

Asthma Phenotypes Defined From Parameters Obtained During Recovery From a Hospital-Treated Exacerbation



Rihuang Qiu, MD^{a,b,*}, Jiaying Xie, MD, PhD^{a,*}, Kian Fan Chung, MD, PhD^{c,d}, Naijian Li, MD^a, Zhaowei Yang, PhD^a, Mengzhang He, MD^a, Jing Li, MD^a, Rongchang Chen, MD^a, Nanshan Zhong, MD^a, and Qingling Zhang, MD, PhD^a
Guangzhou and Ganzhou, China; and London, United Kingdom

What is already known about this topic? Cluster analysis of severe asthma during a period of stability has led to the description of phenotypes on the basis of the presence or absence of airflow obstruction, recurrent exacerbations, and eosinophilic inflammation.

What does this article add to our knowledge? Using parameters obtained during recovery from an exacerbation, we identified 4 asthma phenotypes on the basis of the degree of airflow obstruction and the presence of sputum eosinophilic, neutrophilic, or mixed granulocytic inflammation and smoking.

How does this study impact current management guidelines? Definition of these new clusters of asthma during recovery from an exacerbation may help in targeting specific treatments for these exacerbation phenotypes.

BACKGROUND: Asthma is a heterogeneous disease with diverse clinical manifestations and inflammatory pathologies that is punctuated by exacerbations.

OBJECTIVE: To describe the clinical and inflammatory characteristics of patients with asthma treated in hospital for an acute exacerbation.

METHODS: Data from 320 adult patients receiving treatment for an acute exacerbation of asthma were obtained. In 218 patients with complete data, we used the Ward hierarchical clustering to obtain clusters. Pulmonary function, blood cell counts, sputum cell counts, serum IgE levels, and fractional exhaled nitric oxide were measured on hospital admission. We selected 13 variables with which we performed the Ward minimum-variance hierarchical clustering.

RESULTS: Four clusters were defined. Clusters 1 (24.5%) and 3 (36.7%) were characterized by predominantly female patients with asthma with sputum neutrophilia, with cluster 1 associated with a small degree of airflow obstruction and early-onset asthma and cluster 3 with a moderate degree of reduction in FEV₁. Clusters 2 (22.0%) and 4 (16.5%) were associated with high sputum eosinophilia and severe airflow obstruction. Cluster 4 was made exclusively of male smoking subjects, whereas cluster 2 was made up of predominantly female nonsmoking subjects with the worst FEV₁, forced expiratory flow at 25% to 75% of forced vital capacity (% predicted), and partial pressure of oxygen in arterial blood on admission. There were no differences between clusters in terms of atopy, serum IgE, prevalence of nasal disease, dose of maintenance inhaled corticosteroids, or oral/systemic corticosteroid use and asthma exacerbations.

CONCLUSIONS: The clusters during recovery from an exacerbation of asthma were distinguished by airflow obstruction and a neutrophilic, eosinophilic, or mixed inflammation. Eosinophilic inflammation was found in smoking and nonsmoking patients with asthma during an exacerbation. © 2018 American Academy of Allergy, Asthma & Immunology (J Allergy Clin Immunol Pract 2018;6:1960-7)

Key words: Asthma; Asthma phenotypes; Sputum neutrophils; Sputum eosinophils; Cluster analysis

^aDepartment of Allergy and Clinical Immunology, State Key Laboratory of Respiratory Diseases, National Clinical Research Center for Respiratory Disease, Guangzhou Institute of Respiratory Health, The First Affiliated Hospital of Guangzhou Medical University, Guangzhou, China

^bDepartment of Respiratory Medicine, The Affiliated Ganzhou Hospital of Nanchang University, Ganzhou, China

^cNational Heart and Lung Institute, Imperial College London, London, United Kingdom

^dBiomedical Research Unit, Royal Brompton & Harefield NHS Trust, London, United Kingdom

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Corresponding author: Qingling Zhang, MD, PhD, Department of Allergy and Clinical Immunology, State Key Laboratory of Respiratory Diseases, National Clinical Research Center for Respiratory Disease, Guangzhou Institute of Respiratory Health, The First Affiliated Hospital of Guangzhou Medical University, 151 Yanjiang Rd, Guangzhou 510120, China. E-mail: zqling68@hotmail.com.

*These authors contributed equally to the work and are co-first authors.
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Abbreviations used

FEF₂₅₋₇₅- Forced expiratory flow at 25% to 75% of forced vital capacity

FENO- Fractional exhaled nitric oxide

FVC- Forced vital capacity

INTRODUCTION

Asthma is a heterogeneous disease in terms of both its severity and its inflammatory component. About 5% to 10% of all patients with asthma are considered to have severe asthma, a heterogeneous condition defined by poor therapeutic responses to currently approved asthma medications, particularly, inhaled and oral corticosteroid therapies.¹ These patients experience poor quality of life and have a higher economic burden when compared with those with mild asthma. Severe asthma is not a single disease entity. Patients with severe asthma have a spectrum of characteristics, including degree of chronic airflow obstruction, occurrence of exacerbations, inflammatory features, response to corticosteroid therapy, and disease outcomes.²

