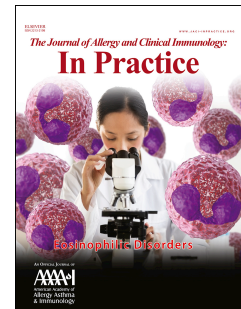


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Cluster analysis of inflammatory biomarker expression in the International Severe Asthma Registry (ISAR)

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ABSTRACT

Background:

Allergy, eosinophilic inflammation, and epithelial dysregulation are implicated in severe asthma pathogenesis.

Objective:

We characterized biomarker expression in adults with severe asthma.

Methods:

Within the International Severe Asthma Registry (ISAR), we analyzed data from 10 countries in North America, Europe and Asia, with pre-specified thresholds for biomarker positivity (serum IgE ≥ 75 kU/L, blood eosinophils ≥ 300 cells/uL, and FeNO ≥ 25 ppb), and with hierarchical cluster analysis using biomarkers as continuous variables.

Results:

Of 1,175 patients; 64% were female, age (mean $\pm$ SD) 53 ± 15 years, body mass index (BMI) 30 ± 8 , post-bronchodilator FEV₁ predicted $74 \pm 20\%$. By pre-specified thresholds, 59% were IgE positive, 57% eosinophil positive, and 58% FeNO positive. There was substantial inflammatory biomarker overlap; 59% were positive for either two or three biomarkers. Five distinct clusters were identified: **Cluster 1** (61%, low-to-medium biomarkers) comprised highly symptomatic, older females with elevated BMI and frequent exacerbations; **Cluster 2** (18%, elevated eosinophils and FeNO) older females with lower BMI and frequent exacerbations; **Cluster 3** (14%, extremely high FeNO) older, highly symptomatic, lower BMI and preserved lung function; **Cluster 4** (6%, extremely high IgE) younger, long duration of asthma, elevated BMI, and poor lung function; **Cluster 5** (1.2%, extremely high eosinophils) younger males with low BMI, poor lung function, and high burden of sino-nasal disease and polyposis.

Conclusion:

There is significant overlap of biomarker positivity in severe asthma. Distinct clusters according to biomarker expression exhibit unique clinical characteristics, suggesting the occurrence of discrete patterns of underlying inflammatory pathway activation and providing pathogenic insights relevant to the era of monoclonal biologics.

Highlight Box

What is already known about this topic?

Asthma is now understood to encompass a variety of distinct clinical phenotypes, likely arising from different pathological mechanisms.

What does this article add to our knowledge?

In a large international severe asthma cohort, distinct clusters according to biomarker expression exhibited unique clinical characteristics, suggesting the occurrence of discrete patterns of underlying inflammatory pathway activation.

How does this study impact current management guidelines?

Understanding more about distinct patterns of underlying inflammatory pathway activation in individual severe asthma patients allows clinicians to tailor targeted severe asthma therapies such as monoclonal biologics, furthering precision medicine for severe asthma.

Key words:

Severe asthma

Biomarkers

Immunoglobulin E

Fractional exhaled nitric oxide

Eosinophils

Abbreviations:

IgE – immunoglobulin E

FeNO - fractional exhaled nitric oxide

ISAR - International Severe Asthma Registry

SD - standard deviation

BMI – body mass index

FEV₁ – Forced expiratory volume in 1 second

FVC – Forced vital capacity

GINA – Global Initiative for Asthma

ACQ - Asthma Control Questionnaire

ACT - Asthma Control Test

AQLQ – Asthma-related Quality of life Questionnaire

NAEPP – National Asthma Education and Prevention Program

ICU – intensive care unit

ATS – American Thoracic Society

ERS – European Respiratory Society

Cells/uL – cells per microliter

Ppb - parts per billion

kU/L - kilounits per liter

ANOVA – analysis of variance

INTRODUCTION

One in ten patients with asthma suffers from severe asthma, which often remains uncontrolled despite treatment with high-dose inhaled corticosteroids plus a second controller.¹ These individuals account for the most healthcare expenditure, experience the greatest morbidity, and face the highest risk of death compared to patients with non-severe asthma.²⁻⁵

A variety of cellular pathways are activated in patients with severe asthma. Allergy, eosinophilic inflammation, and airway epithelial dysregulation have each been implicated in the pathogenesis of severe asthma.⁶⁻¹⁰ Accordingly, increased serum immunoglobulin E (IgE), peripheral blood eosinophils, and fractional exhaled nitric oxide (FeNO), have been used as biomarkers to suggest corresponding activation of these respective inflammatory pathways, and to predict responsiveness to monoclonal biologics such as those targeting IgE, interleukin (IL)-5, and the IL-4/13 receptor.^{1,7,9,11-15}

It appears likely that different inflammatory pathways in severe asthma may be activated to a different extent in different patients. We hypothesized that such differential activation of inflammatory pathways would lead to differential biomarker expression, and would also be manifested by different clinical characteristics.^{16,17}

Previous studies in a general asthma population have indicated substantial overlap in inflammatory biomarkers, but this has not been previously examined in severe asthma patients.¹⁸ We tested this hypothesis using a large cohort of patients with severe asthma, drawn from the International Severe Asthma Registry (ISAR; <http://isaregistries.org/>).¹⁹ We aimed to describe the interrelation between inflammatory biomarker expression in severe asthma in order to characterize the activation of underlying inflammatory pathways. We employed two approaches to distinguish patient groups with different patterns of biomarker activation; firstly, using pre-specified thresholds for each biomarker, and secondly, using cluster analysis. We then compared the clinical characteristics of the patient subgroups derived from these analyses.

