

Sex-Specific Asthma Phenotypes, Inflammatory Patterns, and Asthma Control in a Cluster Analysis



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What is already known about this topic? Cluster analyses can discover and classify the subphenotype and develop novel treatments for asthma.

What does this article add to our knowledge? The sex-specific clusters were identified with physical, clinical, and biological parameters in our study and showed different asthma phenotypes between females and males.

How does this study impact current management guidelines? The study results provide new directions for personalized management of asthma.

BACKGROUND: Asthma is a heterogeneous disease with complex mechanisms and involves many risk factors and *in vivo* cellular molecules. It is notable that sex differences may have a potential effect on asthma phenotype.

OBJECTIVE: To identify sex-specific phenotypes and health outcomes of asthma.

METHODS: We conducted the Taiwanese Adult Asthma Cohorts study to enroll female (n = 421) and male (n = 299) adult patients with stable asthma. Eight variables were selected by a factor analysis. We further performed a 2-step sensitivity cluster analysis to classify asthma clusters. The risks of asthma-related outcomes among the clusters were assessed using simple logistic regressions.

RESULTS: Three different clusters were identified in males and females. In the female clusters, atopy/eosinophil-predominant (cluster 2), and obesity/neutrophil-predominant pattern (cluster 3) had more than a 2-fold risk of asthma exacerbations (odds ratio, 2.51; 95% CI, 1.12-5.59 and odds ratio, 2.22; 95% CI, 1.01-4.93). In the male clusters, current smoker/neutrophilic atopic cluster (cluster 5) and ex-smoker/eosinophil-predominant or mixed inflammatory pattern (cluster 6) also had a higher risk of asthma exacerbations.

CONCLUSIONS: This study identified heterogeneous characteristics between sexes. In females, the analysis showed atopy with eosinophil-predominant and obese with neutrophil-predominant inflammation. Two distinct asthma phenotypes were found in current and ex-smokers in males. Understanding asthma phenotypes and explaining the potentially biological pathways have become important. © 2018 Published by Elsevier Inc. on behalf of the American Academy of Allergy, Asthma & Immunology (J Allergy Clin Immunol Pract 2019;7:556-67)

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Interested researchers may request a deidentified data set from the corresponding author Tsu-Nai Wang.

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INTRODUCTION

Asthma is a common chronic respiratory inflammatory disease with variable airflow obstruction, variable responses to treatment, and complex mechanisms of asthma pathogenesis. Several studies performed an unsupervised cluster approach to determine novel subphenotypes and provide a classification in both adults and children.¹⁻⁵ Among these cluster studies, sex is an essential influential variable related to the characteristic of cluster phenotypes, and many studies also have demonstrated that there are potentially different mechanisms of asthma by sex.^{6,7}

Epidemiological studies have shown evidence that the incidence and prevalence of asthma are higher in boys than in girls before puberty and higher in women than in men in adulthood.⁸ Weight gain was associated with asthma, particularly in women

Abbreviations used

BMI- body mass index
FVC- forced vital capacity
ICS- inhaled corticosteroid
NEA- noneosinophilic asthma
OCS- oral corticosteroid
WHR- waist to hip ratio

but not in men. Sex influences the risk of asthma in age-dependent and inflammatory patterns.⁶ Sex hormones may play a role in the modulation of immunologic inflammation of asthma.⁷ T cells, specific antibodies, and proinflammatory mediators were affected by sex hormones in females and males.⁹ Women displayed more immune responses and had higher levels of all antibody classes except for IgE.¹⁰ However, there have been no cluster analyses stratified by sex to explore the potentially different asthma phenotypes. The mechanism of the relation between sex difference and asthma still remains unclear.¹¹ Therefore, our study included all severities of asthma to understand the clinical and biological phenotypical classification of asthma in males and females.

Two primary asthma phenotypes (early-onset asthma with atopy status, and obese woman with late-onset and non-eosinophilic asthma) were identified in previous cluster studies.^{1,12} Furthermore, Moore et al⁴ used a hierarchical clustering algorithm, and noted that moderate to severe asthma presented neutrophil-predominant or mixed cellular inflammatory patterns.

A 2-step cluster analysis is a method that combines hierarchical and nonhierarchical clustering approaches. The first step uses the hierarchical method to determine the number of clusters, and the second step moves each individual within and between the groups using the k-means clustering method. The first objective of our study was to identify and characterize clusters that represented potential sex-specific phenotypes using a 2-step cluster analysis. Second, we examined the relationships between these potential asthma phenotypes and asthma-related health outcomes by sex.

METHODS

Study population

Taiwanese Adult Asthma Cohorts were enrolled from the Kaohsiung Chang Gung Memorial Hospital in Taiwan, as previously described.^{13,14} Subjects older than 20 years and diagnosed with mild-to-severe asthma by the pulmonary physicians between July 2012 and August 2015 were included in our study. The diagnosis criteria followed the Global Initiative for Asthma (GINA) criteria as follows: (1) history of respiratory symptoms, such as episodic breathlessness, wheezing, cough, and chest tightness, and (2) the patient had at least 12% and 200 mL improvement in FEV₁ after inhaling a bronchodilator. Study subjects who had no changes in asthma medication in the past 4 weeks were eligible for further analysis.

After obtaining written informed consent, the study staff who were trained in standardized methods administered research procedures, such as questionnaire interview, pulmonary function tests, and collection of blood samples. We recruited 757 stable asthmatic patients older than 20 years in our study; however, 9 subjects had

incomplete questionnaire data, 25 had a history of other lung diseases (including chronic obstructive pulmonary disease, tuberculosis, emphysema), and 3 had cancer; these subjects were excluded from this study. Finally, a total of 720 patients were eligible for subsequent analysis (Figure 1).

All subjects provided written informed consent before undergoing the full study, and the detailed protocol was approved by the Institutional Review Board of the Kaohsiung Chang Gung Memorial Hospital.

Questionnaire

Asthma-related questionnaire and anthropometric examination. There are 5 parts of the asthma-related questionnaire: demographic information, asthma symptoms, health care utilization, allergy and medical history, and surrounding environmental factors. The definition of current smoker was the subject who smoked currently and for over a half-year. Ex-smoker was defined as a smoker quitting at least a half-year before the evaluation. We also collected medical history of allergic rhinitis, atopic dermatitis, other lung diseases, and cancers. Height, weight, waist and hip circumference, and waist to hip ratio (WHR) were obtained during a clinical visit. Additional descriptions of Methods are given in this article's Online Repository at www.jaci-inpractice.org.

Inflammatory patterns. In our study, 4 patterns (paucigranulocytic, eosinophil-predominant, neutrophil-predominant, mixed granulocytic) of granulocytes in the blood were identified according to previous studies.^{4,15-17} Nadif et al^{15,16} defined EOS^{hi} as blood cell counts of 250 eosinophils/mm³ or more and NEU^{hi} as blood cell counts of 5,000 neutrophils/mm³ or more, which corresponded to the 75th percentile of distribution.^{15,16} We also applied a sensitivity analysis to test associations with different cutoff points (50th and 75th percentile) for blood eosinophil and neutrophil levels in our asthma cohort. The definition of 4 inflammatory patterns and results are provided in Table E1 and Table E3 in this article's Online Repository at www.jaci-inpractice.org.

Lung function. Pulmonary function was examined by professional respiratory therapists following a standard protocol in which they measured "baseline" prebronchodilator predicted value (%) and postbronchodilator predicted value (%) and assessed for the highest values of forced vital capacity (FVC, %), FEV₁ (%), and FEV₁/FVC ratio at least 3 times.