An unbiased approach to defining phenotypes using clustering techniques has been applied to cohorts of asthma with a range of clinical severities, using clinicophysiological parameters in the Severe Asthma Research Program (SARP),³ and to the UK Leicester cohorts.⁴ Phenotypes that have been described include those patients with little airflow obstruction and activity of disease, those with early age of onset of disease with an atopic background, and a more severe group of patients with asthma associated with adult-onset disease. Similarly, in the European U-BIOPRED cohort, 2 out of 3 clusters of patients with severe asthma were characterized by airflow obstruction in a nonsmoking cohort as well as in a currently smoking or ex-smoking cohort, whereas the third cluster consisted of obese female patients with severe asthma with recurrent exacerbations.⁵ Such clusters have been replicated in Korean and Japanese cohorts.^{6,7} In addition, in a study in which eosinophilic inflammation was examined, an early-onset atopic asthma phenotype with airway dysfunction and eosinophilic inflammation has been reported with increased hospitalizations.⁴

Clustering has so far been performed using clinical and physiological features that have been obtained during a period of clinical stability. Because exacerbations are a common feature of asthma, a better understanding of the phenotypic characteristics of patients with asthma with an acute exacerbation would be important so that more effective treatments can be developed for such exacerbations. Studying patients with asthma during an acute exacerbation may show residual evidence of the inflammatory processes present during the exacerbation and may yield clinically useful insights with regard to tailoring treatment to a given patient group during an exacerbation.

We hypothesize that the clusters obtained during recovery from an acute exacerbation of asthma may be different from those already described in patients with asthma during a stable period, because the degree of inflammation and airflow obstruction would be more prominent. In this study, we have collected clinical, inflammatory, and biomarker data during the recovery from an exacerbation of asthma treated in a university hospital in Guangzhou, China. We used an unbiased clustering approach to define the clusters identified during asthma exacerbations.

METHODS

Patients

An analysis was conducted of patients aged between 18 and 65 years with at least 1 of the respiratory symptoms of dyspnea, cough, sputum production, or wheezing that required hospitalization and who have been diagnosed with an acute exacerbation of bronchial asthma⁸ at the Guangzhou Institute of Respiratory Health between 2012 and 2014. Three hundred twenty patients with asthma were classified as having a severe asthma exacerbation defined by the requirement for a course of systemic corticosteroids and were included in the study (Figure 1). Patients were enrolled and assessed within 3 to 10 days of admission, when they were judged to have recovered sufficiently to undertake spirometric tests and sputum collection. All patients received oral or systemic corticosteroid therapy for more than 3 days, with inhaled salbutamol for as long as needed. The diagnosis of asthma was based on the criteria of the Global Initiative for Asthma guidelines⁹ supported by the demonstration of airway reversibility or bronchial hyperresponsiveness during the past year.¹⁰

Ninety-three patients with any of the following conditions were excluded (Figure 1): (1) serious nonpulmonary diseases such as heart failure, cancer, and severe psychiatric disorders and (2) other pulmonary diseases such as emphysema, chronic obstructive pulmonary disease, bronchiectasis, pneumonia, or lung damage caused by previous medical conditions such as pulmonary tuberculosis.

Arterial blood gas analysis and blood cell counts were performed on admission. The subjects underwent a standardized assessment for age, body mass index, duration of asthma (determined by patient recall of the first time experiencing symptoms of asthma), presence of nasal disease, smoking status, pulmonary function, sputum and blood analysis, levels of serum total IgE and specific IgE, and fractional exhaled nitric oxide within 3 to 10 days of admission (Figure 1).

Lung function

Lung function was measured with a spirometer (MasterScreen PFT, Care Fusion, Hoechstberg, Germany) according to international recommendations.¹¹ The measurements included forced vital capacity (FVC), FEV₁, peak expiratory flow rate, and the average forced expiratory flow at 25% to 75% of FVC (FEF₂₅₋₇₅). Postbronchodilator measurements were taken 10 to 15 minutes after inhalation of 400 µg of salbutamol.

Inflammatory parameters

Levels of nitric oxide in exhaled breath (FENO) were measured with a portable rapid response chemoluminescent analyzer (NIOX System, Aerocrine, Solna, Sweden) at an expiratory flow rate of 50 mL/s.¹² Eosinophil and neutrophil counts in blood were measured using an automated hematology analyzer (UniCel DxH 800, Beckman Coulter, Miami, Fla). Sputum induction was performed as previously described.¹³ In brief, albuterol (200 µg) was inhaled first, followed 15 minutes later by inhalation of 2 concentrations of saline (0.9% and 3%, each inhaled for 7 minutes). Sputum was then processed and a differential count on 400 cells was performed. We accepted a percentage count of squamous cells of less than 30%. By taking cutoff points of eosinophil of 3% or more and neutrophils of 61% or more,^{14,15} we defined 4 inflammatory phenotypes: neutrophilic (≥61% neutrophils and <3% eosinophils), eosinophilic (≥3% eosinophils and <61% neutrophils), mixed granulocytic (≥61% neutrophils and ≥3% eosinophils), and paucigranulocytic (<61% neutrophils and <3% eosinophils).

