments: PGG, JLS, XYZ. Performed the experiments and data collection: All authors and the AMAZES group. Analysed the data and wrote the paper: XYZ. Edited and reviewed manuscript: PGG, JLS, IAY, ALJ, JWU. All authors read and approved the final manuscript.

Conflict of interest

The authors declare no conflict of interest.

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