Transformation and definition of study variables. Initially, a total of 273 variables were collected from the asthma-related questionnaire and medical records, and we edited the questions, processed redundant variables, and excluded variables missing more than 10% data, to simplify variables. Twenty-nine variables were evaluated for further statistical analysis (see Table E2 in this article's Online Repository at www.jaci-inpractice.org). The variable process and definition are provided in this article's Online Repository at www.jaci-inpractice.org.

Statistical analysis

The variables that did not meet normal distribution were log-transformed to improve normality of distribution for subsequent statistical analysis. Regarding the missing data processing, variables with greater than 10% missing data were excluded from this study. If the missing rate was equal to 10% or less, the data were estimated using Markov Chain Monte-Carlo algorithm for multiple imputations based on a Bayesian approach. We created 5 copies of the data

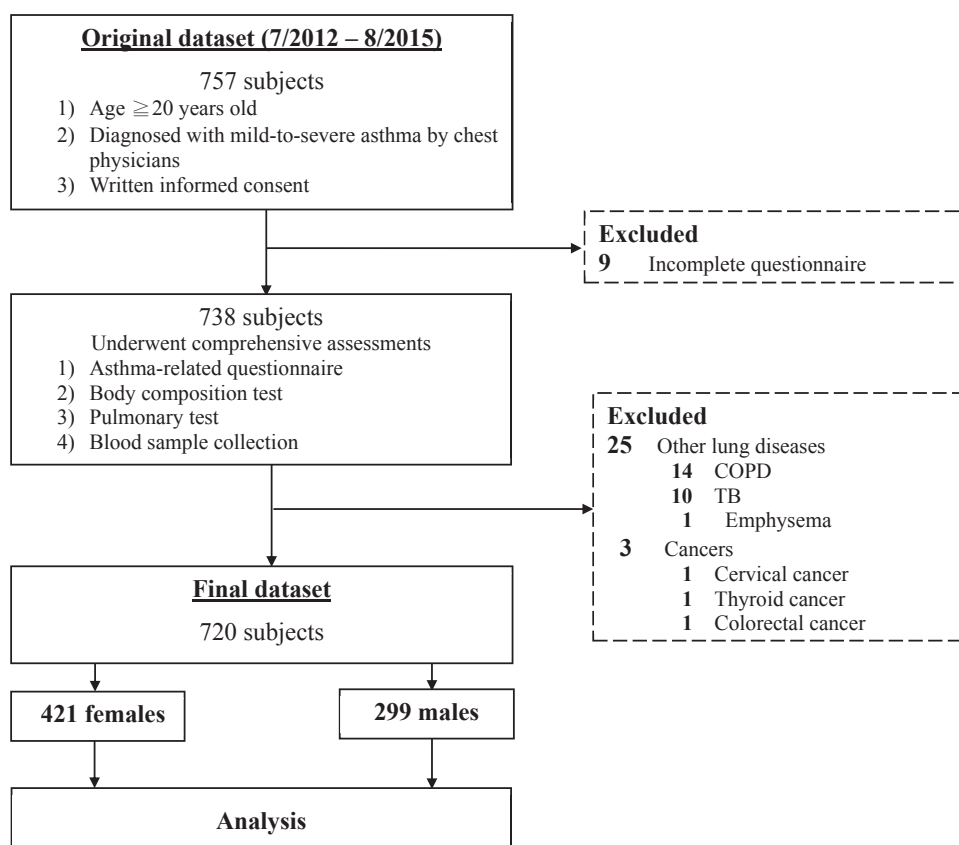


FIGURE 1. Flowchart of study subject selection. COPD, Chronic obstructive pulmonary disease; TB, tuberculosis.

set to replace the missing values by imputed values, and then averaged values were used to fit the model of interest and estimate associations to each of the imputed data sets.¹⁸

Orthogonal varimax factor analysis was performed to reduce and summarize numerous variables to provide variable selection for cluster analysis. We extracted common factors between the variables, with yielded fewer factors instead of the original complex multivariate structure. There were a total of 12 variables (in females and males) consisting of 5 factors, and we selected 1 or 2 representative variables from each factor for the cluster analysis. A total of 8 variables—(1) age, (2) body mass index (BMI), (3) asthma-onset age, (4) prebronchodilator FEV₁ predicted values (%), (5) prebronchodilator FVC predicted values (%), (6) total serum IgE, (7) blood eosinophils (%), and (8) blood neutrophils (%)—were further selected for 2-step cluster analysis in both sexes. In our study, a 2-step cluster analysis that combined Ward's and K-means approaches was performed to classify subjects into larger groups on the basis of similarity or dissimilarity of variables. The first step uses the hierarchical method to determine the numbers of clusters. A hierarchical clustering was performed using an agglomerative approach and Ward's minimum-variance linkage method.^{19,20} At each generation of clusters, the Ward method was used to identify participants who were merged into larger clusters on the basis of variables of interest to minimize the within-cluster sum of squares. The second step moves each individual within and between the groups using the k-means clustering method. The Hartigan-Wong algorithm is the standard algorithm of k-means method,²¹ which aims to partition the points into *k* groups and minimize the Euclidean distances such that the within-cluster

sum of squared errors from points to the assigned cluster centers is minimized. A stepwise discrimination analysis was performed to identify the most influential clustering parameters among 3 clusters by sex. To evaluate the accuracy and performance within the model, we used a leave-one-out cross-validation procedure.

To find differences among 3 clusters in males and females, chi-square and 1-way ANOVA tests were used for data from categorical variables and continuous variables, respectively. The odds ratios and 95% CIs of asthma-related outcomes among clusters were assessed using simple logistic regressions. Data were analyzed using R 3.4.1 (R Foundation, Vienna, Austria) and SPSS 20.0 (IBM Corporation, Armonk, NY), and a *P* value of less than .05 was considered significant.

Sensitivity analysis after removing smokers and ex-smokers

Because there are large differences in smoking history between males and females, we performed a sensitivity analysis after removing smokers and ex-smokers. The results of sensitivity analysis after removing 155 smokers and ex-smokers are provided in Tables E4 to E7 in this article's Online Repository at www.jaci-inpractice.org.

RESULTS

Demographic characteristics and factor analysis

The demographic characteristics and biological factors are presented in Table I. Females had a higher percentage of obesity (BMI, >30 kg/m², 15%) than did males; 45.3% of male subjects had a smoking history. Some descriptions of demographic