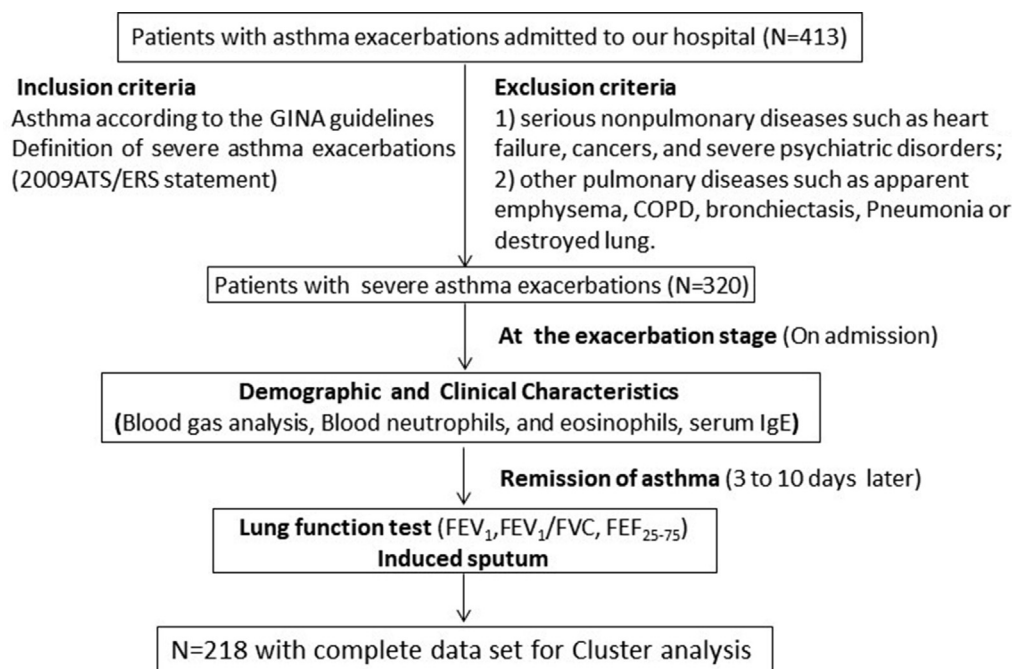


FIGURE 1. Flowchart describing the selection criteria of patients and the clinical data used for analysis. *ATS*, American Thoracic Society; *COPD*, chronic obstructive pulmonary disease; *ERS*, European Respiratory Society; *GINA*, Global Initiative for Asthma.

Atopy

Levels of total IgE and specific IgE to common aeroallergens (*Dermatophagoides pteronyssinus*, *Dermatophagoides farina*, cat and dog dander, mugwort and ragweed pollens, *Blatella germanica*, *Aspergillus fumigatus*, and *Alternaria alternata*) were determined using the ImmunoCAP system (Thermo Scientific, Uppsala, Sweden). Specific IgE level was defined as positive if the measurement was 0.35 IU/mL or higher. Subjects were characterized as atopic if they had at least 1 positive specific IgE.

Data analysis

Chi-square test was used to compare categorical variables, and independent Student *t* test was used to compare continuous variables. The Mann-Whitney *U* test was used to compare continuous variables that did not follow a normal distribution.

Missing values in variables with less than 10% missing data were imputed by using a K-NNimpute algorithm.¹⁶ Of the 73 variables initially considered, 24 variables including postbronchodilator FEV₁, FENO, and total and specific serum IgE were excluded because of an excess of missing data defined as in more than 10% of subjects. Of the remaining 49 variables, 28 variables were excluded on the basis of being clinically irrelevant for the clustering and of being represented by other variables. Eventually, the 21 variables that were selected (Table I) were then evaluated by factor analysis with orthogonal varimax rotation. On the basis of the pattern of loading, 10 factors were identified with an eigenvalue of more than 1, which accounted for 72.91% of the variance in the factor analysis. The most influential factors (factors 1-2) were lung function parameters and inflammatory cell percentage in sputum. We selected 1 parameter representative for each factor on the basis of the highest loading. Because sex and age of asthma onset were important clinical characteristics, and the inclusion of inflammatory cell percentage in sputum and blood was the primary purpose of this cluster analysis, we selected sex, age of asthma onset, and blood eosinophil

percentage as cluster parameters. In all, 13 variables were used in cluster analysis. The Ward minimum-variance hierarchical clustering method was used by applying an agglomerative (bottom-up) approach and the Ward linkage. At each generation of clusters, samples were merged into larger clusters to minimize the within-cluster sum of squares or to maximize the between-cluster sum of squares.^{3,17} Stepwise discriminant analysis was used to identify the most influential variables in cluster assignment. To ensure repeatability and stability within the model, the K-means algorithm was repeated 99 times in a leave-one-out validation model. To compare differences between clusters, ANOVA, the Kruskal-Wallis test, and chi-square test were used for parametric continuous, nonparametric continuous, and categorical variables, respectively.

RESULTS

Characteristics of patients with asthma with asthma exacerbations

The characteristics of the 218 patients with asthma are presented in Table II, and these were compared with the characteristics of the other 102 patients who were not included in the clustering because of incomplete data and lack of sputum results. The clinical characteristics of these 2 groups were not different except that the patients in the group being clustered were slightly younger than those in the excluded group and were on a higher dose of maintenance inhaled corticosteroids (Table II).

Discriminant analysis and cluster analysis

We identified 4 clusters in the 218 subjects. The differences between the clusters were highlighted by the degree of airflow obstruction and sputum inflammatory characteristics (Table III; Figure 2).

By using stepwise discriminant analysis, the 3 most influential variables for cluster assignment were smoking history ($F = 524.42$; $P < .001$), induced sputum eosinophil percentages

TABLE I. Factor analysis using orthogonal varimax rotation of 21 variables

Variable	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6	Factor 7	Factor 8	Factor 9	Factor 10
Sex*	0.020	−0.074	0.102	−0.732‡	−0.072	−0.110	0.177	−0.306	−0.055	0.137
Body mass index*	0.088	−0.164	0.212	0.127	0.215	0.104	0.024	−0.106	−0.006	−0.686‡
Age at enrollment*	−0.251	−0.013	0.748‡	0.004	0.102	0.278	0.027	−0.249	0.105	0.224
Age of asthma onset*	−0.049	0.072	0.977‡	0.075	0.014	0.059	−0.009	−0.035	0.041	−0.014
Smoking history* (>10 packs/y)	−0.061	0.063	0.050	0.869‡	0.054	0.001	0.038	−0.049	−0.127	0.053
Asthma exacerbations in past year	−0.215	0.098	−0.160	−0.036	0.135	0.046	−0.055	−0.070	−0.423	0.119
Nasal disease	−0.042	0.365	−0.002	−0.028	0.175	0.047	−0.319	−0.032	0.052	0.043
Atopy	−0.029	−0.031	−0.084	−0.032	0.064	0.004	0.284	0.619	0.063	−0.070
Dose of maintenance ICS (BDP equivalent, µg/d)	−0.269	0.099	−0.221	−0.040	0.416	−0.087	−0.114	−0.015	0.313	0.226
Oral/systemic steroid usage in previous 3 mo	0.058	0.031	−0.097	−0.157	0.220	−0.435	−0.018	−0.012	0.069	−0.187
Days after admission for lung function	0.176	0.402	−0.011	0.150	0.237	0.099	0.441	−0.120	0.127	−0.072
FEV ₁ (% predicted)*	0.910‡	−0.010	−0.132	0.020	0.039	−0.026	0.550	0.011	0.062	−0.027
FEV ₁ /FVC (%)	0.891	−0.101	−0.028	−0.088	0.051	0.031	0.526	−0.027	0.045	−0.028
FEF ₂₅₋₇₅ (% predicted)*	0.845	0.005	0.041	−0.050	0.043	0.001	0.687‡	−0.033	0.036	−0.004
Blood eosinophil (%)*	−0.098	0.244	−0.004	−0.054	−0.728‡	−0.066	−0.151	−0.085	−0.094	0.093
Blood neutrophil (%)*	0.052	−0.206	0.091	0.052	0.758‡	−0.067	0.018	−0.008	−0.165	0.014
Total serum IgE level*	−0.129	0.036	−0.006	0.029	0.008	0.009	−0.329	0.704‡	−0.002	0.162
Paco ₂ (mm Hg)	−0.252	0.027	0.140	−0.092	0.298	0.253	0.189	0.158	0.014	0.369
Pao ₂ (mm Hg)*	0.058	0.105	−0.008	−0.052	−0.289	−0.730‡	0.002	0.185	−0.068	0.082
Sputum eosinophil (%)*	−0.003	0.871‡	0.048	0.053	−0.199	0.015	−0.074	0.045	−0.558	−0.068
Sputum neutrophil (%)*	−0.169	−0.828	−0.065	−0.034	0.263	0.097	−0.017	0.070	0.646‡	0.063
Variance explained by individual factor	3.18	2.42	2.31	1.90	1.36	1.28	1.15	1.11	1.02	1.01

BDP, Beclomethasone dipropionate; ICS, inhaled corticosteroid; Paco₂, partial pressure of carbon dioxide in arterial blood; Pao₂, partial pressure of oxygen in arterial blood.

*Final variable used in the cluster analysis.

‡Variable close to the highest loading, selected because of clinical importance.

‡Variable with the highest loading.

($F = 220.88$; $P < .001$), and FEV₁ (% predicted) ($F = 161.07$; $P < .001$; Table IV). Other variables, including age of asthma onset, peripheral blood eosinophil percentages, FEF₂₅₋₇₅, and induced sputum neutrophil percentages, contributed less toward cluster assignment.

Cluster 1 ($n = 54$; 24.5% of subjects, neutrophilic and mixed granulocytic inflammation) was made up of the youngest patients (mean age, 35.78 years). This cluster had the earliest age of asthma onset (mean, 22.88 years) and the highest FEV₁ of 75.6 (% predicted), but a reduced FEF₂₅₋₇₅ of 46.83 (% predicted). Sputum neutrophilia (median, 72.5%) was the highest in this cluster, and sputum eosinophilia was the lowest (median, 2.75%).

Cluster 2 ($n = 48$; 22.0% of subjects, mostly eosinophilic inflammation) was female-predominant (68.8%), with the most severe airflow obstruction (FEV₁, 58.08% predicted) and severe reduction in FEF₂₅₋₇₅ (mean, 22.35% predicted), together with the highest sputum and blood eosinophil percentage (mean, 53.5% and 10.35%, respectively). Cluster 2 was also the most hypoxemic group (mean partial pressure of oxygen in arterial blood of 77.9 mm Hg).