METHODS

The study was designed, implemented and reported in compliance with the European Network Centres for Pharmacoepidemiology and Pharmacovigilance (ENCePP) Code of Conduct (EUPAS30430) and with all applicable local and international laws and regulation. Governance was provided by The Anonymous Data Ethics Protocols and Transparency (ADEPT) committee (registration number: ADEPT1019). All data collection sites in ISAR have obtained regulatory agreement in compliance with specific data transfer laws, country-specific legislation, and relevant ethical boards and organizations.

This cross-sectional study included adults with severe asthma enrolled in ISAR with serum IgE, blood eosinophil count (BEC), and FeNO available at registry enrollment. ISAR requests that the enrollment biomarker measurements be baseline measurements for patients who have multiple measurements available; for those with multiple baseline measurements, the

highest measurement was used. At the time of this analysis (July 2019), this multi-country data repository included data from the baseline visits of patients with severe asthma from 10 countries, including the United States, the United Kingdom, Canada, Greece, Italy, Ireland, South Korea, Bulgaria, Kuwait, and Spain. Patients with severe asthma enrolled in ISAR are aged ≥ 18 years and have either uncontrolled asthma at Global Initiative for Asthma (GINA) Step 4 treatment OR are receiving GINA Step 5 treatment; all were on high-dose inhaled corticosteroids. Uncontrolled asthma was defined as at least one of the criteria outlined in *Table 1*.

The biomarkers examined were total serum IgE (kU/L), BEC (cells/uL), and FeNO (ppb) at the point of enrollment in ISAR. For the dichotomous analysis, biomarker positivity was pre-defined as total IgE ≥ 75 kU/L,¹⁵ BECs ≥ 300 cells/uL, and FeNO ≥ 25 ppb.²⁰ Patients were classified categorically according to ISAR enrollment biomarker status. Due to lack of international consensus on biomarker thresholds, sensitivity analyses were performed for pre-specified alternate biomarker thresholds (IgE ≥ 30 , ≥ 100 , ≥ 300 , ≥ 400 , ≥ 700 ; BEC ≥ 150 and ≥ 400 ; FeNO ≥ 50), allergic sensitization rather than IgE (defined by positive skin prick testing [wheal > 3 mm] or serum specific IgE of ≥ 0.1 kU/L to at least one perennial allergen), and the presence of oral corticosteroids at baseline. The distribution of each biomarker, overlap of biomarkers, and combinations of positive biomarkers was described.

Pre-specified biomarker combinations

The eight possible biomarker combinations were described (positive: IgE, BEC, FeNO, IgE and BEC, IgE and FeNO, BEC and FeNO, all three, or none positive). For each, the characteristics at enrollment, including demographics, lung function, asthma symptoms, exacerbations, presence of comorbidities and asthma medications were described. Exacerbations were defined according to American Thoracic Society/European Respiratory Society criteria.^{1,21} Exacerbations were recorded over the 12-month period prior to enrollment in the ISAR. Asthma control was determined by standardized and validated asthma control questionnaires, Asthma Control Test²², Asthma Control Questionnaire²³ or GINA guidelines.^{24,25} Comorbidities were defined by a clinician diagnosis after relevant diagnostic testing and review of medical records, and recorded as current, past, or never. Comorbidities examined were allergic rhinitis, chronic rhinosinusitis, eczema, and nasal polyps.

Comparison between groups was performed with Pearson's chi-squared test or one-way ANOVA with the post hoc Tuckey test. P-value < 0.05 was considered significant. Adjustment for multiple comparisons was not performed.

A post-hoc analysis was performed describing characteristics of the biomarker groups who were on biologic medications.

Cluster analysis

The distributions of IgE, BEC, and FeNO were examined using histograms and standardized for analysis using z-scores. A pre-specified primary cluster analysis was then performed with the biomarkers as continuous variables via Ward's minimum variance hierarchical cluster analysis method with an agglomerative approach and Ward's linkage. A dendrogram was generated to estimate the number of clusters within the study population. K-means cluster

analysis was used to test the consistency of the model using multiple different cluster numbers. Detailed cluster analysis methods are available in the Online Repository Text, Section 1. Comparisons between clusters were performed with Pearson's chi-squared test for categorical variables (presented as % of total) and one-way ANOVA for continuous variables (presented as mean $\pm$ standard deviation).

RESULTS

Total cohort

Of 6270 adults with severe asthma, 1175 with available biomarkers were included in the study. Characteristics at enrollment are described in *Table 2*. The majority of patients were from the United States (49%) and the United Kingdom (31%). Patients were predominantly White (81%) and female (64%), with a mean age of 53 years and mean body mass index (BMI) of 30kg/m².

The majority of patients (80%) had uncontrolled asthma symptoms, and experienced a mean of four asthma exacerbations in the 12 months prior to enrollment. Mean pre-bronchodilator Forced Expiratory Volume in first second (FEV₁) was 72% predicted, pre-bronchodilator Forced Vital Capacity (FVC) of 88% predicted, and FEV₁/FVC 69%.

Patient sub-groups according to pre-specified biomarker thresholds

Overall, using pre-determined biomarker thresholds, 57% were BEC positive, 58% FeNO positive, and 59% IgE positive, with substantial overlap (*Figure 1*). The likelihood that one biomarker was positive when another was positive is presented in *Figure 2*.

Classification according to positivity or negativity to each biomarker yielded eight patient sub-groups; triple biomarker-positive (27% of the total cohort), BEC and FeNO positive (14%), BEC and IgE positive (8%), FeNO and IgE positive (10%), only BEC positive (8%), only FeNO positive (7%), only IgE positive (14%), and triple biomarker negative (12%).

Demographic & clinical characteristics

Characteristics of each biomarker group are shown in *Table 2*. There were significantly more females among triple negative biomarker patients (74%) than among triple biomarker positive patients (57%, $p=0.01$). BMI was lower in triple biomarker positive and BEC/FeNO positive patients compared to those patients who were only IgE positive. Patients with BEC/FeNO positivity, FeNO positivity, and triple biomarker negativity were significantly older than patients who were FeNO/IgE positive and IgE positive only. There were no significant between-group differences for asthma symptoms, exacerbations, or FEV₁% predicted.

Despite changing the biomarker thresholds for the sensitivity analyses, there was still considerable biomarker overlap between groups, although the characteristics of the groups did change. These results are presented in the Online Repository Text, Section 3. A post-hoc analysis was performed describing those in each biomarker group on biologic medications and those who had known information on biomarkers being measured prior to biologic medication initiation; these results are presented in the Online Repository Text Table 3e.

In one sensitivity analysis, replacing total IgE with allergic sensitization as a more relevant

marker of atopy, the largest patient group was triple biomarker-positive (34%), whereas only 5% were triple negative. In this analysis, the triple biomarker-negative group also had the highest BMI (35 versus 30 in the whole group). No significant difference was found in age, sex, or lung function between groups in this analysis, although there was a trend toward more uncontrolled disease and less allergic rhinitis and chronic sinusitis in the triple biomarker-negative group.

Cluster analysis

Histograms showed a large range of distributions for all three biomarkers (serum IgE, BEC and FeNO). Extreme outliers were excluded from the IgE and BEC groups. Biomarker values were then standardized using z-scores for inclusion in the cluster analysis.

The dendrogram used for estimation of the number of clusters within the studied population is shown in the Online Repository Text, Figure E1a. K-means cluster analysis was performed with the five distinctive clusters identified via the Hierarchical cluster approach and also repeated with four and six clusters to assess the stability of clusters. The consistency of the two different cluster analysis methods was high, with similar patient numbers in each cluster using the two methods. The stability of smaller clusters was also found to be high when using different numbers of clusters.

Final results identified five clusters within the severe asthma cohort (*Figure 3, Table 3*), a summary is presented below with mean biomarker values for each group presented in brackets. Baseline characteristics of each cluster are shown in *Figure 4 and Table 3*. There was stability of clusters when repeating cluster analysis with greater and fewer numbers of clusters and different cluster techniques.

Cluster One: older, symptomatic, obese females (61%)

The biomarker profile in this group showed relatively low mean serum IgE (167 kU/L), BEC (40 cells/uL), and FeNO (23 ppb), although many patients in this group would still have “positive” biomarkers based on traditional pre-specified biomarker thresholds. Clinically, this large cluster was comprised predominantly of older, obese females who had poor asthma control and frequent exacerbations. Lung function was mildly impaired with a mean FEV₁ % predicted of 71%.

Cluster Two: older, eosinophilic, exacerbating females (18%)

The biomarker profile showed high mean BEC (911 cells/uL), elevated FeNO (51 ppb), and relatively low IgE (187 kU/L). Clinically, this large cluster comprised mainly older females with lower BMI, with a relatively shorter duration of asthma. Despite preserved lung function and fewer patients with poor symptom control, this cluster had the highest number of exacerbations of any cluster.

Cluster Three: older, symptomatic, high FeNO, preserved lung function (14%)

The biomarker profile was characterized by very high FeNO (166 ppb), and moderately elevated IgE (358 kU/L) and BEC (509 cells/uL). Clinically, this cluster comprised the oldest patients with a significantly lower BMI, a relatively short duration of asthma, and the best lung function. Despite a relatively low number of exacerbations, asthma symptom control remained poor.