TABLE I. Characteristics of the study population in females and males

Characteristic	Total (N = 720)	Females (N = 421; 58.47%)	Males (N = 299; 41.52%)	P value
Age (y)	53.63 ± 14.53	53.29 ± 13.90	54.11 ± 15.37	.463
Age of asthma onset (y)	46.77 ± 17.72	46.77 ± 16.82	46.78 ± 18.95	.990
Onset <16 y, n (%)	50 (6.9)	20 (4.8)	30 (10.0)	.005
Asthma duration (y)	6.86 ± 12.16	6.53 ± 11.49	7.33 ± 13.06	.875
Obese indicators				
BMI	25.44 ± 4.34	25.40 ± 4.77	25.50 ± 3.64	.745
BMI ≥ 30 kg/m ² , n (%)	93 (12.9)	63 (15.0)	30 (10.0)	.032
Body fat (%)	31.11 ± 9.02	35.95 ± 7.66	24.29 ± 5.81	<.0001
Waistline (cm)	86.39 ± 11.54	84.23 ± 12.28	89.44 ± 9.63	<.0001
WHR	0.88 ± 0.10	0.86 ± 0.10	0.92 ± 0.08	.026
Smoking, n (%)				<.0001
No	563 (78.2)	400 (95.2)	163 (54.7)	
Ex-smoker	91 (12.6)	9 (2.1)	82 (27.5)	
Current smoker	64 (8.9)	11 (2.6)	53 (17.8)	
Lung function				
Pre-FEV ₁ , % predicted	85.98 ± 17.53	87.08 ± 17.66	84.42 ± 17.25	.045
Pre-FVC, % predicted	92.72 ± 15.38	94.20 ± 15.98	90.63 ± 14.27	.002
FEV ₁ /FVC ratio	0.77 ± 0.09	0.78 ± 0.08	0.75 ± 0.10	<.0001
Atopy status				
Total IgE* (IU/mL)	190.87 ± 349.00	171.11 ± 318.08	218.69 ± 387.25	.071
Comorbidities, n (%)				
Allergic rhinitis	255 (35.4)	151 (35.9)	104 (34.8)	.413
Atopic dermatitis	30 (4.2)	18 (4.3)	12 (4.0)	.510
Frequency of asthma symptom, past year				
Breathlessness, n (%)				.328
No	202 (28.1)	113 (26.8)	89 (29.8)	
<1 time/wk	133 (47.8)	211 (50.1)	133 (44.5)	
≥1 time/wk	174 (24.2)	97 (23.0)	77 (25.8)	
Breathlessness, during sleeping, n (%)				<.0001
No	406 (56.5)	210 (50)	196 (65.6)	
<1 time/wk	155 (21.6)	107 (25.5)	48 (16.1)	
≥1 time/wk	158 (22.0)	103 (24.5)	55 (18.4)	
ICS, n (%)				<.0001
None	169 (23.5)	102 (24.2)	67 (22.4)	
Low-to-moderate-dose ICS	286 (39.7)	188 (44.7)	98 (32.8)	
High-dose ICS	285 (36.8)	131 (31.1)	134 (44.8)	
OCS, n (%)	114 (15.9)	69 (16.4)	45 (15.1)	.355

Values presented as mean ± SD for continuous variables, and n (%) for categorical variables.

*IgE levels were logarithmically transformed before statistical testing.

characteristics are given in this article's Online Repository at www.jaci-inpractice.org. Orthogonal varimax factor analysis identified 5 dimensions in both females and males (Table II).

Cluster analysis

Cluster 1: (N = 174) Late-onset, normal BMI, non-atopy, low neutrophils, low eosinophils, normal lung function in females. Cluster 1 was the largest group that consisted of slightly older subjects (mean, 57.95 years), and nearly all subjects had late-onset asthma (onset age, >16 years) with normal BMI, body fat, and WHR. Cluster 1 had the highest mean pre-FEV₁ predicted value (%) and FEV₁/FVC ratio and had the lowest total serum IgE, blood eosinophil (%), and blood neutrophil (%) levels among the 3 clusters (Table III).

This group had a lower frequency of asthma symptoms during the past 1 year. Approximately 33.3% of subjects took high-dose inhaled corticosteroids (ICSs), and 12.7% of subjects used oral corticosteroid (OCS), which was less in cluster 1 than that in clusters 2 and 3 (Table IV).

Cluster 2: (N = 117) Young adults with atopy, normal BMI, high blood eosinophils, low neutrophils in females. The females in cluster 2 had a younger mean age (37.56 years) and asthma-onset age (30.28 years), did not have obesity, and had normal lung function. This asthma group had the highest blood eosinophil counts and total serum IgE levels among the 3 clusters. Half (53.0%) of these subjects had self-reported allergic rhinitis (Table III). Approximately 63.8% of

TABLE II. Factor analysis in females and males: Orthogonal varimax rotation

	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5
Female					
BMI*	0.932	−0.077	−0.094	−0.016	−0.033
Waistline	0.910	−0.069	−0.131	−0.126	−0.112
Body fat	0.877	0.059	−0.055	−0.035	−0.071
Blood lymphocytes (%)	−0.008	0.981	0.123	−0.106	0.129
Blood neutrophils (%)*	0.056	−0.981	−0.105	0.104	−0.318
Pre-FEV ₁ (% predicted)*	−0.081	0.095	0.969	−0.054	−0.064
Pre-FVC (% predicted)*	−0.119	0.129	0.961	−0.008	0.040
Asthma-onset age*	0.032	0.105	0.074	−0.916	−0.059
Age*	0.128	0.113	−0.025	−0.904	0.005
Blood eosinophils (%)*	−0.104	0.308	−0.118	0.027	0.824
Blood basophils (%)	−0.114	0.192	0.131	−0.185	0.726
Total IgE*	0.034	0.013	−0.061	0.336	0.487
Male					
BMI*	0.935	−0.014	0.049	−0.082	0.029
Waistline	0.906	0.073	−0.076	0.109	−0.020
Body fat	0.876	−0.034	−0.107	−0.024	−0.051
Blood neutrophils (%)*	−0.059	0.938	−0.002	0.063	−0.190
Blood lymphocytes (%)	0.116	−0.918	0.045	−0.120	−0.011
WBC	0.219	0.625	−0.112	−0.234	0.035
Pre-FVC (% predicted)*	−0.041	−0.029	0.952	−0.063	−0.016
Pre-FEV ₁ (% predicted)*	−0.084	−0.079	0.934	0.037	−0.168
Age*	−0.029	0.005	−0.099	0.920	−0.012
Asthma-onset age*	0.041	−0.022	0.066	0.918	0.004
Total IgE*	0.090	0.207	−0.017	−0.158	0.715
Blood basophils (%)	−0.081	−0.137	−0.074	0.066	0.655
Blood eosinophils (%)*	−0.056	−0.470	−0.132	0.109	0.652

Bold indicates features that most distinguish one cluster from others.

*Final variables were selected for the cluster analysis.

subjects had the symptom of sneezing. Only 24.8% of subjects used high-dose ICS; however, 21.4% of subjects used OCS, which is the highest level of the 3 clusters (Table IV).

Cluster 3: (N = 130): Late-onset, obesity, high neutrophils, low eosinophils and IgE in females. Cluster 3 had the oldest mean age, and 94.6% of subjects had late-onset asthma (asthma-onset age, ≥ 16 years) and obesity. This group had the highest BMI, body fat, and waistline. Subjects had decreased lung function, with the lowest mean pre-FEV₁ predicted value (%) and FEV₁/FVC ratio in the female clusters. This group expressed significantly different inflammatory patterns compared with cluster 2. This group had low total serum IgE and blood eosinophil counts; however, the blood neutrophil level was the highest among the 3 clusters (Table III). The use of more than 2 asthma controller therapies was the highest among the 3 groups (Table IV).

Cluster 4: (N=112) Late-onset, with low IgE and blood eosinophils, normal BMI, normal lung function in males. Cluster 4 subjects were older and not obese. All subjects had late-onset asthma (onset age, >16 years). Compared with other male clusters, cluster 4 had the highest mean pre-FEV₁ (%) and FEV₁/FVC ratio. The total serum IgE and blood eosinophil percentages in cluster 4 were significantly lower than those in cluster 6 (Table III). This group demonstrated less

frequent (28.6%) asthma symptoms during sleep. Only 9.8% of subjects in cluster 4 used OCSs (Table IV).

Cluster 5: (N = 116) Young adults with atopy, current smoking, and high blood neutrophils in males. Cluster 5 had the youngest onset age and a higher mean BMI. Compared with other male clusters, this group had the highest frequency (28.4%) of current smokers. Approximately 40.5% of subjects had self-reported allergic rhinitis, and this group showed high total serum IgE levels (mean, 277.83 IU/mL). Subjects in cluster 5 had the highest neutrophil and lowest eosinophil levels among the 3 male clusters (Table III). More than half (62.1%) of the subjects were treated with ICSs, and 23.5% required OCSs (Table IV).

Cluster 6: (N = 71) Late-onset, ex-smokers, high blood eosinophils in males. Cluster 6 consisted of older subjects (mean, 65.56 years), and most were ex-smokers (43.7%). Only 1 subject was obese (BMI, ≥ 30 kg/m²). The subjects in cluster 6 had the lowest pre-FEV₁ (%) and FEV₁/FVC ratio values. Cluster 6 also had the highest blood eosinophils, and total serum IgE levels (252.21 IU/mL) were moderately elevated. According to the inflammatory cellular patterns, this group had the highest percentage of eosinophil-predominant or mixed inflammatory pattern among the 3 male clusters (Table III).