Cluster 3 ($n = 80$; 36.7% of subjects, predominantly neutrophilic with mixed granulocytic asthma) was female-predominant (73.8%), with the highest age (mean, 53.64 years) and moderate to severe airflow obstruction (mean FEV₁, 67.45% predicted). The sputum neutrophil count was the highest (median, 76.75%).

Cluster 4 ($n = 36$; 16.5% of subjects, eosinophilic with mixed granulocytic inflammation) consisted exclusively of male patients

with asthma (100%) who were smokers, with moderate to severe airflow obstruction (mean FEV₁, 62.73% predicted), severely reduced FEF₂₅₋₇₅ (28.44% predicted), and sputum eosinophilic, mixed eosinophilic, and neutrophilic inflammation (median eosinophilia, 22.0%).

There were no differences among the clusters regarding atopy, serum IgE, prevalence of nasal disease, dose of maintenance inhaled corticosteroids, oral/systemic steroid use, and asthma exacerbations (Table III).

Validation

To determine the repeatability and stability within the model, we carried out the K-means algorithm 99 times by the leave-one-out method with a within-repeatability accuracy of 86.2%.

DISCUSSION

Asthma has been increasingly considered as a heterogeneous multidimensional disease¹⁸ and cluster analysis has been used to determine asthma phenotypes in adults and children in several studies.¹⁹⁻²¹ In this study, we analyzed 13 clinical, physiological, and inflammatory variables from 218 patients with asthma recruited from patients admitted for the treatment of a severe exacerbation of asthma. We described 4 asthma clusters differentiated by sputum inflammatory cell patterns and the degree of airflow obstruction. We found that clusters 1 and 3 were similar with neutrophilic and mixed granulocytic inflammation except for the difference in FEV₁, with cluster 1 having a mild degree and cluster 3 a more severe degree of airflow obstruction; in

TABLE II. Demographic and clinical characteristics of subjects with acute exacerbations of asthma

Characteristic	Used in cluster (n = 218)	Not used in cluster (n = 102)	P value*
Sex, female (%)	57.3	57.8	.932
Age (y), mean $\pm$ SD	47.43 $\pm$ 13.56	51.5 $\pm$ 12.77	.011
Body mass index, mean $\pm$ SD	23.15 $\pm$ 3.54	23.44 $\pm$ 3.52	.393
Obesity (%)	6.9	6.9	.995
Age of asthma onset (y), mean $\pm$ SD	39.44 $\pm$ 15.99	40.37 $\pm$ 15.01	.666
Duration of asthma (y), median (IQR)	5.0 (2-10)	6.5 (2-12)	.239
Presence of nasal disease (%)	11.9	10.8	.680
Smoking history (>10 packs/y) (%)	18.3	17.6	.879
Atopic status (%)	50.0	57.8	.190
Dose of maintenance ICS (BDP equivalent, μ g/d), median (IQR)	800 (400-1000)	400 (400-800)	.023
Oral/systemic steroid usage in previous 3 mo (%)	29.8	31.2	.781
Asthma exacerbations in past year (%)			.970
0	47.7	49.0	
1-2	39.4	37.3	
≥ 3	12.8	13.7	
Days after admission for lung function, d (IQR)	5 (5-7)	5 (5-8)	.262
FEV ₁ (% predicted), mean $\pm$ SD	66.48 $\pm$ 22.62	69.62 $\pm$ 23.15 (n = 100)	.734
FEV ₁ /FVC (%), mean $\pm$ SD	61.81 $\pm$ 15.64	62.52 $\pm$ 15.05 (n = 100)	.387
FEF ₂₅₋₇₅ (% predicted), mean $\pm$ SD	32.55 $\pm$ 23.32	34.71 $\pm$ 24.22 (n = 100)	.297
Postbronchodilator FEV ₁ (% predicted), mean $\pm$ SD	78.08 $\pm$ 20.86 (n = 137)	75.52 $\pm$ 18.20 (n = 55)	.315
Arterial blood gases, median (IQR)			
PaO ₂ (mm Hg)	79.8 (72.5-90.9)	82.6 (73.8-97.0)	.055
PaCO ₂ (mm Hg)	37.2 (34.4-41.1)	38.0 (34.9-40.7)	.487
Induced sputum (%), median (IQR)			
Eosinophil	11.75 (2.00-44.75)	ND	
Neutrophil	58.25 (28.25-82.13)	ND	
Inflammatory subtypes (%)			
Eosinophilic asthma	41.7	ND	
Neutrophilic asthma	24.8	ND	
Mixed granulocytic asthma	24.3	ND	
Paucigranulocytic asthma	9.2	ND	
Peripheral blood cell count (%), median (IQR)			
Neutrophil	60.9 (51.91-75.99)	62.5 (51.07-76.80)	.509
Eosinophil	3.35 (0.20-8.28)	3.55 (0.22-8.32)	.077
Serum total IgE (IU/mL), median (IQR)	155.00 (67.80-312.75)	147.25 (54.50-378.78) (n = 62)	.664
FENO, median (IQR)	40 (25.51-73.26) (n = 99)	41 (27.65-73.00) (n = 51)	.552
Medication during hospitalization (%)			
Oral or systemic corticosteroid	100	100	1.000
Antibiotics	43.2	45.1	.453
Short-acting β agonist	100	100	1.000
LTRA	74.4	73.0	.584
Theophylline	41.0	42.4	.853

BDP, Beclomethasone dipropionate; ICS, inhaled corticosteroid; IQR, interquartile range; LTRA, leukotriene receptor antagonist; ND, not determined; PaCO₂, partial pressure of carbon dioxide in arterial blood; PaO₂, partial pressure of oxygen in arterial blood.

