Exploring the neutrophilic pattern in asthma: are “intermediate” and “high” neutrophilic patients the same population?

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To the Editor:

Asthma is a heterogeneous disease, generally characterised by chronic inflammation of the airways. Phenotyping has recently become a core tool to optimise its management and treatment [1] and biomarkers identification is now even more crucial. Blood eosinophils count is the most widely available inflammatory biomarker used for both therapeutic guidance and monitoring treatment response. Induced sputum (IS) is the gold-standard for a non-invasive assessment of airway inflammatory cell distribution. Four patterns have been identified based on the percentage of eosinophils and neutrophils in IS: eosinophilic, neutrophilic, mixed granulocytic and pauci-granulocytic [2].

Recently, interest around type-2-low asthma has grown significantly, due to the limited therapeutic approaches available and without a well-established definition [3]. This asthma phenotype is mainly characterised by neutrophilic and pauci-granulocytic airway inflammation [4]. Asthma patients with neutrophilic airway inflammation have been identified as a cluster with a greater disease severity [5], correlated with low post-bronchodilator forced expiratory volume in 1 s [6], and as less responsive to inhaled corticosteroids and/or reflecting their overuse [7, 8]. However, this pattern definition is not always concordant among studies, although an overall agreement exists on the cut-off for the eosinophilic group, that for neutrophils still ranges from 40% to 76% [9, 10]. Whether patients’ clinical and/or functional characteristics may change according to different levels of neutrophilic airway inflammation is a matter of debate. Our hypothesis is that within the neutrophilic pattern, asthma patients with a higher neutrophil percentage in IS may be more clinically and functionally severe than patients with a lower neutrophil percentage.

The aim of our study is to investigate the clinical, biological and functional characteristics of neutrophilic asthma patients, comparing “high” and “intermediate” neutrophilic groups. We retrospectively reviewed electronic medical records dating from 2017 to 2024 of all consecutive adult asthma patients with IS performed according to ERS guidelines [11] in an Italian asthma reference centre. Asthma was diagnosed and stratified for severity according to GINA reports (www.ginasthma.org). Current smokers and/or patients undergoing maintenance oral corticosteroids and/or patients with COPD were excluded. We also did not include patients with reported respiratory infections/exacerbations the month before IS collection. Only patients with an isolated neutrophilic pattern in the airways, defined as both neutrophils ⩾61% and eosinophils <3% [2], were included. Neutrophilic patients were divided into two groups: high neutrophilic (HN) (⩾78%) and intermediate neutrophilic (IN) (⩾61% and <78%) based on the median neutrophil percentage in this cohort [12]. Study approval was granted from the local ethics committee (N.0004596/24).

Data normality distribution was assessed using Shapiro–Wilk’s test and graphical representation (QQ-plots). Mann–Whitney U and Chi-square tests (with Yates’ continuity correction) were used to compare the IN and the HN patients. Correlations between IS neutrophil and other continuous variables were assessed by Spearman’s rank coefficients. The Benjamini–Hochberg method was adopted to correct multiple testing. Statistical threshold was set at 0.05. Analyses were performed using R software (version 4.2.3).

Of 212 patients who were assessed for all eligibility criteria, 61 neutrophilic patients were included in the analysis based on IS evaluation (table 1). Based on the median value of IS neutrophil percentage (78%), the study population was subsequently divided into two groups: 31 (51%) patients were defined as HN and

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TABLE 1 Sample characteristics and comparison of intermediate *versus* high neutrophilic groups

Variables	N	Intermediate neutrophilic	N	High neutrophilic	p-value	Benjamini–Hochberg adjusted p-value	Effect size (95% CI)	All patients
Age (years)	30	59.5 (57.3 to 66.8)	31	58.0 (50.0 to 71.0)	0.740[#]	>0.90	0.05 (−0.27 to 0.35)[+]	59.0 (51.0 to 71.0)
Male	30	15 (50.0%)	31	14 (45.2%)	0.903[¶]	>0.90	−0.85 (−0.96 to −0.72)[§]	29 (47.5%)
BMI ($kg \cdot m^{-2}$)	30	27.4 (24.9 to 29.5)	31	25.2 (21.9 to 28.2)	0.072[#]	0.336	0.27 (−0.03 to 0.53)[+]	26.6 (23.1 to 29.0)
Smoking habit	30		31		0.529[¶]	0.837	0.11 (0.00 to 0.36)[§]	
Former		15 (50.0%)		12 (38.7%)				27 (44.3%)
Never		15 (50.0%)		19 (61.3%)				34 (55.7%)
Atopy	30	17 (56.7%)	31	20 (64.5%)	0.715[¶]	>0.90	0.08 (0.00 to 0.33)[§]	37 (60.7%)
CRSwNP	30	7 (23.3%)	31	8 (25.8%)	>0.90[¶]	>0.90	0.03 (0.00 to 0.26)[§]	15 (24.6%)
Allergic rhinitis	30	10 (33.3%)	31	15 (48.4%)	0.350[¶]	0.712	0.15 (0.00 to 0.40)[§]	25 (41.0%)
GORD	30	15 (50.0%)	31	15 (48.4%)	>0.90[¶]	>0.90	0.02 (0.00 to 0.21)[§]	30 (49.2%)
Arterial hypertension	30	12 (40.0%)	31	13 (41.9%)	>0.90[¶]	>0.90	0.02 (0.00 to 0.23)[§]	25 (41.0%)
Bronchiectasis	30	5 (16.7)	31	8 (25.8%)	0.576[¶]	0.853	0.11 (0.00 to 0.36)[§]	13 (21.3%)
Emphysema	30	2 (6.7%)	31	5 (16.1%)	0.449[¶]	0.757	0.15 (0.00 to 0.40)[§]	7 (11.5%)
OSA	30	10 (33.3%)	31	5 (16.1%)	0.207[¶]	0.436	0.20 (0.00 to 0.45)[§]	15 (24.6%)
Beclometasone HFA equivalent (µg)	30	360.0 (200.0 to 600.0)	31	368.0 (200.0 to 400.0)	>0.90[#]	>0.90	0.00 (−0.31 to 0.30)[+]	368.0 (200.0 to 600.0)
Any exacerbations in previous year	30	15 (50.0%)	31	12 (38.7%)	0.529[¶]	0.837	0.11 (0.00 to 0.36)[§]	27 (44.3%)
Number of exacerbations in previous year	30	0.5 (0.0 to 1.0)	31	0.0 (0.0 to 1.5)	0.780[#]	>0.90	0.04 (−0.21 to 0.32)[+]	0.0 (0.0 to 1.0)
ACQ 6	30	0.8 (0.2 to 1.1)	31	0.7 (0.2 to 1.1)	0.722[#]	>0.90	0.05 (−0.24 to 0.36)[§]	0.8 (0.2 to 1.2)
FEV_1 (L)	30	2.7 (2.1 to 3.1)	31	2.4 (1.7 to 3.1)	0.419[#]	0.747	0.12 (−0.18 to 0.41)[+]	2.6 (1.8 to 3.1)
FEV_1 (% predicted)	30	88.5 (81.8 to 99.4)	31	88.9 (68.5 to 100.2)	0.452[#]	0.757	0.11 (−0.18 to 0.40)[+]	88.9 (75.6 to 100.2)
FVC (L)	30	3.3 (2.8 to 4.1)	31	3.2 (2.4 to 4.0)	0.415[#]	0.747	0.12 (−0.18 to 0.40)	3.3 (2.6 to 4.1)
FVC (% pred)	30	91.8 (87.3 to 102.9)	31	92.7 (82.8 to 101.7)	0.581[#]	0.853	0.08 (−0.22 to 0.38)[+]	92.1 (84.3 to 102.6)
FEV_1/FVC (% pred)	30	72.8 (69.0 to 80.9)	31	76.0 (67.5 to 81.3)	0.767[#]	>0.90	−0.05 (−0.33 to 0.25)[+]	73.6 (69.0 to 81.3)
$FEF_{25-75\%}$ ($L \cdot s^{-1}$)	27	2.1 (1.7 to 3.0)	27	1.9 (1.2 to 2.6)	0.174[#]	0.416	0.22 (−0.11 to 0.51)[+]	2.0 (1.4 to 3.0)
$FEF_{25-75\%}$ (% pred)	27	85.4 (70.9 to 120.7)	27	76.2 (51.1 to 95.1)	0.116[#]	0.348	0.25 (−0.06 to 0.54)[+]	84.8 (62.6 to 110.9)
Blood leukocytes count ($cells \cdot \mu L^{-1}$)	30	6.1 (5.5 to 7.6)	28	7.1 (6.3 to 9.0)	0.090[#]	0.336	−0.26 (−0.58 to −0.06)[+]	6.8 (5.5 to 8.1)
Blood neutrophils count ($cells \cdot \mu L^{-1}$)	30	3536.7 (3029.8 to 3802.2)	28	4389.6 (3446.1 to 5152.1)	0.004[#]	0.036	−0.43 (−0.73 to −0.12)[+]	3731.8 (3029.8 to 4696.2)
Blood eosinophils count ($cells \cdot \mu L^{-1}$)	30	176.3 (108.5 to 236.9)	28	145.5 (113.1 to 230.6)	0.669[#]	0.928	−0.07 (−0.23 to 0.39)[+]	169.7 (111.3 to 236.9)

Continued

TABLE 1 Continued

Variables	N	Intermediate neutrophilic	N	High neutrophilic	p-value	Benjamini–Hochberg adjusted p-value	Effect size (95% CI)	All patients
Blood lymphocytes count	30	2144.7 (1839.3 to 2725.0)	28	1895.8 (1575.8 to 2161.4)	0.122[#]	0.348	0.24 (−0.06 to 0.54)[+]	2033.2 (1590.4 to 2554.2)
NLR	30	1.6 (1.3 to 1.9)	28	2.2 (1.6 to 2.9)	0.007[#]	0.047	−0.41 (−0.68 to −0.13)[+]	1.8 (1.4 to 2.5)
IS total cells (counts per mL ×10^4)	30	170.5 (72.3 to 290.3)	25	320.0 (192.0 to 755.0)	0.011[#]	0.062	−0.40 (−0.68 to −0.11)[+]	235.0 (85.1 to 443.5)
IS vitality (%)	26	89.0 (83.2 to 91.6)	24	92.1 (85.0 to 97.2)	0.080[#]	0.336	−0.29 (−0.60 to 0.05)[+]	89.8 (83.2 to 94.0)
IS macrophages (%)	30	23.9 (18.7 to 26.9)	30	7.4 (2.2 to 13.8)	<0.001[#]	<0.001	0.93 (0.84 to 0.98)[+]	16.0 (7.5 to 23.7)
IS eosinophils (%)	30	0.9 (0.3 to 1.9)	31	0.6 (0.0 to 1.3)	0.199[#]	0.436	0.19 (−0.10 to 0.50)[+]	0.7 (0.2 to 1.8)
IS lymphocytes (%)	30	1.5 (0.8 to 2.4)	30	1.2 (0.8 to 1.8)	0.151[#]	0.409	0.22 (−0.09 to 0.50)[+]	1.2 (0.8 to 2.0)
IS epithelial cells (%)	30	2.5 (1.1 to 4.7)	30	0.9 (0.4 to 2.3)	0.004[#]	0.036	0.43 (0.16 to 0.67)[+]	1.6 (0.8 to 3.6)

Continuous variables are described as median (interquartile range); categorical variables are reported as count and percentages. BMI: body mass index; CRSwNP: chronic rhinosinusitis with nasal polyps; GORD: gastro-oesophageal reflux disease; OSA: obstructive sleep apnoea; HFA: extra fine hydrofluoroalkane; ACQ: asthma control questionnaire; FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity; $FEF_{25–75\%}$: forced expiratory flow at 25–75% of FVC; NLR: blood neutrophils/blood lymphocytes; IS: induced sputum. [#]: Mann–Whitney U test; [¶]: Chi-square test; [+]: Cliff's delta; [§]: Cramer's V.