TABLE III. Demographic characteristics of subjects in female and male clusters

Characteristic	Females				Males			
	Cluster 1: Late-onset, normal lung function (N = 174)	Cluster 2: Young adults, eosinophilic atopic (N = 117)	Cluster 3: Obese, neutrophilic (N = 130)	P value	Cluster 4: Late-onset, normal lung function (N = 112)	Cluster 5: Current smokers, neutrophilic atopic (N = 116)	Cluster 6: Ex-smokers, eosinophilic (N = 71)	P value
Age (y)	57.95 ± 10.36	37.56 ± 9.44	61.21 ± 9.41	<.0001	62.45 ± 7.85	39.06 ± 10.75	65.56 ± 9.95	<.0001
Age of asthma onset (y)	53.94 ± 12.83	30.28 ± 10.70	52.01 ± 15.94	<.0001	59.55 ± 7.80	29.30 ± 13.43	55.20 ± 17.22	<.0001
Onset <16 y, n (%)	1 (0.6)	12 (10.3)	7 (5.4)	.001	0 (0.0)	26 (22.4)	4 (5.6)	<.0001
Asthma duration (y)	4.02 ± 8.37	7.29 ± 11.13	9.20 ± 14.42	<.0001	2.89 ± 6.11	9.76 ± 14.72	10.37 ± 16.14	<.0001
Obese indicators								
BMI	25.10 ± 4.19	23.86 ± 4.56	27.17 ± 5.14	<.0001	25.38 ± 3.14	26.16 ± 4.46	24.60 ± 2.57	.016
BMI ≥ 30 kg/m ² , n (%)	20 (11.5)	13 (11.1)	30 (23.1)	.008	9 (8.0)	20 (17.2)	1 (1.4)	<.0001
Body fat (%)	35.96 ± 7.01	33.86 ± 7.38	37.834 ± 8.28	<.0001	24.26 ± 5.93	24.55 ± 5.90	23.92 ± 5.50	.769
Waistline (cm)	83.81 ± 11.79	79.05 ± 11.34	89.47 ± 11.70	<.0001	88.99 ± 8.96	89.33 ± 11.67	90.30 ± 6.55	.663
WHR	0.86 ± 0.10	0.87 ± 0.10	0.81 ± 0.07	<.0001	0.92 ± 0.05	0.90 ± 0.09	0.94 ± 0.10	.002
Smoke, n (%)				.011				<.0001
No	167 (96.0)	104 (89.7)	129 (99.2)		65 (58.6)	67 (57.8)	31 (43.7)	
Ex-smoking	3 (1.7)	6 (5.2)	0 (0.0)		35 (31.5)	16 (13.8)	31 (43.7)	
Current smoking	4 (2.3)	6 (5.2)	1 (0.8)		11 (9.9)	33 (28.4)	9 (12.7)	
Lung function								
Pre-FEV1, % predicted	99.18 ± 11.99	87.28 ± 13.46	70.71 ± 13.86	<.0001	94.59 ± 10.71	86.22 ± 14.66	65.43 ± 14.00	<.0001
Pre-FVC, % predicted	104.54 ± 11.19	95.56 ± 11.88	79.15 ± 12.72	<.0001	98.85 ± 9.22	92.35 ± 11.99	74.86 ± 11.40	<.0001
FEV ₁ /FVC ratio	0.79 ± 0.06	0.78 ± 0.09	0.75 ± 0.10	<.0001	0.76 ± 0.09	0.78 ± 0.10	0.69 ± 0.10	<.0001
Atopy status								
Total IgE* (IU/mL)	80.79 ± 149.67	312.55 ± 483.43	164.72 ± 244.98	<.0001	136.19 ± 190.95	277.83 ± 522.60	252.21 ± 337.76	.020
Comorbidities, n (%)								
Allergic rhinitis	55 (31.6)	62 (53.0)	34 (26.2)	<.0001	37 (33.0)	47 (40.5)	20 (28.2)	.200
Atopic dermatitis	4 (2.3)	12 (10.3)	2 (1.5)	.001	4 (3.6)	8 (6.9)	0 (0.0)	.060
Blood sample inflammatory cells								
WBC count	7.05 ± 2.96	8.00 ± 6.08	7.67 ± 2.80	.127	6.64 ± 2.08	8.10 ± 2.81	7.59 ± 3.23	<.0001
Neutrophil (%)	58.77 ± 11.20	59.81 ± 8.36	63.64 ± 10.28	<.0001	60.76 ± 9.15	62.14 ± 12.53	60.74 ± 11.82	.579
Eosinophil (%)*	2.34 ± 2.16	3.70 ± 2.92	2.78 ± 3.32	<.0001	2.92 ± 2.68	2.70 ± 2.34	5.46 ± 4.99	<.0001
Monocyte (%)	5.52 ± 1.71	5.17 ± 1.56	5.17 ± 1.57	.102	5.80 ± 1.68	5.66 ± 2.24	6.02 ± 1.70	.458
Lymphocyte (%)	32.70 ± 10.49	30.57 ± 7.75	27.94 ± 9.71	<.0001	29.75 ± 8.77	28.77 ± 10.81	27.08 ± 9.86	.205
Basophil (%)*	0.45 ± 0.34	0.43 ± 0.26	0.43 ± 0.36	.179	0.55 ± 0.87	0.39 ± 0.29	0.50 ± 0.36	.100

(continued)

TABLE III. (Continued)

Characteristic	Females			Males			P value
	Cluster 1: Late-onset, normal lung function (N = 174)	Cluster 2: Young adults, eosinophilic atopic (N = 117)	Cluster 3: Obese, neutrophilic (N = 130)	Cluster 4: Late-onset, normal lung function (N = 112)	Cluster 5: Current smokers, neutrophilic atopic (N = 116)	Cluster 6: Ex-smokers, eosinophilic (N = 71)	
Blood granulocyte patterns (%) [†]							<.001
Mixed granulocyte	5 (2.9)	15 (12.8)	11 (8.5)	7 (6.2)	13 (11.2)	8 (11.3)	
Neutrophil-predominant	35 (20.1)	22 (18.8)	40 (30.8)	14 (12.5)	38 (32.8)	13 (18.3)	
Eosinophil-predominant	21 (12.1)	31 (26.5)	18 (13.8)	19 (17.0)	20 (17.2)	29 (40.8)	
Paucigranulocytic	113 (64.9)	49 (41.9)	61 (46.9)	72 (64.3)	45 (38.8)	21 (29.6)	

BMI, Body mass index; FEV₁, forced expiratory volume in 1 second; FVC, forced vital capacity; IgE, immunoglobulin E; WBC, white blood cell; WHR, waist to hip ratio.

Values presented as mean ± SD for continuous variables, and n (%) for categorical variables.

*IgE levels were logarithmically transformed before statistical testing.

[†]Four blood granulocyte patterns were defined by blood cell counts of ≥ 250 EOS/mm³ and blood cell counts of ≥ 5000 NEU/mm³ as cutoff points.

Stepwise discrimination analysis

A stepwise discriminant analysis using the same 29 variables showed the 8 and 6 strongest discriminatory variables for cluster assignment in females and males, respectively. In both sexes, age and prebronchodilator FEV₁ (%) were the first and second most influential variables (Table V).

Clusters and asthma-related outcomes

Among female and male clusters, cluster 1 and cluster 4 were selected as a reference group, which was based on the lowest frequency of asthma exacerbations, the lowest scores of Taiwan version Asthma Quality of Life Questionnaire, and the highest scores of Asthma Control Test (Table VI). In female clusters, both cluster 2 (young adult asthma, atopy) and cluster 3 (late-onset asthma, obesity) had significantly more than 2 times the risk of asthma exacerbations (Figure 2). In male clusters, cluster 5 (current smokers, atopy) and cluster 6 (ex-smoker, eosinophil) had a borderline significant effect on the risk of asthma exacerbations. Cluster 5 had a significantly increased risk of poor asthma quality of life (Figure 3). A leave-one-out cross-validation model was implemented for validation. This showed a within-repeatability accuracy of 92.2% for females and 92.6% for males, respectively.

DISCUSSION

In this study, we applied 8 predictors to an unsupervised cluster approach, and 3 distinct clusters with significantly different characteristics were identified within and between females and males. The inflammatory patterns were mainly influenced by obesity in females and smoking status in males. The different clinical features, physiological variables, and patterns of cellular inflammation in 6 clusters suggested potential information regarding the underlying mechanisms of asthma by sex.

Female clusters

Cluster 1 was the reference group in females and showed the best control of asthma and was also mainly composed of a paucigranulocytic pattern. The characteristics of cluster 2 were youngest onset age and high levels of total IgE and blood eosinophils (%). According to these characteristics, cluster 2 was considered eosinophilic asthma. Eosinophilic asthma is related to the T_H2 inflammatory asthma pathway, which is primarily related to T_H2 immune responses involving the cytokines IL-4, IL-5, and IL-13.²² Moreover, B cells were promoted by cytokines such as IL-4 to produce IgE, which binds to mast cells and then releases histamine, causing airway inflammation.²³ High IgE and eosinophils were also observed in cluster 2. Furthermore, we found that cluster 2 had a higher frequency of asthma exacerbations and worse asthma quality of life. The results were consistent with those of previous studies that showed an increased risk of exacerbations and poor asthma control in patients who had increasing eosinophils *in vivo*.²⁴ A previous study indicated that patients with eosinophilic asthma improved with use of inhaled fluticasone, whereas there was a nonsignificant improvement in patients with noneosinophilic asthma (NEA).²⁵ Importantly, the subjects in cluster 2 had persistent blood eosinophilia despite requiring treatment with high doses of ICSs or OCSs (21.4%), and this cluster might also represent a specific eosinophilic subpopulation.²⁶

TABLE IV. Asthma symptoms and medication of subjects in female and male clusters

Characteristic	Females			P value	Males			P value
	Cluster 1: Late-onset, normal lung function (N = 174)	Cluster 2: Young adults, eosinophilic atopic (N = 117)	Cluster 3: Obese, neutrophilic (N = 130)		Cluster 4: Late-onset, normal lung function (N = 112)	Cluster 5: Current smokers, neutrophilic atopic (N = 116)	Cluster 6: Ex-smokers, eosinophilic (N = 71)	
Frequency of asthma symptoms, past year								
Breathlessness, n (%)				.004				.58
No	59 (33.9)	24 (20.5)	30 (23.1)		37 (33.0)	29 (25.0)	23 (32.4)	
<1 time/wk	72 (41.4)	60 (51.3)	79 (60.8)		47 (42.0)	53 (45.7)	33 (46.5)	
≥1 time/wk	43 (24.7)	33 (28.2)	21 (16.2)		28 (25.0)	34 (29.3)	15 (21.1)	
Breathlessness, during sleeping, n (%)				.430				.19
No	93 (53.8)	53 (45.3)	64 (49.2)		80 (71.4)	71 (61.2)	45 (63.4)	
<1 time/wk	36 (20.8)	35 (29.9)	36 (27.7)		13 (11.6)	19 (16.4)	16 (22.5)	
≥1 time/wk	44 (25.4)	29 (24.8)	30 (23.1)		19 (17.0)	26 (22.4)	10 (14.1)	
Sneezing and nasal congestion, n (%)				.034				.61
No	90 (51.7)	42 (36.2)	60 (46.2)		49 (43.8)	47 (40.5)	34 (47.9)	
Yes	84 (48.3)	74 (63.8)	70 (53.8)		63 (56.3)	69 (59.5)	37 (52.1)	
ICS, n (%)				.095				.26
None	45 (25.9)	35 (29.9)	22 (16.9)		54 (48.2)	44 (37.9)	36 (50.7)	
Low-to-moderate-dose ICS	71 (40.8)	53 (45.3)	64 (49.2)		37 (33.0)	39 (33.6)	22 (31.0)	
High-dose ICS	58 (33.3)	29 (24.8)	44 (33.8)		21 (18.8)	33 (28.4)	13 (18.3)	
OCS, n (%)	22 (12.7)	25 (21.4)	22 (16.9)	.147	11 (9.8)	27 (23.5)	7 (9.9)	.01
Controllers, n (%)								
≥2 controllers	45 (25.9)	19 (16.2)	39 (30.0)	.036	8 (7.1)	5 (4.3)	5 (7.0)	.61
Antihistamine, n (%)	69 (39.9)	69 (59.0)	42 (32.3)	<.0001	36 (32.1)	41 (35.7)	19 (26.8)	.45

All values presented as n (%) for categorical variables.

The high-dose ICS for a year is equivalent to >800 µg/d for budesonide and >500 µg/d for fluticasone.

Controllers include ICSs, OCSs, theophylline, and leukotrienes.

TABLE V. Cluster assignment using stepwise discriminant analysis in females and males

Step	Variable	Partial R^2	F value	P value
Females				
1	Age	0.497	211.9	<.0001
2	Pre-FEV ₁ (% predicted)*	0.266	195.5	<.0001
3	Blood eosinophils	0.231	149.6	<.0001
4	Total IgE	0.210	122.8	<.0001
5	BMI	0.195	104.5	<.0001
6	Pre-FVC (% predicted)	0.185	91.0	<.0001
7	Blood neutrophils (%)	0.177	80.9	<.0001
8	Asthma-onset age	0.172	72.6	<.0001
Males				
1	Age	0.384	237.1	<.0001
2	Pre-FEV ₁ (% predicted)*	0.219	167.6	<.0001
3	Asthma-onset age	0.182	131.7	<.0001
4	Pre-FVC (% predicted)	0.167	106.0	<.0001
5	Blood eosinophils	0.155	89.9	<.0001
6	BMI	0.15	76.9	<.0001

*Prebronchodilator FEV₁ % predicted values were selected with the greater value of 3 tests.

The female subjects in cluster 3 were characterized by old age, late-onset asthma, obesity, and poor asthma control, which was

also found in previous cluster studies.^{1,12} Adipose tissue does not only impinge on chest volumes, leading to reduced lung volumes in the airway, but also secretes proinflammatory elements and adipokines. The characteristics of cluster 3 resemble NEA. Another study found that obese asthmatic patients had more neutrophilic inflammation in both blood and sputum, decreased lung function, and worse response to medication than did nonobese asthmatic patients, but only in females.⁶ This subgroup was mainly composed of neutrophil-predominant and mixed granulocyte patterns and showed worse lung function and high medication usage similar to a previous study.² A possible explanation for the increased neutrophilic inflammation may be due to adipose tissue in obese patients with asthma.^{11,27} Adipose tissue produces proinflammatory mediators including leptin, TNF- α , and IL-6. Leptin stimulates monocytes to release TNF- α , which activates neutrophils and can gather neutrophils to the airways. The activated neutrophil will release mediators such as neutrophil elastase and create human airway smooth muscle shrinkage, leading to airway narrowing.²⁷ Patients with NEA have expressed poor response to corticosteroid treatment despite receiving high doses.²⁵ These results support our findings that cluster 3 had a high percentage of ICS use and worse asthma health outcomes. The previous study also indicated that the concentrations of neutrophils may be affected by different body compositions between females and males. Compared with males, females had more subcutaneous adipose that secretes more leptin

TABLE VI. Asthma control–related outcomes in female and male clusters

Characteristic	Females				Males			
	Cluster 1: Late-onset, normal lung function (N = 174)	Cluster 2: Young adults, eosinophilic atopic (N = 117)	Cluster 3: Obese, Neutrophilic (N = 130)	P value	Cluster 4: Late-onset, normal lung function (N = 112)	Cluster 5: Current smokers, neutrophilic atopic (N = 116)	Cluster 6: Ex-smokers, eosinophilic (N = 71)	P value
Asthma exacerbations, n (%)	11 (6.32)	17 (14.53)	17 (13.08)	.048	4 (3.6)	12 (10.3)	8 (11.3)	.08
TAQLQ	50.45 ± 16.70	53.96 ± 16.34	50.54 ± 18.61	.181	43.87 ± 14.64	49.03 ± 16.16	45.45 ± 15.08	.04
ACT	21.33 ± 2.9	21.05 ± 3.18	20.43 ± 3.42	.078	21.78 ± 2.78	21.16 ± 3.30	21.77 ± 3.29	.25

ACT, Asthma Control Test (used for assessing asthma control during the past 4 wk); TAQLQ, Taiwanese version of the Asthma Quality of Life questionnaire (used for assessing quality of life of patients with asthma during the past 2 wk).

Asthma exacerbations were defined as at least 1 hospitalization or emergency department visit during the past year.

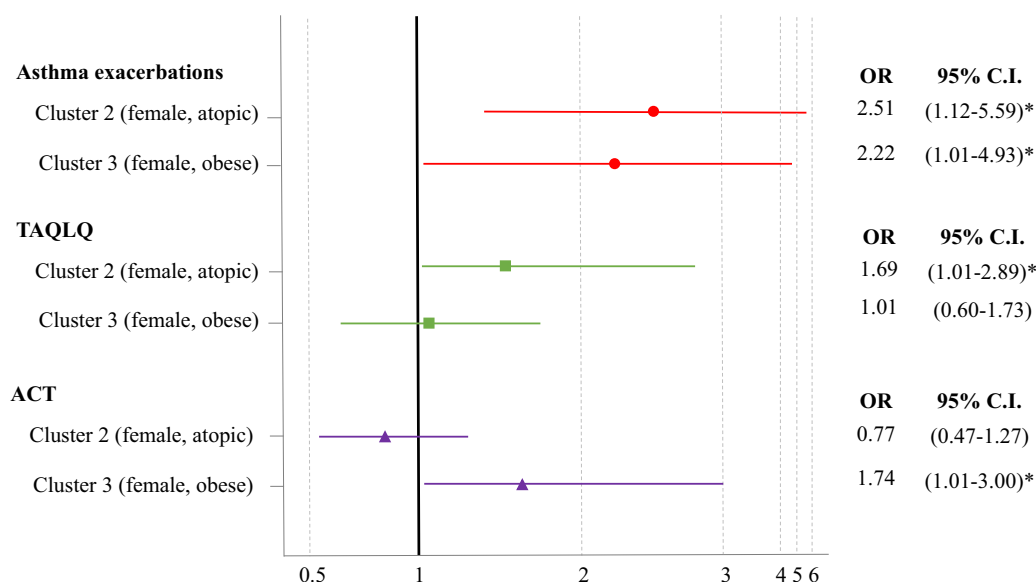


FIGURE 2. OR for the association between asthma-related outcome in female clusters. The definition of outcome variables: Asthma exacerbations (hospitalization or emergency department visit), TAQLQ (a cutoff score of >61 reflected poor quality of life), and ACT (a cutoff score of <19 reflected poor asthma control). The reference group for simple logistic regression analysis was cluster 1 (late-onset, normal lung function), which was based on the lowest TAQLQ mean scores and the highest ACT mean scores. ACT, Asthma Control Test; OR, odds ratio; TAQLQ, Taiwan version Asthma Quality of Life Questionnaire.

than abdominal adipose. In addition, obese women have higher plasma leptin concentrations than do men, leading to increased neutrophilic inflammation.¹¹ Thus, our results suggest that asthma phenotypes may be affected by sex.

Male clusters

Cluster 4 was the reference group in males and showed the best control of asthma. Cluster 5 had a high current smoking rate, slightly high blood neutrophils (%), and low blood eosinophils (%). A previous study indicated that current smokers have a predominately noneosinophilic phenotype.²⁸ The males in cluster 6 had eosinophil-predominant or mixed inflammatory pattern, the highest ex-smoking rate (43.7%), and lowest FEV₁/FVC ratio (mean, 69%). Male adult-onset asthma patients have been reported to frequently have high blood eosinophil counts with higher rates of exacerbations and less well-controlled asthma, similar to characteristics observed in cluster 6.²⁹ To

date, many previous studies have indicated that principally 2 different biological pathways have been found in asthmatic patients including eosinophilic asthma (type 2 asthma) and NEA (type 2-low asthma).^{22,30} Eosinophils and neutrophils contribute to the different pathobiology of asthma.^{15,31} The evidence shows that patients with NEA are often associated with adult-onset age and higher neutrophilic percentage, which is modified by obesity and smoking status.¹²

However, the mechanism of NEA is still unclear. A previous study suggested that cigarette smoking was associated with neutrophilic inflammation but did not aggravate eosinophilic inflammation.²⁸ Cigarette smoke partly induced T_H17-type inflammation, and the cytokines of T_H17 recruited neutrophils in the airway and activated IL-8 from epithelial cells.³² The previous studies indicated that current smokers had a lower percentage of sputum eosinophils and poor outcome than did never-smokers,²⁸ which supported our findings in cluster 5.

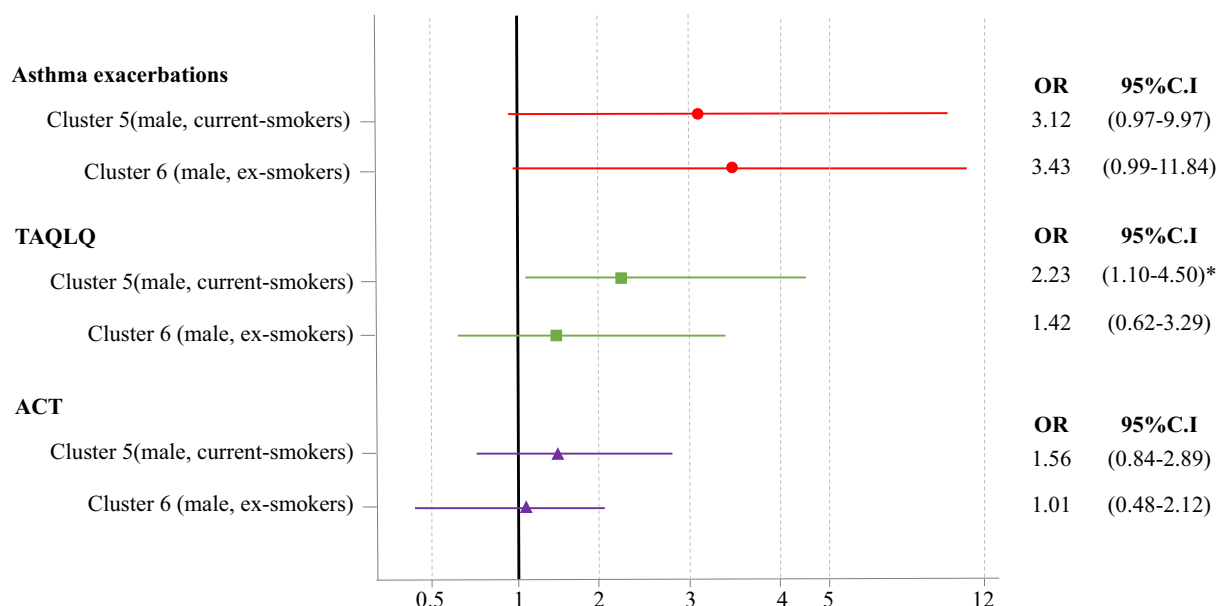


FIGURE 3. OR for association between asthma-related outcome in male clusters. The definition of outcome variables: Asthma exacerbations (hospitalization or emergency department visit), TAQLQ (a cutoff score of >61 reflected poor quality of life) and ACT (a cutoff score of <19 reflected poor asthma control). The reference group for simple logistic regression analysis was cluster 4 (late-onset, normal lung function), which was based on the lowest TAQLQ mean scores and the highest ACT mean scores. *ACT*, Asthma Control Test; *OR*, odds ratio; *TAQLQ*, Taiwan version Asthma Quality of Life Questionnaire.

However, other studies also found that cigarette smoking may maintain eosinophilic airway inflammation, particularly in elderly smokers.^{32,33} The mechanism underlying persistent eosinophilic inflammation in current and ex-smokers is mostly explained by the induction of thymic stromal lymphopoietin, which is induced by smoking and then promoted by T_H2 immune responses.³⁴ We found that cluster 6 subjects had the worst lung function, which may be explained by older age and smoking status. A fall in chest wall compliance and decreased supporting tissue, such as elastic fibers around alveolar ducts, may impair pulmonary function in the elderly.³⁵ A cohort study indicated that current smoking and asthma have an additive effect on the decline in FEV₁ % predicted value.³⁶

Study strengths and limitations

This is the first study using a cluster analysis to identify asthmatic phenotypes stratified by sex. This current study has some potential limitations. First, there were large differences in smoking history between sexes in Taiwan. In our study, 45.3% of male patients, in contrast to only 4.7% of females, had a smoking history. The smoking rate in several countries is also high in males and low in females, especially in Asian countries, such as Indonesia (57%, 4%), China (45%, 2%), Korea (36%, 4%), Cambodia (42%, 4%), Malaysia (38%, 1%), Thailand (37%, 2%), Vietnam (41%, 2%), and Bangladesh (44%, 2%). The findings may be limited to populations with similar smoking rates.³⁷ The inclusion of smokers in our study is similar to several previous cluster studies.^{2,38} Wide inclusion of patients with asthma, including all severities of asthma and smokers, suggests that the results are clinically significant and generalize to the general population.³⁸ However, because of large differences in smoking history between sexes and increasing the robustness of

using FEV₁ and FVC as classifying variables, we further performed sensitivity analysis after removing 155 smokers and ex-smokers and found that the characteristics of 3 clusters in males are also different from those in females. Second, the study is a cross-sectional study, and we could not observe the temporal variability in blood inflammatory cell counts in each cluster. Third, we measured inflammatory cell counts in peripheral blood. Previous studies indicated that sputum eosinophils are more accurate than blood eosinophils in measuring asthmatic airway inflammation. We did not include other measures of type 2 inflammation, such as sputum eosinophils and exhaled nitric oxide. Induced sputum cell counts are the criterion standard test for assessing inflammatory phenotypes. However, access to the method of induced sputum cell counts is limited. Several studies have suggested that peripheral blood cell counts may be a noninvasive and reliable method to evaluate airway inflammation in asthma for clinical practice.³⁹⁻⁴² Both blood and sputum eosinophils can reflect the clinical response of an acute exacerbation of asthma.⁴³ Nadif et al¹⁵ defined 4 inflammatory patterns according to blood eosinophil and neutrophil count cutoff points, and found marked differences in asthma phenotype characteristics among 4 blood inflammatory patterns.¹⁵ In recent years, more and more studies have suggested that blood counts of eosinophils and neutrophils may be a useful method to detect airway inflammatory phenotype and could also be predictive biomarkers for asthma outcomes.^{15,16,39,44-46} Fourth, most of the environmental and clinical symptoms in our study were obtained by questionnaires, and this approach might have recall bias. Fifth, the mean age of the asthma cohort was rather high (average of 53 years). Unlike in asthma, airflow limitation in chronic obstructive pulmonary disease is often progressive and is not fully reversible. Even though asthma can usually be

distinguished from chronic obstructive pulmonary disease, in some individuals it is difficult to distinguish the 2 diseases. In general, chronic obstructive pulmonary disease is defined by fixed airway obstruction as measured by postbronchodilator FEV₁/FVC ratio of less than 0.70.⁴⁷ Heavy current and ex-smokers were allowed if the patient had more than 12% increase in FEV₁ after inhaling a bronchodilator and a normal diffusion capacity.⁴⁸

Moore et al¹ used 34 variables and unsupervised hierarchical cluster analysis to identify 5 clusters for severe asthma, and observed that female and male asthmatic patients were mixed together from 53% to 80% females in these 5 clusters. Wu et al⁴⁹ applied 112 clinical, physiologic, and inflammatory variables to perform unsupervised machine learning approaches, and also found that female and male asthmatic patients were mixed together from 40% to 84% females in their 6 clusters. Among these cluster studies, sex is an essential influential variable related to the characteristic of cluster phenotypes. However, this is a first sex-specific cluster analysis to explore the potentially different asthma phenotypes between males and females. After elimination of redundant data and factor analysis, we selected 8 variables—age, BMI, asthma-onset age, prebronchodilator FEV₁ predicted values (%), prebronchodilator FVC predicted values (%), total serum IgE, blood eosinophils (%), and blood neutrophils (%)—to identify sex-specific clusters by using 2-step cluster analysis. In practice, developing a simple, accessible clinical approach is important for the classification and management of asthma. Exploring these sex-specific subphenotypes may be useful for understanding the underlying mechanisms of adult asthma and may provide new directions for personalized management.

CONCLUSIONS

This study identified 3 clusters with heterogeneous characteristics in each sex stratum and showed different asthma phenotypes between females (clusters 1-3) and males (clusters 4-6). The sex-specific clusters were identified with physical, clinical, and biological parameters in our study. In females, these showed an atopic, eosinophil-predominant pattern and an obese, neutrophilic-predominant pattern. There were 2 distinct asthma phenotypes in current and ex-smokers in males.

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ONLINE REPOSITORY

Methods

Asthma-related questionnaire and anthropometric examination. The asthma-related questionnaire, including demographic information, asthma symptoms, health care utilization, allergy and medical history, and surrounding environmental factors, data were collected. There are 4 questions on asthma symptoms, involving the frequency of breathlessness, cough, sneezing, and nasal congestion during the previous year. We also collected medical history of allergic rhinitis, atopic dermatitis, other lung diseases, and cancers. Physiological tests including height, weight, waist and hip circumference, and WHR were examined during a clinical visit. Body fat was measured by bioelectrical impedance analysis using a Tanita body fat analyzer (Tanita BF-720; Tanita Corporation, Tokyo, Japan). BMI was calculated using the following standard formula: weight (kg)/height (m)². Waistline and hipline were obtained by staff using a tape measure. The formula of WHR was waist circumference divided by hip circumference.

The 5-point 27-item Taiwan version Asthma Quality of Life Questionnaire (TAQLQ) was simplified from the original 7-point 32-item Asthma Quality of Life Questionnaire^{E1,E2} and provides acceptable precise estimates for patients with asthma, especially less educated patients with asthma in southern Taiwan.^{E2} A higher score indicates a worse quality of life in patients with asthma. There are 4 levels based on the cutoff score (61, 89, 106, and 120) as follows: quite mild, mild, moderate, or severe asthma.^{E2} Before the statistical analysis, the total scores were transformed into a binary variable according to the score of 61, which represented well (<61) and not-well (≥61) quality of life. The Asthma Control Test (ACT) is a patient-based 5-point 5-item questionnaire used to assess asthma control during the past 4 weeks.^{E3} A cutoff score of less than 19 reflects poorly controlled asthma, which is defined by GINA.^{E4} In our study, we transformed the scores into a binary variable to represent poorly controlled asthma (<19) and well-controlled asthma (≥19) groups. Asthma health outcome was evaluated as the following variables: (1) asthma exacerbations included at least 1 hospitalization or emergency department visit in the past year. (2) TAQLQ assessed the score of asthma quality of life during the previous 2 weeks, and the cutoff score of 61 was used to define well and not-well quality of life, and (3) ACT was determined by the subjects' self-reported asthma control in the past 4 weeks, and the cutoff score of 19 was used to define well and poor control of asthma. TAQLQ, ACT, and asthma exacerbations were selected as outcome variables.

Sensitivity analysis with different cutoff points for inflammatory patterns. We conducted a sensitivity analysis with different cutoff points for inflammatory patterns. In our study, 4 patterns of granulocytes in the blood were identified according to Nadif et al,^{E5,E6} and also 50th (median) and 75th percentile of eosinophil and neutrophil levels in our asthma cohort. The definitions of 4 inflammatory patterns and results are provided in Tables E1 and E3.

Transformation and definition of study variables. Initially, a total of 273 variables were collected from the asthma-related questionnaire and medical records, and we edited

the questions, processed redundant variables, and excluded variables missing more than 10% data, to simplify variables. Twenty-nine variables were evaluated for further statistical analysis (see Table E2). The variable process and definition are as follows. Childhood asthma was defined as onset age of less than 16 years, and obesity was defined as BMI of 30 kg/m² or more. Self-reported physician-diagnosed allergic rhinitis, atopic dermatitis, and asthma symptoms were binary variables. BMI, WHR, body fat, age of asthma onset, asthma duration, pre-bronchodilator FEV₁ predicted values (%), prebronchodilator FVC predicted values (%), FEV₁/FVC ratio, total IgE, and percentages of neutrophils, monocytes, eosinophils, lymphocytes, and basophils were continuous variables. The definition of high-dose ICSs is equivalent to the medicinal use of more than 800 µg/d for budesonide or more than 500 µg/d for fluticasone. Asthma medication controllers included ICSs, OCSs, theophylline, and leukotriene.

Results

Demographic characteristics of study subjects. Fifty-eight percent of the patients were female. Most subjects (93.1%) had adult-onset asthma in our study. Females had a higher percentage of obesity (BMI, >30 kg/m², 15%) than did males; 45.3% of male subjects had a smoking history; in contrast, 4.7% of females had a smoking history. The frequency of asthma symptoms during the past year was higher in females than in males; 44.7% of female subjects used low-to-moderate-dose ICSs, whereas male subjects had a higher percentage (44.8%) of high-dose ICS use.

The heat maps with dendrograms. Figures E1 and E2 show the heat maps with dendrograms generated by Ward's hierarchical clustering analysis in female and male asthmatic patients. Hierarchical clustering analysis was performed for pre-evaluation of the number of clusters. A Euclidean distance and Ward's minimum-variance linkage clustering approach were used. In this study, 3 major clusters were identified for female and male asthma, respectively. Then K-means analysis was conducted by using the prespecified number of clusters from hierarchical clustering analysis.

Sensitivity analysis with different cutoff points for inflammatory patterns. We observed that 4 blood granulocyte phenotypes defined by above 3 criteria were significantly different among 3 clusters in female and male asthma, and were associated with asthma phenotype characteristics (see Table III, Table E3, and Figures E3-E5).

Sensitivity analysis after removing 155 smokers and ex-smokers. After removing 155 smokers and ex-smokers (135 males and only 20 females), in our sensitivity analysis, the characteristics of 3 clusters in females showed no change. However, we found a nonspecific group with late-onset/normal lung function asthma, a young adults with atopic cluster, and a mixed granulocyte cluster with lower lung function in males that were different from the female clusters. In nonsmoking males, we found significant differences in lung function among 3 clusters and did not find these clusters related to BMI and percent body fat (Table E5). The late-onset/normal lung function asthma (cluster 4, n = 86) was identified, and this group had better lung function and did not have more frequency of symptoms or asthma exacerbations in the past year. The cluster

with young adults with eosinophilic atopic asthma (cluster 5, n = 47) had higher levels of IgE, frequency of allergic rhinitis, high-dose use of ICS, and more frequent asthma exacerbations. Asthma with mixed granulocyte inflammatory pattern (cluster 6, n = 30) had higher levels of IgE, higher frequency of controller

use, and the lowest lung function. This cluster also has poor life quality. Therefore, the characteristics of 3 clusters in males were different from those in females. However, the asthma outcomes were minimally different between male clusters (see [Tables E4-E7](#)).

TABLE E1. The definitions of 4 inflammatory patterns based on different cutoff points in blood

Inflammatory patterns	Definitions
Definition 1	The cutoff points based on Nadif et al ^{E5,E6}
Paucigranulocytic	$<250 \text{ EOS/mm}^3$ and $<5,000 \text{ NEU/mm}^3$
Eosinophil-predominant	$\geq 250 \text{ EOS/mm}^3$ and $<5,000 \text{ NEU/mm}^3$
Neutrophil-predominant	$<250 \text{ EOS/mm}^3$ and $\geq 5,000 \text{ NEU/mm}^3$
Mixed granulocytic	$\geq 250 \text{ EOS/mm}^3$ and $\geq 5,000 \text{ NEU/mm}^3$
Definition 2	The criteria based on 50th (median) percentile
Paucigranulocytic	$<150 \text{ EOS/mm}^3$ and $<4,020 \text{ NEU/mm}^3$
Eosinophil-predominant	$\geq 150 \text{ EOS/mm}^3$ and $<4,020 \text{ NEU/mm}^3$
Neutrophil-predominant	$<150 \text{ EOS/mm}^3$ and $\geq 4,020 \text{ NEU/mm}^3$
Mixed granulocytic	$\geq 150 \text{ EOS/mm}^3$ and $\geq 5,000 \text{ NEU/mm}^3$
Definition 3	The criteria based on 75th percentile
Paucigranulocytic	$<270 \text{ EOS/mm}^3$ and $<5,330 \text{ NEU/mm}^3$
Eosinophil-predominant	$\geq 270 \text{ EOS/mm}^3$ and $<5,330 \text{ NEU/mm}^3$
Neutrophil-predominant	$<270 \text{ EOS/mm}^3$ and $\geq 5,330 \text{ NEU/mm}^3$
Mixed granulocytic	$\geq 270 \text{ EOS/mm}^3$ and $\geq 5,330 \text{ NEU/mm}^3$

EOS, Eosinophils; NEU, neutrophils.

TABLE E2. List of the 29 variables used in the factor analysis

Number	Variable	Subvariable	Definition
1	Demographic characteristics	Age	
2		Age of asthma onset	
3		Early-onset asthma	Onset ≥ 16 y (0); <16 (1)
4		Asthma duration	Age, asthma-onset age
5		BMI	Weight (kg)/height (m^2)
6		Obesity	BMI < 30 kg/ m^2 (0); ≥ 30 kg/ m^2 (1)
7		Body fat	
8		Waistline	
9		WHR	Waist circumference/hip circumference
10	Lung function	Smoking history	None (0); ex-smoker (1); current smoker (2)
11		FEV ₁ , % predicted	Prebronchodilator spirometry and assessed for the highest values at least 3 times
12		FVC, % predicted	
13	Atopy status	FEV ₁ /FVC ratio	
14		Total IgE	
15		Allergic rhinitis	None (0); Yes (1)
16		Atopic dermatitis	None (0); