Original Article

Cluster Analysis of Inflammatory Biomarker Expression in the International Severe Asthma Registry

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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.

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Abbreviations used
BEC- Blood eosinophil count
BMI- Body mass index
FeNO- Fractional exhaled nitric oxide
FEV_1- Forced expiratory volume in one second
GINA- Global Initiative for Asthma
IgE- Immunoglobulin E
ISAR- International Severe Asthma Registry

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 prespecified thresholds for biomarker positivity (serum IgE ≥ 75 kU/L, blood eosinophils ≥ 300 cells/μL, and FeNO ≥ 25 ppb), and with hierarchical cluster analysis using biomarkers as continuous variables.
RESULTS: Of 1,175 patients; 64% were female, age (mean ± SD) 53 ± 15 years, body mass index (BMI) 30 ± 8, postbronchodilator forced expiratory volume in 1 second (FEV_1) predicted 72% ± 20%. By prespecified thresholds, 59% were IgE positive, 57% eosinophil positive, and 58% FeNO positive. There was substantial inflammatory biomarker overlap; 59% were positive for either 2 or 3 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 sinonasal disease and polyposis.
CONCLUSIONS: 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. **(J Allergy Clin Immunol Pract 2021;9:2680-8)**

Key words: *Severe asthma; Biomarkers; Immunoglobulin E; Fractional exhaled nitric oxide; Eosinophils*

INTRODUCTION

One in 10 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 health care expenditure, experience the greatest morbidity, and face the highest risk of death compared with patients with nonsevere 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-5, and the interleukin-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]

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TABLE I. Definition of uncontrolled asthma, participants with at least 1 of the following criteria present

Criteria	Definition
Poor symptom control	Asthma Control Questionnaire > 1.5, OR Asthma Control Test < 20, OR having 3 or more of the following during the past 4 weeks (National Asthma Education and Prevention Program [NAEPP]/GINA guidelines)[20,21]: daytime symptoms more than twice/wk, night waking due to asthma, reliever needed more than twice/wk, activity limitation due to asthma.
Evidence of airflow limitation	FEV_1 < 80% predicted, in the face of reduced FEV_1/FVC following a withhold of short- and long-acting bronchodilators (ie, prebronchodilator)
Evidence of serious exacerbations	At least 1 hospitalization, ICU stay, or mechanical ventilation for asthma in the previous year
Evidence of frequent severe asthma exacerbations	Two or more 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 three or more days or a hospitalization or emergency department visit due to asthma requiring systemic steroids

FEV_1, Forced expiratory volume in one second; *FVC*, forced vital capacity; *ICU*, intensive care unit.

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 biomarkers expression in severe asthma in order to characterize the activation of underlying inflammatory pathways. We employed 2 approaches to distinguish patient groups with different patterns of biomarker activation; first, using prespecified thresholds for each biomarker, and second, 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. The 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 multicountry 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 or older 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 1 of the criteria outlined in Table I.

The biomarkers examined were total serum IgE (kU/L), BEC (cells/μL), and FeNO (ppb) at the point of enrollment in ISAR. For the dichotomous analysis, biomarker positivity was predefined as total IgE of 75 kU/L or greater,[15] BECs of 300 cells/μL or greater, and FeNO of 25 ppb or greater.[22] Patients were classified categorically according to ISAR enrollment biomarker status. Owing to lack of international consensus on biomarker thresholds, sensitivity analyses were performed for prespecified alternate biomarker thresholds (IgE $\geq$ 30, $\geq$ 100, $\geq$ 300, $\geq$ 400, and $\geq$ 700; BEC $\geq$ 150 and $\geq$ 400; and FeNO $\geq$ 50), and allergic sensitization rather than IgE (defined by positive skin prick testing [wheal > 3 mm] or serum specific IgE of 0.1 kU/L or greater to at least 1 perennial allergen). The distribution of each biomarker, overlap of biomarkers, and combinations of positive biomarkers was described.

Prespecified biomarker combinations

The 8 possible biomarker combinations were described (positive: IgE, BEC, FeNO, IgE and BEC, IgE and FeNO, BEC and FeNO, all 3, 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.[1,23] 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 Test,[24] Asthma Control Questionnaire,[25] or GINA guidelines.[20,21] 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 chi-squared test or one-way analysis of variance with the *post hoc* Tuckey test. A *P* value less than .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 prespecified primary cluster analysis was then performed with the biomarkers as continuous variables via Ward minimum variance hierarchical cluster analysis method with an agglomerative approach and Ward linkage. A dendrogram was generated to estimate the number of clusters within the study population. K means cluster

TABLE II. Patient demographics, asthma control, exacerbations, lung function, and comorbidities both overall and with each combination of biomarker positivity*

Group	Total (1,175)	Triple positive 27% (317)	Eos+FeNO+ 14% (163)	Eos+ IgE+ 8% (96)	FeNO+ IgE+ 10% (118)	Eos+ 8% (94)	FeNO+ 7% (81)	IgE+ 14% (165)	Triple negative 12% (141)	*P* value
Sex % female (n)	64 (752)	57† (181)	67 (109)	57 (55)	62 (73)	68 (64)	73 (59)	64 (106)	74† (104)	.01
BMI mean (SD)	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)	.004
Age mean (SD)	53 (±15) n = 1,099	53 (±15)	57† (0.002) ‡(0.03) (±14)	52 (±15)	49†§(0.01) ‖(0.03) (±16)	51 (±15)	57‡ (±15)	51† (±15)	55‖ (±15)	<.001
Asthma control¶: poor % of group (n)	80 (510)	73 (131)	84 (81)	77 (43)	75 (52)	78 (39)	84 (37)	91 (71)	85 (56)	.2
Exacerbations mean (±SD)	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 (.6)
FEV_1 pre %predicted mean (±SD)	72 (±22) n = 1,095	71 (±22)	74 (±21)	67 (±25)	73 (±22)	71 (±20)	69 (±18)	69 (±21)	75 (±22)	NS (.07)
Allergic rhinitis: current % group (n)	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 (n)	66 (354)	66 (92)	62 (48)	68 (27)	73 (37)	62 (31)	80 (31)	65 (46)	65 (42)	NS (n = 533)
Eczema: current % group (n)	10 (104)	7 (20)	9 (14)	12 (10)	10 (11)	7 (6)	11 (8)	12 (19)	13 (16)	NS (n = 1,069)
Nasal polyps: current % group (n)	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 % (n)	46 (541)	44 (138)	50 (81)	51 (49)	42 (49)	50 (47)	52 (42)	44 (72)	45 (63)	
Anti-IgE % (n)	17 (194)	23 (72)	9 (14)	29 (28)	21 (25)	3 (3)	5 (4)	24 (40)	6 (8)	
Anti-IL-5 % (n)	29 (338)	33 (104)	28 (45)	27 (26)	31 (37)	27 (25)	40 (32)	23 (38)	22 (31)	

Eos, blood eosinophils; *IL-5*, interleukin 5; *NS*, not significant.