Note. Arterial blood gas analysis and blood cell counts were performed on admission to hospital and the lung function was performed when the patients had recovered from the exacerbations within 3 to 10 d of admission. Arterial blood gas analysis was performed while they were on additional oxygen.

*A total of 218 cases of patients who had induced sputum performed and with complete data sets were compared with the other 102 patients who did not have induced sputum performed and with incomplete data sets. P values from *t* test, Mann-Whitney *U* test, or χ^2 tests in the 2 groups.

addition, the differentiating factors were that cluster 1 represented the relatively younger group with the earliest onset of asthma, whereas cluster 3 represented the oldest group with late onset of asthma. Clusters 2 and 4 were similar in terms of eosinophilic inflammation and moderate to severe airflow obstruction, with cluster 2 having the highest level of sputum and blood eosinophil counts (exclusively eosinophilic) and the

most reduced FEV₁ and FEF₂₅₋₇₅. It was the most hypoxemic group on admission to hospital. Cluster 2 was predominantly female, whereas cluster 4 was made exclusively of male subjects who were smokers. Thus, the analysis has yielded clusters distinguished by the type of inflammation (eosinophilic, neutrophilic, or mixed granulocytic) and the degree of airflow obstruction. In addition, we describe a cluster of highly

TABLE III. Demographic and clinical characteristics of 218 subjects in the 4 clusters

Characteristic	Cluster 1 (n = 54 [24.5%])	Cluster 2 (n = 48 [22.0%])	Cluster 3 (n = 80 [36.7%])	Cluster 4 (n = 36 [16.5%])	P value
Age (y), mean $\pm$ SD	35.78 $\pm$ 11.89	47.92 $\pm$ 15.10	53.64 $\pm$ 10.00	50.47 $\pm$ 9.38	<.001
Sex, female (%)	61.1	68.8	73.8	0.0	<.001
Body mass index, mean $\pm$ SD	22.47 $\pm$ 3.15	21.36 $\pm$ 3.90	24.01 $\pm$ 3.92	23.76 $\pm$ 3.14	<.001
Smoking history (>10 pack/y) (%)	1.9	2.1	2.5	100	<.001
Age of asthma onset (y), mean $\pm$ SD	22.88 $\pm$ 12.07	39.90 $\pm$ 15.54	46.62 $\pm$ 10.52	43.53 $\pm$ 10.48	<.001
Nasal disease (%)	7.4	10.4	16.3	13.9	.461
Atopy (%)	57.4	52.1	46.3	44.4	.539
Dose of maintenance ICS (BDP equivalent; μ g/d), median (IQR)	800 (400-800)	800 (400-800)	800 (400-1000)	800 (400-950)	.519
Oral/systemic steroid usage in previous 3 mo (%)	20.4%	39.6%	28.7%	33.3%	.191
Asthma exacerbations in past year (%)					.658
0	53.7%	37.5%	50.0%	47.2%	
1-2	31.5%	50.0%	38.8%	38.9%	
≥ 3	14.8%	12.5%	11.3%	13.9%	
Days after admission for lung function, median (IQR)	5 (4.75-7.00)	7 (5.25-7.00)	6 (5.00-7.00)	6 (5.00-7.75)	.310
FEV ₁ (% predicted), mean $\pm$ SD	75.67 $\pm$ 23.38	58.08 $\pm$ 20.98	67.45 $\pm$ 21.22	62.73 $\pm$ 21.88	.001
FEV ₁ /FVC (%), mean $\pm$ SD	70.12 $\pm$ 15.62	55.21 $\pm$ 15.24	62.22 $\pm$ 14.19	57.23 $\pm$ 13.89	<.001
FEF ₂₅₋₇₅ (% predicted), mean $\pm$ SD	46.83 $\pm$ 30.99	22.35 $\pm$ 18.53	30.88 $\pm$ 17.51	28.44 $\pm$ 16.84	<.001
Sputum neutrophil (%), median (IQR)	72.5 (55.87-86.97)	22.00 (11.00-39.50)	76.75 (51.50-82.50)	51.00 (23.37-80.87)	.003
Sputum eosinophil (%), median (IQR)	2.75 (0.50-18.65)	53.5 (45.87-76.12)	3.5 (1.00-11.00)	22.0 (2.50-46.00)	<.001
Inflammatory subtypes (%)					<.001
Eosinophilic asthma	18.5	91.7	23.7	50.0	
Neutrophilic asthma	38.9	2.1	33.8	13.9	
Mixed granulocytic asthma	29.6	2.1	33.8	25.0	
Paucigranulocytic asthma	13.0	4.2	8.7	11.1	
Blood eosinophil (%), median (IQR)	1.55 (0.10-5.27)	10.35 (5.80-16.24)	2.00 (0.10-6.35)	3.30 (0.20-7.33)	<.001
Blood neutrophil (%), median (IQR)	68.30 (57.27-78.21)	52.75 (43.24-62.62)	62.17 (53.04-73.62)	63.20 (54.88-80.46)	<.001
Total serum IgE (IU/mL), median (IQR)	151.85 (45.31-224.00)	153.00 (66.07-600.00)	156.00 (86.10-323.50)	139.25 (62.65-347.70)	.235
Arterial blood gases, median (IQR)					
Paco ₂ (mm Hg)	37.10 (34.75-38.80)	38.10 (34.40-40.98)	37.70 (34.55-41.30)	37.70 (34.80-40.75)	.660
Pao ₂ (mm Hg)	84.45 (71.40-94.00)	77.90 (71.05-90.15)	81.50 (72.35-86.30)	80.30 (71.90-84.65)	.050

BDP, Beclomethasone dipropionate; ICS, inhaled corticosteroid; IQR, interquartile range; Paco₂, partial pressure of carbon dioxide in arterial blood; Pao₂, partial pressure of oxygen in arterial blood.

Note. P values from ANOVA, Kruskal-Wallis, or χ^2 tests among 4 clusters.

eosinophilic inflammation with airflow obstruction in a group of male smokers.

It is of interest to compare these clusters obtained from patients with asthma recovering from an acute exacerbation of asthma with the clusters that have been described in stable patients with asthma. The SARP group identified 5 asthma phenotypes separated on the basis of severity: mild allergic asthma, mild-moderate allergic asthma, more severe older onset asthma, severe variable allergic asthma, and severe fixed airflow asthma.³ In a subsequent analysis with measurements of sputum granulocytic inflammation, the SARP investigators found that most of the patients with moderate to severe asthma (>83%) clustered in a group with low baseline lung function associated with sputum neutrophilia either alone or with concurrent sputum eosinophilia.¹⁷ Interestingly, the European U-BIOPRED cohort identified a cluster of late-onset severe asthma with a history of smoking and chronic airflow obstruction, whereas another cluster with chronic airflow obstruction was composed of nonsmokers, with both clusters having evidence of higher sputum eosinophilia, but without sputum neutrophilia.⁵

Thus, the smoking cluster bears some similarities with our smoking cluster 4, with both groups characterized by eosinophilic inflammation. In addition, in a Japanese asthma cohort,²² a cohort of late-onset asthma, predominantly smokers, or ex-smokers showed high blood and sputum eosinophilia together with high levels of FENO and fixed airflow obstruction. Therefore, the clusters we have described during recovery from an exacerbation bear some similarities with the clusters described in patients with asthma during a period of stability.

We identified a group characterized by predominantly patients with severe asthma with severe airflow obstruction associated with an eosinophilic inflammation, sometimes with a mixed granulocytic inflammation. We described 2 clusters associated with high levels of neutrophilic inflammation, with one associated with near-normal lung function (cluster 1) and the other with a moderate degree of airflow obstruction (cluster 3). We also described 2 clusters associated with more severe airflow obstruction and sputum eosinophilia, distinguished by the smoking history. These data are consistent with those of Wang et al²³ who described a

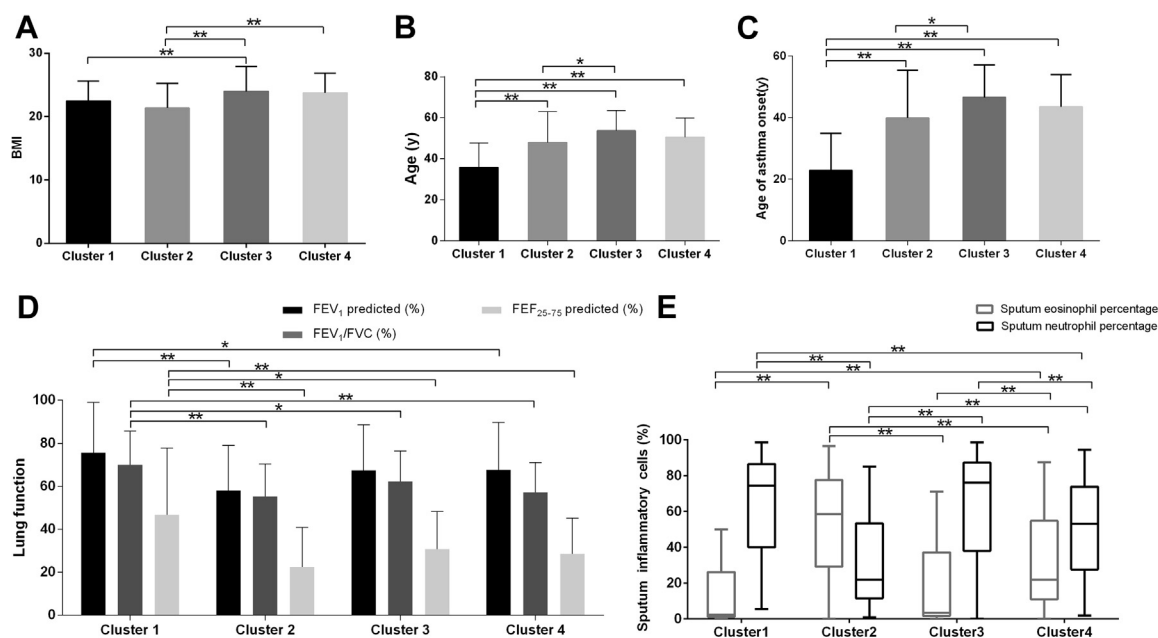


FIGURE 2. A, BMI; B, age; C, age of asthma onset; D, lung function: FEV₁ (% predicted), FEV₁/FVC (%), and FEF₂₅₋₇₅ (% predicted); and E, sputum inflammatory cells: neutrophils (%) and eosinophils (%) between the 4 clusters as determined by cluster analysis. Data shown as mean $\pm$ SD (Fig 2, A-D) and median (interquartile range) (Fig 2, E). BMI, Body mass index. * $P < .05$; ** $P < .01$.

TABLE IV. Important variables for clustering based on stepwise discriminant analysis

Step	Variable	Partial R^2	F value	P value
1	Smoking history (>10 packs/y) (%)	0.120	524.42	<.0001
2	Induced sputum eosinophil (%)	0.059	220.88	<.0001
3	FEV ₁ (% predicted)	0.039	161.07	<.0001
4	Blood eosinophil (%)	0.034	123.27	<.0001
5	Age of asthma onset (y)	0.029	100.39	<.0001
6	FEF ₂₅₋₇₅ (% predicted)	0.027	85.59	<.0001
7	Induced sputum neutrophil (%)	0.024	75.26	<.0001

neutrophilic inflammation in induced sputum within a few hours of admission to hospital of patients with acute exacerbations of asthma. However, there is a high degree of heterogeneity in the association of sputum neutrophilia and eosinophilia with the degree of airflow obstruction. Our data also show that smoking in patients with asthma may be more likely associated with eosinophilic inflammation during an exacerbation. The mechanistic basis for this observation remains unclear.

Woodruff et al²⁴ found that neutrophil numbers in induced sputum from subjects with asthma across a wide range of severity were associated with more severe airflow obstruction. This relationship between sputum neutrophils and airflow obstruction in asthma has been confirmed and extended recently in a study by Shaw et al²⁵ in which both sputum neutrophil and eosinophil numbers were associated with a low prebronchodilator FEV₁, but only high neutrophil numbers were associated with a low post-bronchodilator FEV₁. In the SARP cohort, use of the inflammatory phenotypes as assessed by sputum cell counts led to the

identification of 4 clusters, with 2 clusters of mild to moderate early-onset allergic asthma with paucigranulocytic or eosinophilic sputum inflammatory cell patterns and 2 other clusters with either neutrophilic or mixed inflammatory patterns with moderate to severe asthma requiring frequent health care use despite treatment with high doses of inhaled or oral corticosteroids and also with reduced lung function.¹⁷ A similar report from a cohort in Newcastle, Australia, described patients with neutrophilic inflammation having a greater frequency of primary care doctor visits for asthma exacerbations and a high prevalence of chest infections in the previous 12 months of rhinosinusitis and of gastroesophageal reflux symptoms when compared with eosinophilic asthma.²⁶ In our patients treated with high-dose systemic corticosteroids, one cannot exclude the possibility that the treatment may have been driving the neutrophilic response. Louis et al,²⁷ however, showed that eosinophil numbers in induced sputum were the highest in patients with more severe asthma, as was also shown in the U-BIOPRED cohort.²⁸ Both eosinophilic and neutrophilic inflammation can be associated with airflow obstruction.

A recent study of patients presenting with severe life-threatening asthma exacerbations from Japan found these patients to be heterogeneous, with differences observed in the severity of asthma and the use of inhaled corticosteroids at baseline and the presence of comorbid features of chronic obstructive pulmonary disease with a high frequency of current smokers.²⁹ However, this cluster analysis did not include features associated with the degree of airflow obstruction and inflammatory biomarkers during the exacerbation, thus making it difficult to compare with our current findings.

There are several limitations to consider in this study. First, there is the potential effect of systemic corticosteroids used to

treat the exacerbation on the parameters used such as FEV₁ and sputum eosinophil and neutrophil counts, because such treatment might affect these parameters that have been measured over a varying period of time since admission to hospital. However, it would have been practically difficult to obtain these parameters at admission in these sick patients. At least, these parameters do provide a reflection of the residual severity of the airflow obstruction and lung inflammation at recovery. Second, we studied a relatively small number of exacerbations and we did not analyze a portion of the original cohort, leaving us to study a more severe group of patients with asthma as judged by their higher dose of inhaled corticosteroids. Another limitation is that lung function or induced sputum was not obtained at a time when patients had fully recovered to their baseline state and so we did not know how far the measurements obtained during recovery were different from the baseline values. Some variables such as duration of asthma depend very much on patient recall in contrast with other variables that are more accurately measured. In addition, we have not been able to compare the clusters obtained during recovery from an exacerbation with those obtained in the same patients when they were in a stable state. Cluster analysis also has several limitations, including difficulties in determining the best-fitting number of clusters and also not having examined the longitudinal phenotypic stability of clusters in a clinical setting. Our clusters would also need validation in another independent cohort.

CONCLUSIONS

Despite these limitations, we have shown that patients with asthma during recovery from an acute exacerbation have a neutrophilic, eosinophilic, or mixed granulocytic inflammatory response that is associated with airflow obstruction. The inflammatory response predominant after an exacerbation in patients with asthma who are smokers is an eosinophilic one. These clusters may potentially help us to determine different therapeutic approaches for treating asthma exacerbations depending on the inflammatory response.

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