Cluster Four: younger, high IgE, obese, poor lung function (6%)

The biomarker profile showed very high IgE (1932 kU/L), with moderately elevated BEC (333 cells/uL) and FeNO (38 ppb). Clinically, this cluster comprised the youngest patients (mean age 49 years) with the most even gender balance and the longest duration of asthma of any cluster. BMI was high and lung function was low, yet this cluster had relatively few exacerbations, the best symptom control, and the fewest patients on maintenance oral corticosteroids.

Cluster Five: younger males, eosinophilic, low BMI and lung function with nasal polyps (1.2%)

The biomarker profile was characterized by extremely high BEC (4475 cells/uL), with elevation of FeNO (54 ppb) and IgE (698 kU/L). This was a very small cluster (but highly conserved across different cluster analysis methods and with analysis using different cluster numbers). Clinically, the cluster comprised younger males, with the lowest BMI and the lowest lung function, but the least number of exacerbations, and relatively few patients with poor symptom control or on chronic oral corticosteroids. This cluster had a very high prevalence of allergic rhinitis (82%), chronic rhinosinusitis (75%), and nasal polyps (71%).

DISCUSSION

In this large severe asthma cohort, analysis of biomarker expression by pre-specified threshold analysis and cluster analysis both demonstrated substantial disease heterogeneity, consistent with previous similar findings in a general asthmatic population.¹⁹ According to pre-specified thresholds, there was substantial overlap in biomarker positivity. Cluster analysis of biomarker expression identified five distinct patient clusters. The discrete clustering – highly conserved between cluster analysis methods – suggests that differential inflammatory pathway activation in severe asthma occurs in very specific patterns. Furthermore, each biomarker-derived cluster was also distinguished by distinct clinical characteristics. Taken together, these findings provide new insights into the complexity of severe asthma pathogenesis and response to treatment, and have potential relevance for assisting in delivering precision medicine in the era of targeted monoclonal biologics.

By pre-specified biomarker thresholds, almost 60% of patients were positive for each individual biomarker. These positivity rates exceeded those reported in a general asthma population, where 36% of participants had elevated eosinophils (≥ 300 cells/uL), 18% had high FeNO (≥ 35 ppb), and 26% had low FeNO and blood eosinophils. This finding may indicate that greater inflammatory pathway activation occurs in severe asthma patients.²⁶

Additionally, 59% were positive for either two or three biomarkers. FeNO and blood eosinophil positivity were more likely to occur together than with IgE positivity, even though previous studies have shown that FeNO does not correlate well with blood eosinophils.^{7,9,11} These data mirror findings in a general asthma population, in which considerable overlap in inflammatory biomarkers also occurred.¹⁸ However, the implication in severe asthma is that such patients positive for multiple biomarkers may be suitable for multiple biologic therapies. For this group, new methods are needed to determine the most appropriate choice of targeted therapy.

Our primary and sensitivity analyses also identified a patient group whose clinical needs remain unmet by currently available biologics. This triple biomarker-negative group had significantly more females compared to the triple biomarker-positive group (74% versus 57%), but there were otherwise no significant between-group differences in smoking status, asthma control, exacerbations, lung function, or comorbidities.

Cluster analysis has previously been performed in severe asthma cohorts^{12,27,28} but to our knowledge, this is the largest analysis to date and the first to focus primarily on biomarkers. The discrete clustering according to biomarker expression that we observed implies that specific combinations of inflammatory pathway activation predominate in severe asthma, rather than occurring as an even continuum across all possible combinations. This supports the notion that distinct inflammatory endotypes underpin clinically recognizable severe asthma phenotypes. This characterization of biomarker-derived clusters now identifies new patient sub-populations as a basis to study relevant disease mechanisms in more detail.

Cluster One (older, highly symptomatic obese females with relatively low biomarkers) probably equates to the 'obese non-eosinophilic' cluster described by Halder and colleagues, and the obese severe asthma cluster identified by Moore and colleagues, although biomarker status was not fully described in those patients.^{27,28} Importantly, even though biomarker expression in this cluster was relatively low, many of these patients would be suitable for targeted therapies in countries with conventional biomarker thresholds for biologic eligibility. However, this cluster is likely to include the majority of the 12% of patients that were identified as triple negative in the dichotomous analysis, an important group for targeting future research.

Cluster Two (significantly elevated BEC and FeNO, older females with average BMI) and Cluster Five (extremely high BEC, younger males with sino-nasal disease especially nasal polyposis) have not been previously distinguished in earlier reports of late-onset eosinophilic asthma. Specifically, Cluster Two did not conform to previously reported characteristics of worse lung function and chronic rhinosinusitis, and may represent a novel finding.⁶ Cluster Five also had unique features including a low BMI and, given its small size, may include patients with "asthma-plus" syndromes or hypereosinophilic disorders.

Cluster Three (very high FeNO, older, slight female predominance, preserved lung function and lower BMI) had a larger proportion with poor symptom control but relatively less frequent exacerbations. This appears to be another novel cluster. Cluster Four (very high IgE, equal gender balance, longest duration of asthma, low lung function, high BMI) probably represents the well-recognized clinical phenotype of atopic childhood-onset asthma.²⁷ More than 80% of this group had allergic sensitization. The prevalence of eczema was similar between groups (Table 3) so it is unlikely to be responsible for the IgE elevation. Data on fungal sensitization was not universally available, so raises a potential explanation for extremely high IgE in some patients. The low lung function may be explained by the long asthma duration and repeated exacerbations or impaired lung growth during childhood and adolescence. The high BMI may be as a result of chronic oral corticosteroid exposure or symptoms limiting exercise tolerance.

ISAR is the largest international registry of severe asthma patients, with high-quality, patient level, real-world, standardized data collected from countries across the world. The heterogeneous nature of the data is broader than that collected in randomized controlled trials, and ISAR has the ability to follow patients longitudinally. However, this study has the inherent limitations of an observational disease registry. Of more than 6000 patients enrolled in ISAR, only 1175 had all three biomarkers available at baseline, introducing the possibility of selection bias. Although pre-specified, the biomarker thresholds were arbitrary, and sensitivity analyses showed that applying different threshold changed the characteristics of the groups thus derived. (Online Repository Text, Section 3) Biomarkers were only reported at baseline, so cluster stability in this cohort remains unclear. Previous data does demonstrate the occurrence of biomarker variability over time, and the potential effects of medications including oral corticosteroids and monoclonal biologics do limit the interpretation of this study. However, as a real-world dataset with patients presenting for evaluation having already been commenced on medications these data offer pragmatic insights into inflammatory biomarker patterns. ISAR requests that the biomarker values be taken prior to initiation of biologic medications; however, data on baseline medications were limited in this registry, and although wherever possible the baseline biomarker measurement was prior to initiating biologic medications, this may not have been the case in all patients. Of a sample of 281 subjects who had dates available for both biologic initiation and biomarker measurement, 240 (84%) of these had their biomarkers measured prior to biologic initiation. Some patients may have been on oral corticosteroids at the time of BEC measurement which may have affected the measured BEC level.

To conclude, this study has shown considerable overlap of inflammatory biomarkers in severe asthma suggesting that a more comprehensive approach – rather than relying on simple biomarker threshold positivity – is needed to identify the best therapy for patients. We have also identified and described five severe asthma clusters defined according to biomarker expression. These data suggest the occurrence of discrete patterns of underlying inflammatory pathway activation and may assist in providing pathogenic insights relevant to the era of monoclonal biologics and personalized medicine.

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Table 1: Definition of uncontrolled asthma, participants with at least one of the following criteria present

Criteria	Definition
Poor symptom control	Asthma Control Questionnaire >1.5, OR Asthma Control Test <20, OR Having ≥ 3 of the following during the past 4 weeks [NAEPP/Global Initiative for Asthma guidelines]: ^{24, 25} daytime symptoms >twice/week, night waking due to asthma, reliever needed >twice/week, activity limitation due to asthma.
Evidence of airflow limitation	Forced expiratory volume in one second [FEV ₁] < 80% predicted, in the face of reduced FEV ₁ /forced vital capacity following a withhold of short and long acting bronchodilators, [i.e. Pre-bronchodilator])
Evidence of serious exacerbations	At least one hospitalization, ICU stay or mechanical ventilation for asthma in the previous year
Evidence of frequent severe asthma exacerbations	$\geq$ Two asthma exacerbations defined as per the American Thoracic Society/European Respiratory Society criteria of increased asthma symptoms (cough, shortness of breath, wheeze) necessitating systemic corticosteroids (or an increase in baseline corticosteroids) for ≥ 3 days or a hospitalization or emergency department visit due to asthma requiring systemic steroids ¹

FEV₁ – Forced expiratory volume in one second

Table 2: Patient demographics, asthma control, exacerbations, lung function and comorbidities both overall and with each combination of biomarker positivity using pre-defined cutoffs of blood eosinophils ≥ 300 cells/uL, FeNO ≥ 25 ppb, total IgE ≥ 75 kU/L.

Group	Total	Triple	Eos+	Eos+	FeNO+	Eos+	FeNO+	IgE+	Triple	p-value
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	(1175)	positive 27% (317)	FeNO+ 14% (163)	IgE+ 8% (96)	IgE+ 10% (118)	8% (94)	7% (81)	14% (165)	negative 12% (141)	
Sex % female	64% (752)	57%* (181)	67% (109)	57% (55)	62% (73)	68% (64)	73% (59)	64% (106)	74%* (104)	0.01
BMI mean (st. dev)	30 (±7.5)	29* (0.02) (±6.5)	29† (0.04) (±6.7)	31 (±8.9)	30 (±7)	32 (±7.5)	30 (±6.3)	32*† (±9.3)	31 (±7.6)	0.004
Age mean (st. dev)	53 (±15) N=1099	53 (±15)	57*(0.02) † (0.03) (±14)	52 (±15)	49* ‡(0.01) @ (0.03) (±16)	51 (±15)	57‡ (±15)	51† (±15)	55@ (±15)	0.000
Asthma control¹: poor % of group (number)	80% (510)	73% (131)	84% (81)	77% (43)	75% (52)	78% (39)	84% (37)	91% (71)	85% (56)	0.2
Exacerbations Mean (± st. dev.)	4 (±4)	4 (±4)	3.9 (±4)	4.8 (±4)	4.4 (±4)	4 (±4)	4.5 (±4)	3.7 (±4)	3.3 (±3)	NS (0.6)
FEV₁ pre %predicted Mean (± st. dev.) N = 1095	72% (±22) N = 1095	71% (±22)	74% (±21)	67% (±25)	73% (±22)	71% (±20)	69% (±18)	69% (±21)	75% (±22)	NS (0.07)
Allergic rhinitis: current % group (number)	60% (488)	62% (129)	61% (64)	62% (39)	62% (42)	66% (50)	60% (32)	52% (69)	60% (63)	NS (n=812)
Chronic rhinosinusitis: current % group (number)	66% (354)	66% (92)	62% (48)	68% (27)	73% (37)	62% (31)	80% (31)	65% (46)	65% (42)	NS (n=533)
Eczema: current %	10% (104)	7% (20)	9% (14)	12% (10)	10% (11)	7% (6)	11% (8)	12% (19)	13% (16)	NS (n=1069)

group (number)										
Nasal polyps: current % group (number)	35% (204)	42% (74)	40% (40)	31% (14)	30% (20)	37% (15)	30% (12)	27% (13)	28% (16)	NS (n=577)
Chronic oral corticosteroid use	46% (541)	44% (138)	50% (81)	51% (49)	42% (49)	50% (47)	52% (42)	44% (72)	45% (63)	
Anti-IgE	17% (194)	23% (72)	9% (14)	29% (28)	21% (25)	3% (3)	5% (4)	24% (40)	6% (8)	
Anti-IL5	29% (338)	33% (104)	28% (45)	27% (26)	31% (37)	27% (25)	40% (32)	23% (38)	22% (31)	

*+@ denote significant between group differences. Eos – Blood eosinophils; FeNO – Fractional exhaled nitric oxide; IgE – immunoglobulin E; BMI – body mass index; FEV₁ – Forced expiratory volume in 1 second

¹ Definition of poor asthma control: Asthma Control Questionnaire >1.5, OR Asthma Control Test <20, OR Having ≥3 of the following during the past 4 weeks [NAEPP/Global Initiative for Asthma guidelines]:^{24, 25} daytime symptoms >twice/week, night waking due to asthma, reliever needed >twice/week, activity limitation due to asthma.

Table 3: Characteristics of five clusters identified via hierarchical cluster analysis, p-value refers to significant between group difference.

	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	Total	P-value
Number	669 (61%)	200 (18%)	149 (14%)	66 (6%)	13 (1.2%)	1097	
Female sex	66%	67%	58%	53%	39%	64%	0.01
Age	53 ± 15	54 ± 14	56 ± 15*	49 ± 18*	51 ± 15	53 ± 15	0.03
BMI	31 ± 8*#	28 ± 6#	28 ± 6*	31 ± 8	27 ± 6	30 ± 8	<0.001
FEV₁ %predicted	71 ± 21	73 ± 23	75 ± 23	69 ± 18	68 ± 18	72 ± 22	NS
Blood eosinophils	240 ± 174	911 ± 372	509 ± 310	333 ± 225	4475 ± 1755	452 ± 581	<0.001
IgE	167 ±	187 ±	358 ± 402	1932 ± 1181	698 ± 824	318 ± 584	<0.001

	202	234					
FeNO	23 ± 17	51 ± 23	166 ± 140	38 ± 29	54 ± 44	46 ± 48	<0.001
Allergic sensitisation	78%	84%	72%	81%	60%	78%	NS
Asthma duration	26 ± 17	25 ± 17	26 ± 18	33 ± 17	28 ± 17	26 ± 17	NS
Asthma exacerbations	4.1 ± 3.9	4.5 ± 4.7	3.6 ± 3.2	3.7 ± 3.3	3.4 ± 2	4.1 ± 3.9	NS
Asthma control	84%	72%	80%	68%	71%	79%	0.003
Allergic rhinitis (current)	58%	63%	62%	65%	82%	60%	NS
Chronic rhinosinusitis (current)	66%	70%	62%	64%	75%	67%	NS
Nasal polyps (current)	30%	44%	34%	44%	71%	36%	0.01
Eczema (current)	11%	8%	7%	12%	15%	10%	NS
Baseline oral corticosteroids	47%	48%	45%	38%	39%	46%	NS

BMI – body mass index; FEV₁ – Forced expiratory volume in 1 second; FeNO – Fractional exhaled nitric oxide; IgE – immunoglobulin E;

Figures Legends:

Figure 1: Overlap of baseline positivity of three clinically used biomarkers, blood eosinophils ≥ 300 cells/uL, FeNO ≥ 25 ppb, total IgE ≥ 75 kU/L, in the International Severe Asthma Registry severe asthma cohort.

FeNO – Fractional exhaled nitric oxide; IgE –immunoglobulin E.

Figure 2: Percent likelihood that other biomarkers are positive based on positivity of another biomarker in the International Severe Asthma Cohort.

FeNO – Fractional exhaled nitric oxide; IgE –immunoglobulin E.

Figure 3: Graphical representation of the clinical characteristics of the five severe asthma clusters relating to three biomarker levels identified on Hierarchical cluster analysis performed with blood eosinophils, total IgE and FeNO.

FeNO – Fractional exhaled nitric oxide; IgE –immunoglobulin E; BMI – body mass index; FEV₁ – Forced expiratory volume in 1 second

Figure 4: Clinical characteristics of the five severe asthma clusters relating to three biomarker levels identified on Hierarchical cluster analysis performed with blood eosinophils, total Immunoglobulin E and Fractional Expired Nitric Oxide. Clusters 1-5 along the X axis.

FEV₁ – Forced expiratory volume in 1 second

Figure 1

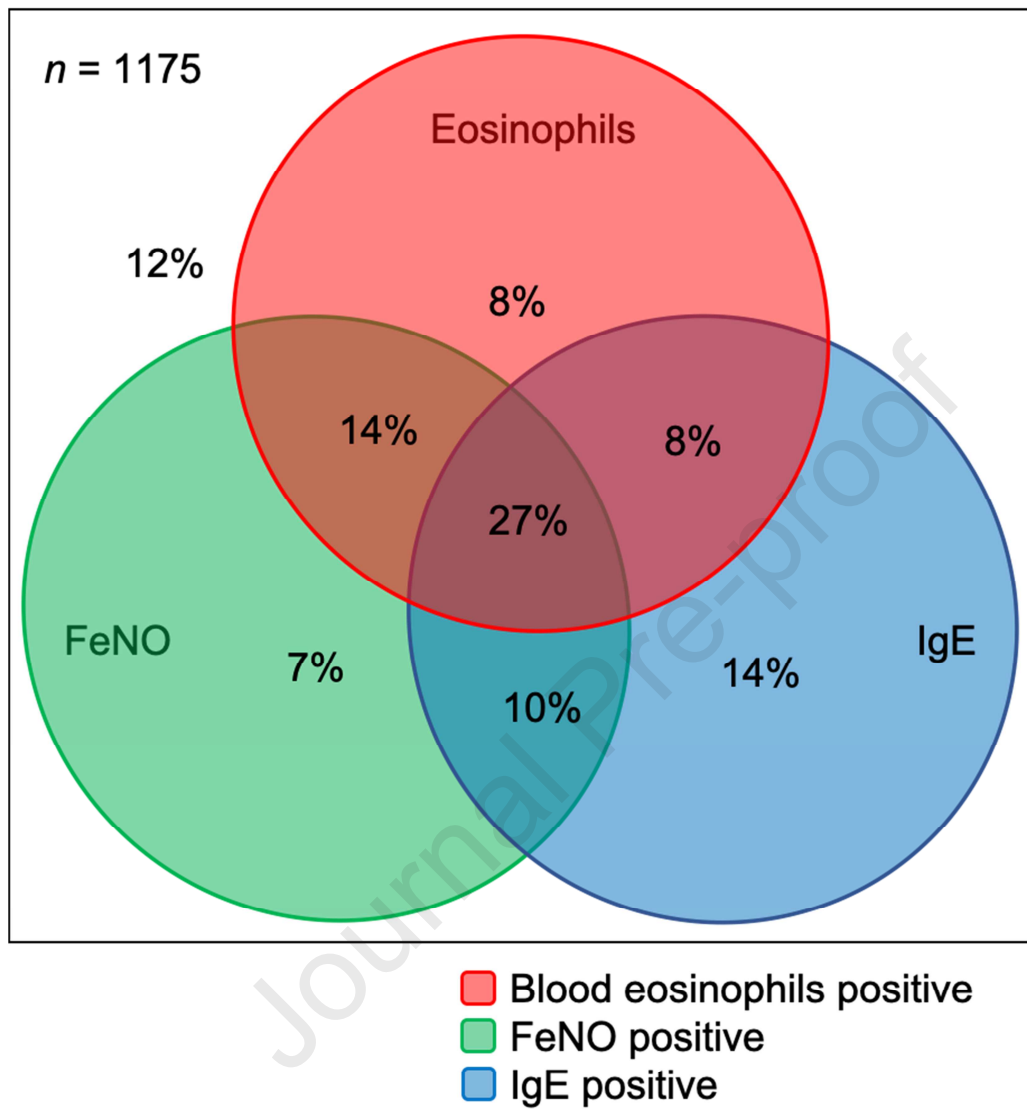


Figure 2

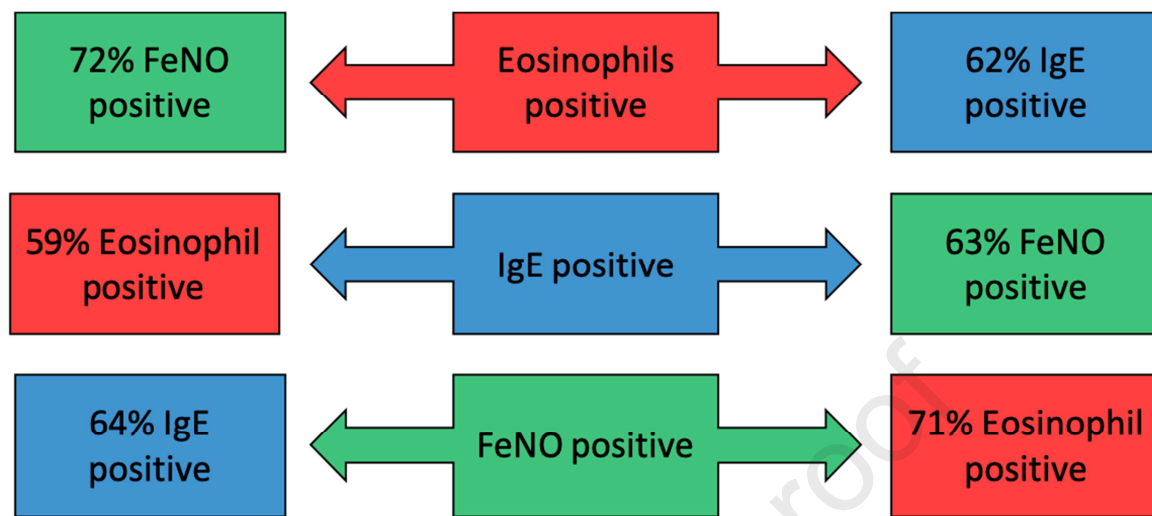


Figure 3

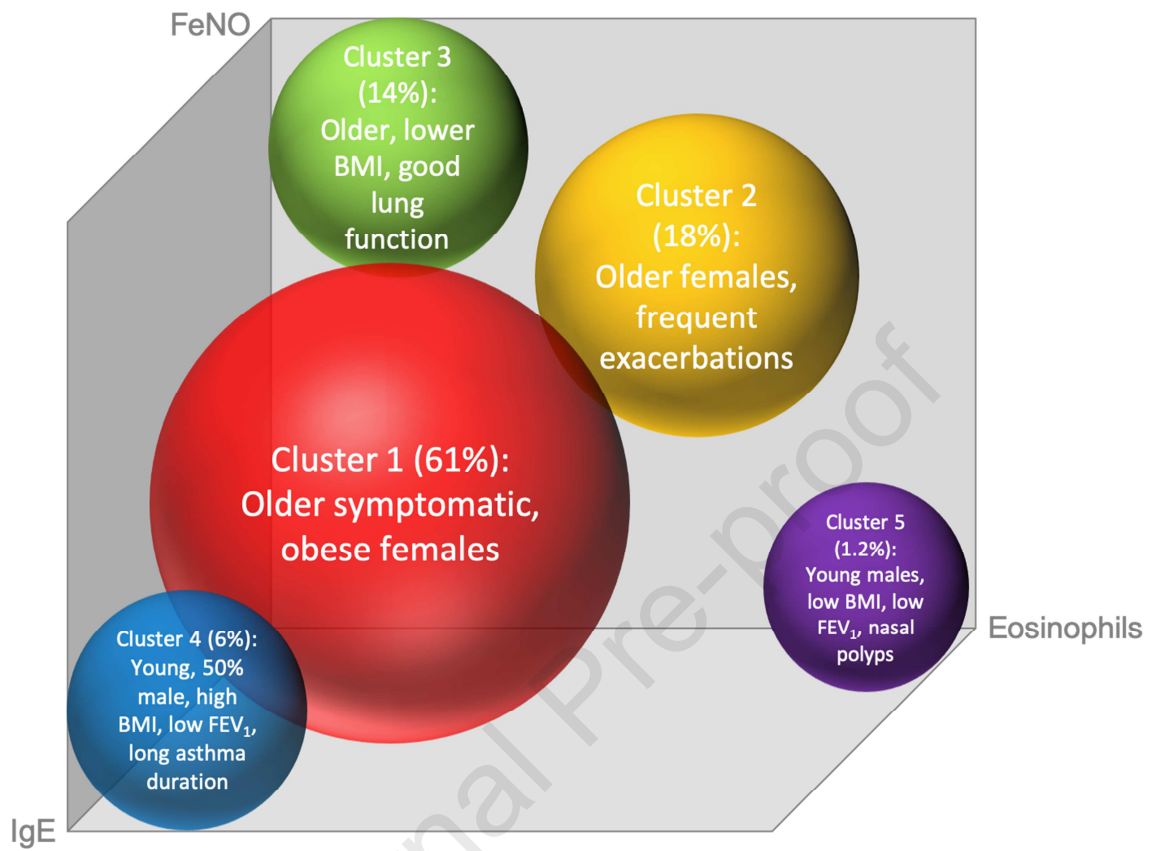


Figure 4