30 (49%) as IN. Overall, the clinical and demographic characteristics were similar in the two groups. No differences were found regarding asthma therapy, asthma control (number of exacerbations and ACQ-6), lung function, smoking habits and comorbidities. From the inflammatory point of view, blood neutrophil count (BNC) and blood neutrophils/blood lymphocytes ratio (NLR) were higher in the HN group (p-adjusted=0.036, and p-adjusted=0.047, respectively), while no significance was found in the blood eosinophils count. Regarding IS, macrophages' percentage was found to be lower in the HN patients (p-adjusted<0.001) as well as the epithelial cells percentage (p-adjusted=0.036). No differences were detected in terms of eosinophilic inflammation. IS neutrophils per cent negatively correlated with IS epithelial cells per cent (r= −0.41; p-adjusted=0.025), and positively with BNC (r=0.35; p-adjusted=0.049) and NLR (r=0.38; p-adjusted=0.036).

To the best of our knowledge, there are no similar studies exploring data on asthma patients with HN pattern through a non-invasive tool such as IS, attempting to re-evaluate the neutrophilic asthma population and the role of ⩾61% as a cut-off to define neutrophilic airway inflammation. The study by Bullone *et al.* [12] is the only one suggesting that HN patients (the majority with mixed eosinophilic/neutrophilic phenotype and defined invasively by inflammatory cell counts in bronchial biopsies) might represent a new cluster showing increased levels of IgE sensitivity to perennial allergens, exacerbation rate and oral corticosteroid dependence.

By contrast, our data indicate that the characteristics of the HN and the IN groups are widely overlapping, confirming the homogeneous characteristics of the neutrophilic cluster. Indeed, no clinical or functional differences were observed in our study. This discrepancy with the study by Bullone *et al.* [12] could be due to different methodologies used to study airway inflammation (IS *versus* biopsies) and/or to the selection criteria applied (excluding *versus* including patients with central eosinophilia).

Overall, the two sub-groups were similar without significant differences in the clinical presentation. Only BNC, NLR, IS total cells count, IS epithelial cells and IS macrophages percentage were different. However, the latter variable should be considered significant only because it represents the largest percentage in sputum besides neutrophils, and thus merely for a mathematical distribution reason.

In our study we highlighted a moderate correlation between blood and sputum neutrophils; the stronger correlation in our neutrophilic cluster in comparison with previous studies [13] may be due to an accurate selection of the population achieved excluding eosinophilic patients, oral corticosteroids users and current smokers. This finding is reflected in the higher BNC, also in the form of NLR, in the HN group. Another

interesting aspect related to IS is the lower percentage of epithelial cells in the HN group in line with the significant association between elevated epithelial cells in non-neutrophilic patterns of other studies [14]. In the end, IS total cells count in the HN group was found to be higher than in the IN asthmatics, probably due to different microbiomes and/or infections/colonisations of the airways, despite clinical presentation [8].

Our study has some limitations: the retrospective-monocentric design, the limited sample-size, and the inclusion requirement of successful sputum induction which may have led to the exclusion of neutrophilic asthmatics unable to expectorate. By contrast, the extent of available data and the comprehensive analysis performed should be regarded as strengths. Moreover, it is worth mentioning that pollutants and comorbidities may cause different airway neutrophils percentage between these two groups, deserving additional studies [8].

In conclusion, our preliminary data contribute to the understanding of the neutrophilic pattern, demonstrating that the neutrophils percentage used in our centre as a cut-off (⩾61%) is able to discriminate an overall homogeneous population without clinical differences between the "high" and "intermediate" neutrophilic clusters. Our exploratory data cannot be considered as a definitive result in the attempt to identify the clinical and functional characteristics of a hypothetical "high" neutrophilic asthma patient and further studies are required to better analyse the correlation between neutrophilia and clinical characteristics to improve asthma management. Moreover, the positive correlation between BNC and IS neutrophils percentage in a selected asthmatic population could allow to use blood neutrophils as a surrogate marker of IS neutrophils. This datum may be useful independently of asthma severity, since even in well-controlled asthmatics inflammation plays an important role in the natural history of the disease. Airway neutrophilia could result in a decreased response to inhaled corticosteroids [7], reflect their overuse [8], and may lead to irreversible airway remodelling [15] suggesting the need for more in-depth phenotyping for further therapeutic opportunities. Our results might be useful to implement precision medicine in clinical care of patients with complex airway diseases, however, further studies are required to improve asthma management of neutrophilic patients.

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