*Using predefined cutoffs of blood eosinophils ≥ 300 cells/μL, FeNO ≥ 25 ppb, total IgE ≥ 75 kU/L.

†,‡,§,‖Denote significant between group differences.

¶Definition of poor asthma control: Asthma Control Questionnaire > 1.5, OR Asthma Control Test < 20, OR having three or more of the following during the past 4 weeks (National Asthma Education and Prevention Program [NAEPP]/GINA guidelines)[20,21]: daytime symptoms more than twice/wk, night waking due to asthma, reliever needed more than twice/wk, activity limitation due to asthma.

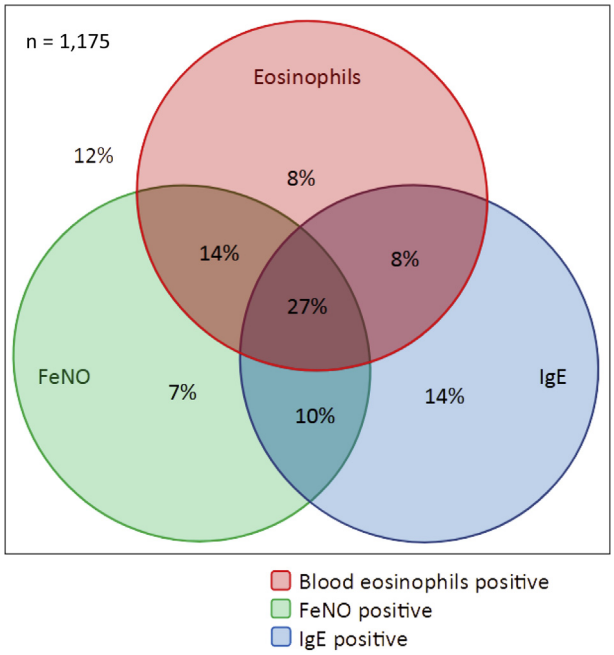

FIGURE 1. Overlap of baseline positivity of 3 clinically used biomarkers, blood eosinophils ≥ 300 cells/μL, FeNO ≥ 25 ppb, total IgE ≥ 75 kU/L, in the ISAR severe asthma cohort.

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, including Figure E1 and Table E1 (available in this article's Online Repository at www.jaci-inpractice.org). Comparisons between clusters were performed with Pearson chi-squared test for categorical variables (presented as % of total) and one-way analysis of variance for continuous variables (presented as mean ± SD).

RESULTS

Total cohort

Of 6,270 adults with severe asthma, 1,175 with available biomarkers were included in the study. Characteristics at enrollment are described in Table II. 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 30 kg/m^2.

The majority of patients (80%) had uncontrolled asthma symptoms, and experienced a mean of 4 asthma exacerbations in the 12 months prior to enrollment. Mean prebronchodilator forced expiratory volume in 1 second (FEV_1) was 72% predicted, prebronchodilator forced vital capacity (FVC) of 88% predicted, and FEV_1/FVC 69%.

Patient subgroups according to prespecified biomarker thresholds

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

Classification according to positivity or negativity to each biomarker yielded 8 patient subgroups; 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 and clinical characteristics

Characteristics of each biomarker group are shown in Table II. There were significantly more females among triple negative biomarker patients (74%) than among triple biomarker positive patients (57%; $P = .01$). The BMI was lower in triple biomarker positive and BEC/FeNO positive patients than in 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 FEV_1 % 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 2, Tables E2-E5. 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 Table E5 and Figure E2 (available in this article's Online Repository at www.jaci-inpractice.org).

In 1 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 vs 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 3 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 E1, *A* (available in this article's Online Repository at www.jaci-inpractice.org). K means cluster analysis was performed with the 5 distinctive clusters identified via the hierarchical cluster approach and also repeated with 4 and 6 clusters to assess the stability of clusters. The consistency of the 2 different cluster analysis methods was high, with similar patient numbers in each cluster using the 2 methods. The stability of smaller clusters was also found to be high when using different numbers of clusters.

Final results identified 5 clusters within the severe asthma cohort (Figure 3, Table III, and Figure E1, *B*) (available in this article's Online Repository at www.jaci-inpractice.org); a summary is presented later with mean biomarker values for each

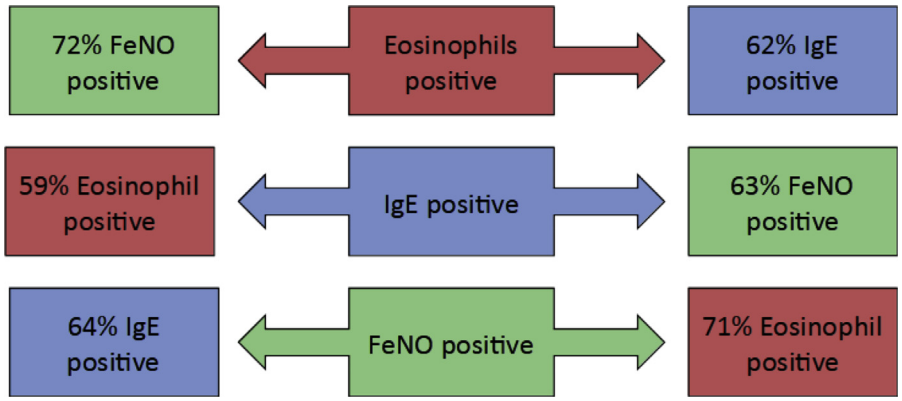

FIGURE 2. Percent likelihood that other biomarkers are positive based on positivity of another biomarker in the ISAR.

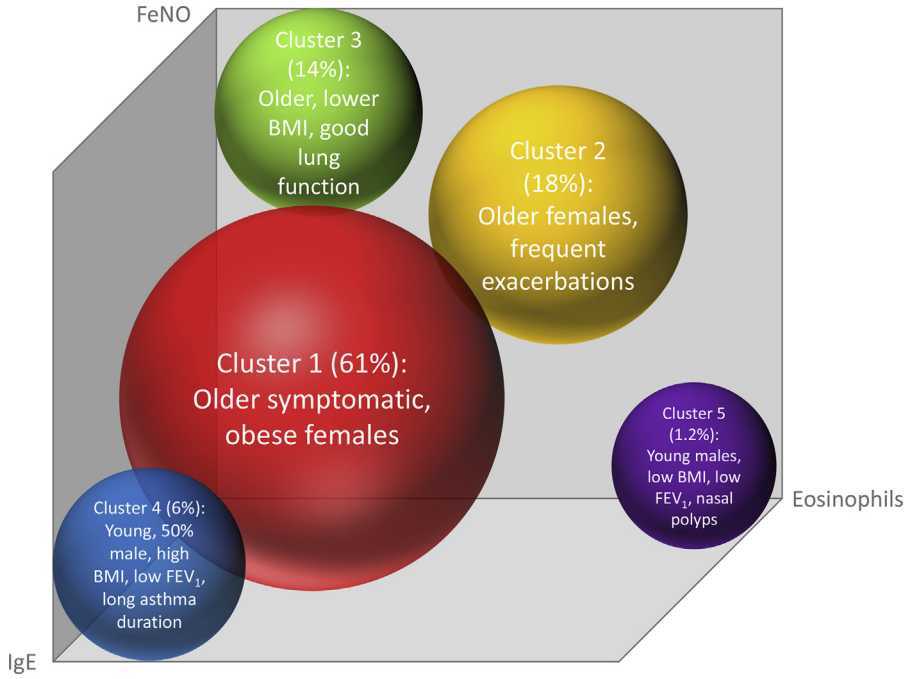

FIGURE 3. Graphical representation of the clinical characteristics of the 5 severe asthma clusters relating to 3 biomarker levels identified on hierarchical cluster analysis performed with blood eosinophils, total IgE, and FeNO.

group presented in parentheses. Baseline characteristics of each cluster are shown in Figure 4 and Table III. There was stability of clusters when repeating cluster analysis with greater and fewer numbers of clusters and different cluster techniques.

Cluster 1: Older, symptomatic, obese females (61%). The biomarker profile in this group showed relatively low mean serum IgE (167 kU/L), BEC (40 cells/μL), and FeNO (23 ppb), although many patients in this group would still have positive biomarkers based on traditional prespecified biomarker thresholds. Clinically, this large cluster was composed predominantly of older, obese females who had poor asthma control and frequent exacerbations. Lung function was mildly impaired with a mean FEV_1 % predicted of 71%.

Cluster 2: Older, eosinophilic, exacerbating females (18%). The biomarker profile showed high mean BEC (911 cells/μL), elevated FeNO (51 bpp), 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 3: 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/μL). Clinically, this cluster comprised the oldest patients with a significantly lower

TABLE III. Characteristics of 5 clusters identified via hierarchical cluster analysis

Characteristic	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%)	1,097	
Female sex	**66%***	**67%***	58%	53%	**39%***	64%	.01
Age	53 ± 15	54 ± 14	**56** ± 15†	**49** ± 18†	51 ± 15	53 ± 15	.03
BMI	**31** ± 8†‡	**28** ± 6‡	**28** ± 6†	31 ± 8	**27** ± 6	30 ± 8	<.001
FEV_1 % predicted	71 ± 21	73 ± 23	75 ± 23	69 ± 18	68 ± 18	72 ± 22	NS
Blood eosinophils	240 ± 174	**911** ± 372	509 ± 310	333 ± 225	**4,475** ± 1,755	452 ± 581	<.001
IgE	167 ± 202	187 ± 234	358 ± 402	**1,932** ± 1,181	**698** ± 824	318 ± 584	<.001
FeNO	23 ± 17	51 ± 23	**166** ± 140	38 ± 29	**54** ± 44	46 ± 48	<.001
Allergic sensitization	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%	.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%	.01
Eczema (current)	11%	8%	7%	12%	15%	10%	NS
Baseline oral corticosteroids	47%	48%	45%	38%	39%	46%	NS

NS, not significant.
Bold indicates statistical significance ($P < .05$).
*,†,‡Denotes significant between-group difference.

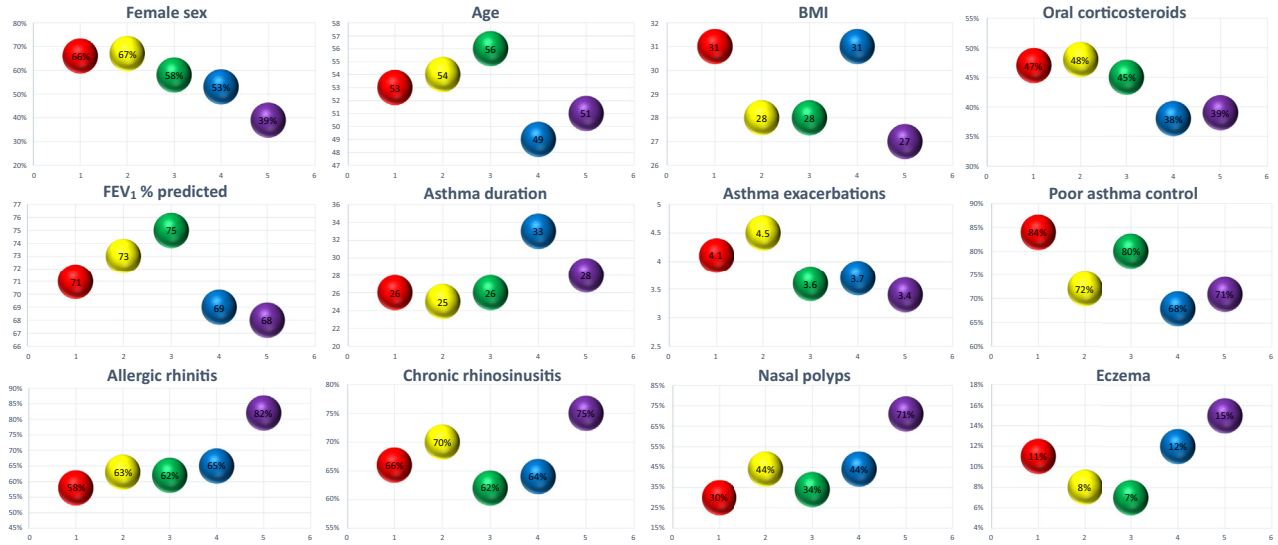

FIGURE 4. Clinical characteristics of the 5 severe asthma clusters relating to 3 biomarker levels identified on hierarchical cluster analysis performed with blood eosinophils, total IgE and FeNO. Clusters 1 to 5 along the x axis.

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 4: Younger, high IgE, obese, poor lung function (6%). The biomarker profile showed very high IgE (1932 kU/L), with moderately elevated BEC (333 cells/μL) 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. The 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 5: Younger males, eosinophilic, low BMI and lung function with nasal polyps (1.2%). The biomarker profile was characterized by extremely high BEC (4,475 cells/μL), 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 prespecified threshold analysis and cluster analysis both demonstrated substantial disease heterogeneity, consistent with previous similar findings in a general asthmatic population.[19] According to prespecified thresholds, there was substantial overlap in biomarker positivity. Cluster analysis of biomarker expression identified 5 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 prespecified 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/μL), 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]

In addition, 59% were positive for either 2 or 3 biomarkers. The 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 than the triple biomarker positive group (74% vs 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,[12,27-29] 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 subpopulations as a basis to study relevant disease mechanisms in more detail.

Cluster 1 (older, highly symptomatic obese females with relatively low biomarkers) probably equates to the obese non-eosinophilic cluster described by Halder and colleagues[27] and the obese severe asthma cluster identified by Moore and colleagues,[28] although biomarker status was not fully described in those patients. 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 2 (significantly elevated BEC and FeNO, older females with average BMI) and cluster 5 (extremely high BEC, younger males with sinonasal disease especially nasal polyposis) have not been previously distinguished in earlier reports of late-onset eosinophilic asthma. Specifically, cluster 2 did not conform to previously reported characteristics of worse lung function and chronic rhinosinusitis and may represent a novel finding.[6] Cluster 5 also had unique features including a low BMI and, given its small size, may include patients with asthma-plus syndromes or hypereosinophilic disorders.

Cluster 3 (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 4 (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 III) so it is unlikely to be responsible for the IgE elevation. Data on fungal sensitization were not universally available, so this 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.

The 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 6,000 patients enrolled in ISAR, only 1,175 had all 3 biomarkers available at baseline, introducing the possibility of selection bias. Although prespecified, 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 2). Biomarkers were only reported at baseline, so cluster stability in this cohort remains unclear. Previous data do 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. The 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 of these (84%) 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, however a repeat analysis excluding those on oral corticosteroids and biologic medications showed a similar biomarker overlap to the overall cohort (Online Repository Text, Section 3, Figure E2).

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 5 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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ONLINE REPOSITORY

SECTION 1: CLUSTER ANALYSIS DETAILED METHODS

Statistical package

SPSS version 24 (IBM, United States) and Microsoft Excel were used for the cluster analysis and associated analyses.

Cluster analysis methods

The primary cluster analysis methodology was performed using Ward's minimum variance hierarchical cluster analysis method with an agglomerative approach and Ward's linkage. This was used to generate a dendrogram for estimation of the number of clusters within the studied population (Figure E1, *A*).

K means cluster analysis was used to test the consistency of the model. This was performed with the number of clusters identified via the dendrogram (5) and also 1 less and more than this to assess the stability of clusters. Using this method the stability of clusters was found to be high with similar patient numbers in each cluster using the 2 methods.

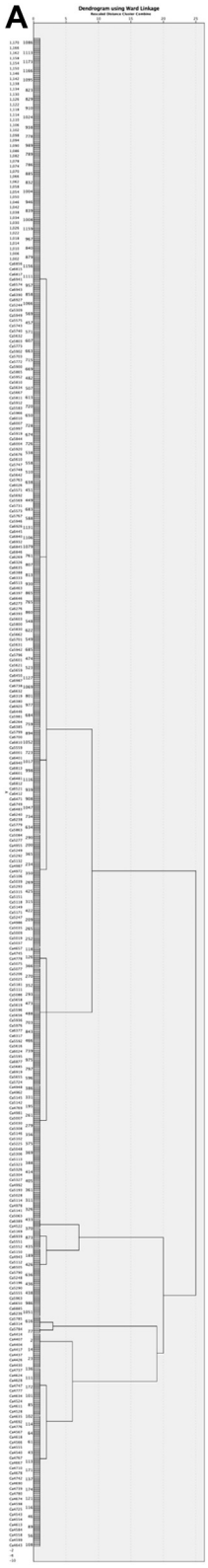
Selection of variables for analysis

Because this study focused on 3 clinically used biomarkers—immunoglobulin E, blood eosinophils, and fractional exhaled nitric oxide—it was prespecified that these 3 continuous variables would be used as the basis for determining clusters in this population. The distribution of these 3 variables was examined using histograms with all 3 biomarkers showing a large range of distributions. Extreme outliers were excluded from the immunoglobulin E and blood eosinophil groups. The 3 biomarkers were then standardized using Z-scores.

TABLE E1. Point prevalence of biomarker groups

Biomarker group	Eos+ FeNO+ IgE+	Eos+ FeNO+	Eos+ IgE+	FeNO+ IgE+	Eos+	FeNO+	IgE+	None+	Total
Eos $\geq$ 150 % (n)	31 (367)	17 (195)	13 (150)	6 (68)	14 (163)	4 (49)	9 (111)	6 (72)	1,175
Eos $\geq$ 400 % (n)	22 (259)	11 (130)	5 (58)	15 (176)	6 (65)	10 (114)	17 (203)	15 (170)	1,175
FeNO $\geq$ 50 % (n)	18 (209)	8 (93)	17 (204)	5 (64)	14 (164)	3 (30)	19 (219)	16 (192)	1,175
IgE $\geq$ 30 % (n)	35 (405)	6 (75)	11 (127)	13 (147)	5 (63)	4 (52)	19 (223)	7 (83)	1,175
IgE $\geq$ 100 % (n)	25 (289)	16 (191)	7 (77)	8 (99)	10 (113)	9 (100)	13 (151)	13 (155)	1,175
IgE $\geq$ 300 % (n)	14 (163)	27 (317)	3 (36)	5 (56)	13 (154)	12 (143)	7 (84)	19 (222)	1,175
IgE $\geq$ 400 % (n)	11 (124)	30 (356)	3 (29)	4 (51)	14 (161)	13 (148)	6 (70)	20 (236)	1,175
IgE $\geq$ 700 % (n)	6 (68)	35 (412)	1 (14)	2 (28)	15 (176)	15 (171)	2 (27)	24 (279)	1,175
Allergic sensitization % (n)	34 (276)	10 (81)	13 (107)	16 (130)	2 (17)	6 (49)	14 (109)	5 (39)	808
Baseline oral corticosteroids % (n)	26 (138)	15 (81)	9 (49)	9 (49)	9 (47)	8 (42)	13 (72)	12 (63)	541

Eos, blood eosinophils; *FeNO*, fractional exhaled nitric oxide; *IgE*, immunoglobulin E.



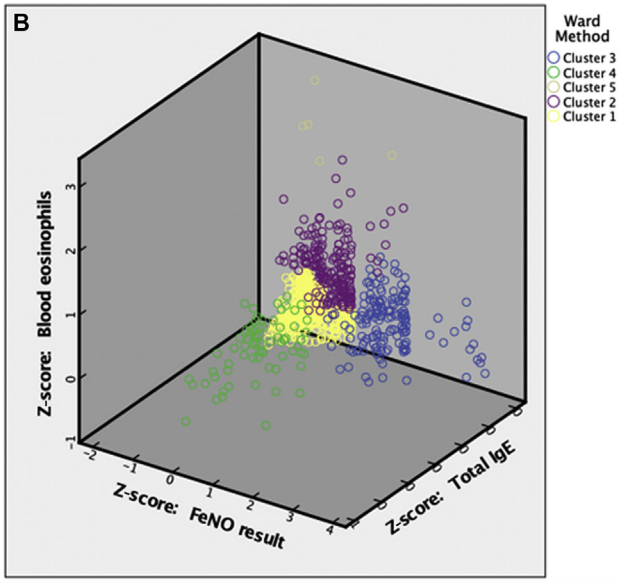


FIGURE E1. (***CONTINUED***).

FIGURE E1. (**A**) Dendrogram to estimate the number of clusters in the studied population based on biomarkers total IgE, blood eosinophil count and fractional exhaled nitric oxide. (**B**) Clusters identified during hierarchical cluster analysis with an agglomerative approach and Ward's linkage.

SECTION 2: CLINICAL CHARACTERISTICS OF PATIENTS USING ALTERNATIVE BIOMARKER CUT-OFFS

TABLE E2. Describe biomarker groups demographics: age, sex, body mass index (BMI)

Age in years (overall mean 53 ± 15)

Biomarker group	Eos+ FeNO+ IgE+	Eos+ FeNO+	Eos+ IgE+	FeNO+ IgE+	Eos+	FeNO+	IgE+	None+	Total	*P* value
Eos ≥ 150 mean (SD)	53 (±15)	57 (±14)	52 (±15)	47 (±18)	53 (±15)	57 (±15)	51 (±15)	56 (±16)	1,099	< .001
Eos ≥ 400 mean (SD)	54 (±14)	56 (±14)	53 (±14)	51 (±16)	50 (±16)	58 (±15)	51 (±15)	55 (±15)	1,099	< .001
FeNO ≥ 50 mean (SD)	54 (±15)	58 (±13)	52 (±14)	51 (±16)	52 (±15)	54 (±16)	50 (±16)	56 (±15)	1,099	< .001
IgE ≥ 30 mean (SD)	54 (±14)	57 (±15)	52 (±14)	50 (±16)	51 (±15)	59 (±16)	52 (±16)	57 (±14)	1,099	< .001
IgE ≥ 100 mean (SD)	53 (±15)	57 (±14)	51 (±15)	48 (±17)	52 (±14)	57 (±15)	51 (±15)	55 (±15)	1,099	< .001
IgE ≥ 300 mean (SD)	52 (±15)	56 (±14)	50 (±16)	47 (±17)	52 (±14)	55 (±15)	50 (±16)	54 (±15)	1,099	< .001
IgE ≥ 400 mean (SD)	52 (±16)	56 (±14)	50 (±16)	46 (±17)	52 (±14)	55 (±15)	49 (±17)	54 (±15)	1,099	< .001
IgE ≥ 700 mean (SD)	51 (±16)	55 (±14)	51 (±16)	44 (±16)	51 (±14)	54 (±16)	48 (±18)	54 (±15)	1,099	.002
Allergic sensitization mean (SD)	55 (±16)	52 (±17)	53 (±15)	54 (±17)	57 (±15)	48 (±18)	57 (±14)	53 (±16)	767	.1

Female sex

Biomarker group	Eos+ FeNO+ IgE+	Eos+ FeNO+	Eos+ IgE+	FeNO+ IgE+	Eos+	FeNO+	IgE+	None+	Total	*P* value
Eos ≥ 150 % (n)	58 (214)	69 (134)	59 (89)	60 (41)	70 (114)	69 (34)	64 (71)	75 (54)	1,175	.03
Eos ≥ 400 % (n)	58 (149)	67 (89)	53 (31)	60 (106)	63 (41)	69 (79)	64 (129)	75 (127)	1,175	.007
FeNO ≥ 50 % (n)	56 (116)	69 (64)	59 (121)	59 (38)	67 (109)	73 (22)	64 (140)	73 (141)	1,175	.008
IgE ≥ 30 % (n)	60 (241)	67 (50)	58 (73)	65 (95)	73 (46)	71 (37)	65 (145)	77 (64)	1,175	.03
IgE ≥ 100 % (n)	57 (165)	66 (126)	53 (41)	58 (57)	69 (78)	75 (75)	62 (94)	74 (115)	1,175	.001
IgE ≥ 300 % (n)	54 (88)	64 (203)	53 (19)	46 (26)	65 (100)	74 (106)	60 (50)	72 (159)	1,175	< .001
IgE ≥ 400 % (n)	52 (64)	64 (227)	55 (16)	47 (24)	64 (103)	73 (108)	60 (42)	71 (167)	1,175	.001
IgE ≥700 % (n)	49 (33)	63 (258)	50 (7)	57 (16)	64 (112)	68 (116)	59 (16)	69 (193)	1,175	.06
Allergic sensitization % (n)	59 (164)	57 (46)	69 (74)	65 (84)	65 (11)	49 (24)	69 (75)	64 (25)	808	.2

Body mass index

Biomarker group	Eos+ FeNO+ IgE+	Eos+ FeNO+	Eos+ IgE+	FeNO+ IgE+	Eos+	FeNO+	IgE+	None+	Total	*P* value
Eos ≥ 150 mean (SD)	29 (±6)	29 (±7)	31 (±9)	30 (±8)	31 (±7)	29 (±7)	31 (±9)	31 (±8)	1,170	.007
Eos ≥ 400 mean (SD)	29 (±6)	28 (±6)	30 (±8)	30 (±7)	32 (±8)	31 (±7)	32 (±9)	31 (±8)	1,170	< .001
FeNO ≥ 50 mean (SD)	29 (±6)	29 (±7)	30 (±8)	30 (±7)	31 (±7)	30 (±7)	31 (±9)	30 (±7)	1,170	.03
IgE ≥ 30 mean (SD)	29 (±7)	29 (±7)	31 (±8)	30 (±7)	33 (±8)	29 (±7)	32 (±9)	30 (±7)	1,170	.001
IgE ≥ 100 mean (SD)	29 (±7)	29 (±7)	30 (±9)	30 (±7)	32 (±8)	30 (±6)	32 (±9)	31 (±8)	1,170	.003
IgE ≥ 300 mean (SD)	30 (±7)	29 (±7)	31 (±9)	30 (±8)	31 (±8)	30 (±6)	32 (±10)	31 (±8)	1,170	.001
IgE ≥ 400 mean (SD)	30 (±7)	29 (±7)	30 (±8)	30 (±8)	31 (±8)	29 (±6)	32 (±9)	31 (±8)	1,170	.003
IgE ≥ 700 mean (SD)	29 (±7)	29 (±7)	32 (±8)	30 (±9)	31 (±8)	30 (±6)	31 (±10)	31 (±8)	1,170	.006
Allergic sensitization mean (SD)	30 (±7)	28 (±5)	29 (±7)	30 (±7)	30 (±7)	30 (±5)	31 (±8)	35 (±8)	808	< .001

Eos, blood eosinophils; *FeNO*, fractional exhaled nitric oxide; *IgE*, immunoglobulin E.

TABLE E3. Asthma status: control, exacerbations, lung function

Asthma control (percent poorly controlled)

Biomarker group	Eos+ FeNO+ IgE+	Eos+ FeNO+	Eos+ IgE+	FeNO+ IgE+	Eos+	FeNO+	IgE+	None+	Total	*P* value
Eos ≥ 150 % (n)	74 (153)	84 (95)	83 (69)	73 (30)	81 (70)	85 (23)	88 (45)	83 (25)	639	.2
Eos ≥ 400 % (n)	72 (107)	82 (61)	74 (23)	76 (76)	82 (32)	86 (57)	88 (91)	82 (63)	639	.07
FeNO ≥ 50 % (n)	74 (84)	88 (45)	73 (90)	75 (30)	79 (75)	86 (12)	87 (93)	84 (81)	639	.2
IgE ≥ 30 % (n)	76 (177)	81 (35)	74 (55)	75 (66)	84 (27)	92 (23)	89 (93)	85 (34)	639	.1
IgE ≥ 100 % (n)	75 (122)	80 (90)	76 (34)	74 (43)	79 (48)	84 (46)	92 (68)	84 (59)	639	.4
IgE ≥ 300 % (n)	76 (69)	77 (143)	67 (16)	76 (28)	81 (66)	80 (61)	98 (43)	84 (84)	639	.2
IgE ≥ 400 % (n)	76 (51)	77 (161)	70 (14)	76 (25)	79 (68)	80 (64)	97 (34)	85 (93)	639	.3
IgE ≥ 700 % (n)	71 (27)	78 (185)	63 (5)	74 (14)	79 (77)	80 (75)	93 (14)	88 (113)	639	.3

Exacerbations

Biomarker group	Eos+ FeNO+ IgE+	Eos+ FeNO+	Eos+ IgE+	FeNO+ IgE+	Eos+	FeNO+	IgE+	None+	Total	*P* value
Eos ≥ 150 mean (SD)	4 (±4)	4 (±4)	4 (±4)	4 (±4)	4 (±3)	4 (±4)	4 (±4)	4 (±4)	541	>.99
Eos ≥ 400 mean (SD)	4 (±4)	3 (±3)	5 (±5)	4 (±3)	4 (±4)	5 (±5)	4 (±4)	3 (±3)	541	.4
FeNO ≥ 50 mean (SD)	4 (±3)	5 (±5)	5 (±5)	4 (±4)	4 (±3)	6 (±3)	4 (±4)	4 (±3)	541	.2
IgE ≥ 30 mean (SD)	4 (±4)	3 (±4)	5 (±4)	4 (±3)	4 (±4)	6 (±4)	4 (±4)	4 (±4)	541	.5
IgE ≥ 100 mean (SD)	4 (±4)	4 (±4)	5 (±5)	4 (±4)	4 (±4)	4 (±4)	4 (±4)	4 (±3)	541	.7
IgE ≥ 300 mean (SD)	4 (±3)	4 (±5)	4 (±4)	5 (±4)	5 (±4)	4 (±3)	4 (±4)	3 (±3)	541	.6
IgE ≥ 400 mean (SD)	4 (±3)	4 (±5)	3 (±3)	5 (±4)	5 (±4)	4 (±3)	4 (±4)	4 (±3)	541	.4
IgE ≥ 700 mean (SD)	4 (±3)	4 (±4)	4 (±3)	6 (±4)	5 (±4)	4 (±3)	4 (±4)	4 (±4)	541	.4
Allergic sensitization mean (SD)	3 (±4)	2 (±2)	4 (±4)	4 (±4)	2 (±1)	3 (±3)	4 (±4)	3 (±3)	229	.5

FEV_1 %predicted

Biomarker group	Eos+ FeNO+ IgE+	Eos+ FeNO+	Eos+ IgE+	FeNO+ IgE+	Eos+	FeNO+	IgE+	None+	Total	*P* value
Eos ≥ 150 mean (SD)	72 (±22)	73 (±21)	68 (±23)	73 (±23)	72 (±21)	71 (±18)	69 (±18)	75 (±22)	1,095	.3
Eos ≥400 mean (SD)	72 (±22)	74 (±20)	70 (±30)	71 (±22)	72 (±21)	71 (±20)	68 (±20)	74 (±22)	1,095	.2
FeNO ≥ 50 mean (SD)	72 (±22)	74 (±20)	69 (±23)	71 (±22)	72 (±21)	63 (±19)	71 (±21)	74 (±21)	1,095	.08
IgE ≥ 30 mean (SD)	72 (±21)	76 (±21)	69 (±24)	72 (±22)	70 (±20)	70 (±18)	72 (±22)	72 (±21)	1,095	.6
IgE ≥ 100 mean (SD)	72 (±22)	73 (±21)	68 (±26)	72 (±21)	70 (±20)	71 (±21)	69 (±21)	74 (±22)	1,095	.4
IgE ≥ 300 mean (SD)	71 (±22)	73 (±21)	69 (±26)	73 (±18)	69 (±22)	71 (±22)	72 (±20)	72 (±22)	1,095	.7
IgE ≥ 400 mean (SD)	71 (±22)	73 (±21)	66 (±22)	73 (±19)	70 (±23)	71 (±22)	72 (±20)	72 (±22)	1,095	.7
IgE ≥ 700 mean (SD)	68 (±19)	73 (±22)	64 (±14)	72 (±16)	69 (±23)	71 (±22)	75 (±20)	71 (±22)	1,095	.4
Allergic sensitization mean (SD)	74 (±21)	80 (±22)	75 (±23)	73 (±19)	75 (±18)	74 (±23)	73 (±20)	70 (±24)	744	.4

Eos, blood eosinophils; *FeNO*, fractional exhaled nitric oxide; *FEV_1*, forced expiratory volume in 1 second; *IgE*, immunoglobulin E.

TABLE E4. Comorbidities: allergic rhinitis, chronic rhinosinusitis, eczema, nasal polyps

Allergic rhinitis (current)

Biomarker group	Eos + FeNO + IgE +	Eos + FeNO +	Eos + IgE +	FeNO + IgE +	Eos +	FeNO +	IgE +	None +	Total	*P* value
Eos ≥ 150 % (n)	63 (150)	62 (77)	64 (68)	60 (21)	63 (76)	56 (19)	44 (40)	62 (37)	812	.4
Eos ≥ 400 % (n)	63 (112)	63 (52)	63 (24)	60 (59)	69 (35)	58 (44)	53 (84)	60 (78)	812	.5
FeNO ≥ 50 % (n)	64 (90)	53 (31)	61 (78)	63 (20)	68 (83)	70 (14)	54 (91)	58 (81)	812	.4
IgE ≥ 30 % (n)	62 (165)	62 (28)	64 (57)	63 (53)	64 (32)	55 (21)	55 (99)	55 (33)	812	.9
IgE ≥ 100 % (n)	64 (124)	58 (69)	64 (30)	65 (37)	64 (59)	57 (37)	50 (60)	61 (72)	812	.6
IgE ≥ 300 % (n)	65 (70)	60 (123)	52 (11)	61 (17)	66 (78)	61 (57)	55 (37)	55 (95)	812	.8
IgE ≥ 400 % (n)	62 (54)	62 (139)	53 (9)	59 (16)	66 (80)	61 (58)	56 (32)	55 (100)	812	.8
IgE ≥ 700 % (n)	65 (31)	61 (162)	67 (6)	57 (8)	64 (83)	61 (66)	63 (15)	54 (117)	639	.6
Allergic sensitization % (n)	76 (73)	88 (7)	78 (32)	70 (28)	80 (4)	100 (3)	73 (30)	25 (1)	238	.7

Chronic rhinosinusitis (current)

Biomarker group	Eos + FeNO + IgE +	Eos + FeNO +	Eos + IgE +	FeNO + IgE +	Eos +	FeNO +	IgE +	None +	Total	*P* value
Eos ≥ 150 % (n)	68 (112)	66 (61)	63 (35)	68 (17)	62 (50)	78 (18)	69 (38)	67 (23)	533	.6
Eos ≥ 400 % (n)	68 (85)	64 (41)	70 (16)	68 (44)	54 (20)	73 (38)	65 (57)	68 (53)	533	.4
FeNO ≥ 50 % (n)	66 (61)	61 (27)	67 (58)	76 (22)	63 (52)	100 (12)	66 (61)	66 (61)	533	.03
IgE ≥ 30 % (n)	65 (121)	63 (19)	62 (38)	73 (45)	69 (20)	82 (23)	65 (64)	65 (24)	533	.2
IgE ≥ 100 % (n)	69 (85)	59 (55)	59 (17)	71 (30)	67 (41)	79 (38)	65 (42)	65 (46)	533	.2
IgE ≥ 300 % (n)	70 (48)	62 (92)	50 (7)	76 (19)	67 (51)	75 (49)	74 (23)	62 (65)	533	.1
IgE ≥ 400 % (n)	68 (39)	63 (101)	55 (6)	75 (18)	66 (52)	76 (50)	77 (20)	62 (68)	533	.2
IgE ≥ 700 % (n)	68 (23)	64 (117)	75 (3)	73 (8)	64 (55)	76 (60)	73 (11)	64 (77)	533	.3
Allergic sensitization % (n)	55 (46)	50 (7)	47 (18)	47 (14)	60 (3)	100 (4)	43 (15)	33 (1)	212	.6

Eczema (current)

Biomarker group	Eos + FeNO + IgE +	Eos + FeNO +	Eos + IgE +	FeNO + IgE +	Eos +	FeNO +	IgE +	None +	Total	*P* value
Eos ≥ 150 % (n)	8 (26)	8 (14)	10 (14)	8 (5)	9 (13)	17 (8)	14 (15)	14 (9)	1069	.3
Eos ≥ 400 % (n)	8 (19)	7 (8)	10 (5)	7 (12)	9 (5)	13 (14)	13 (24)	11 (17)	1,069	0.7
FeNO ≥ 50 % (n)	7 (14)	7 (6)	9 (16)	5 (3)	10 (14)	11 (3)	13 (27)	12 (21)	1,069	.7
IgE ≥ 30 % (n)	8 (28)	8 (6)	12 (13)	10 (14)	5 (3)	10 (5)	13 (27)	11 (8)	1,069	.5
IgE ≥ 100 % (n)	7 (19)	9 (15)	12 (8)	11 (10)	8 (8)	10 (9)	13 (18)	12 (17)	1,069	.4

(continued)

TABLE E4. *(Continued)*

Eczema (current)										
Biomarker group	Eos + FeNO + IgE +	Eos + FeNO +	Eos + IgE +	FeNO + IgE +	Eos +	FeNO +	IgE +	None +	Total	*P* value
IgE ≥ 300 % (n)	8 (12)	8 (22)	10 (3)	14 (7)	9 (13)	9 (12)	16 (13)	11 (22)	1,069	.3
IgE ≥ 400 % (n)	8 (9)	8 (25)	8 (2)	13 (6)	10 (14)	9 (13)	19 (13)	10 (22)	1,069	.2
IgE ≥ 700 % (n)	8 (5)	8 (29)	17 (2)	12 (3)	9 (14)	10 (16)	27 (7)	11 (28)	1,069	.07
Allergic sensitization % (n)	3 (6)	3 (2)	5 (4)	9 (10)	0 (0)	4 (2)	10 (8)	3 (1)	682	.007
Nasal polyps (current)										
Biomarker group	Eos + FeNO + IgE +	Eos + FeNO +	Eos + IgE +	FeNO + IgE +	Eos +	FeNO +	IgE +	None +	Total	*P* value
Eos ≥ 150 % (n)	41 (82)	38 (45)	32 (20)	29 (12)	33 (24)	29 (7)	23 (7)	26 (7)	577	.5
Eos ≥ 400 % (n)	43 (61)	37 (30)	33 (9)	33 (33)	46 (15)	36 (22)	27 (18)	24 (16)	577	.2
FeNO ≥ 50 % (n)	41 (47)	39 (22)	38 (41)	37 (15)	38 (33)	31 (5)	24 (18)	28 (23)	577	.1
IgE ≥ 30 % (n)	41 (95)	41 (19)	33 (21)	29 (25)	35 (8)	32 (7)	29 (20)	25 (9)	577	.4
IgE ≥ 100 % (n)	43 (67)	39 (47)	28 (10)	28 (16)	38 (19)	31 (16)	26 (11)	29 (18)	577	.1
IgE ≥ 300 % (n)	40 (34)	42 (80)	24 (5)	32 (12)	37 (24)	28 (20)	35 (8)	25 (21)	577	.2
IgE ≥ 400 % (n)	44 (26)	40 (88)	29 (5)	36 (12)	35 (24)	27 (20)	38 (8)	25 (21)	577	.2
IgE ≥ 700 % (n)	51 (18)	40 (96)	33 (2)	33 (7)	34 (27)	29 (25)	44 (4)	26 (25)	577	.2
Allergic sensitization % (n)	34 (84)	32 (26)	31 (29)	14 (16)	18 (3)	29 (14)	15 (11)	23 (9)	717	< .001

Eos, blood eosinophils; *FeNO*, fractional exhaled nitric oxide; *IgE*, immunoglobulin E.

TABLE E5. Information about biologic medications

Biologic medications										
Group	Total	Triple positive	Eos + FeNO +	Eos + IgE +	FeNO + IgE +	Eos +	FeNO +	IgE +	Triple negative	*P* value
Anti-IgE	194	37% (72)	7% (14)	14% (28)	13% (25)	2% (3)	2% (4)	21% (40)	4% (8)	< .001
Anti-IL5	338	31% (104)	13% (45)	8% (26)	11% (37)	7% (25)	10% (32)	11% (38)	9% (31)	.06
Biomarker before biologic	142	39% (55)	16% (22)	11% (16)	5% (7)	9% (13)	5% (7)	9% (12)	7% (10)	.008

Eos, blood eosinophils; *FeNO*, fractional e1xhaled nitric oxide; *IgE*, immunoglobulin E; *IL-5*, interleukin-5.

SECTION 3: BIOMARKER OVERLAP IN THOSE NOT ON ORAL CORTICOSTEROIDS OR ANTI-INTERLEUKIN-5

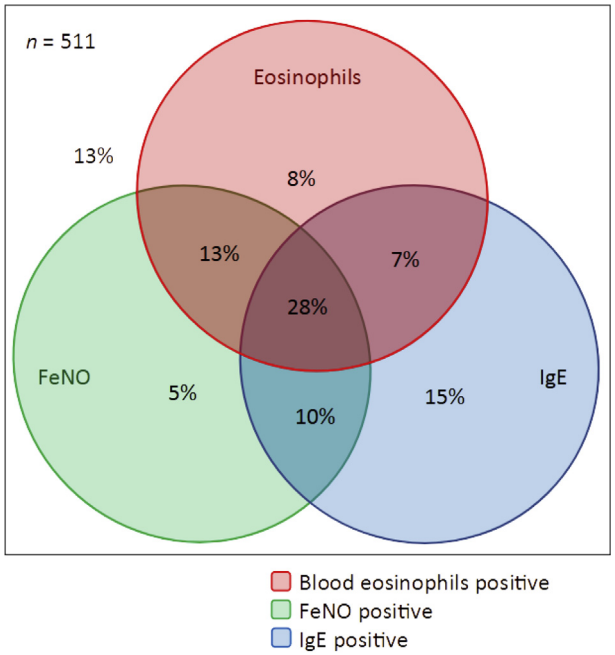


FIGURE E2. Overlap of inflammatory biomarkers in those not on oral corticosteroids or anti-interleukin-5 medications. *FeNO*, fractional exhaled nitric oxide; *IgE*, immunoglobulin E.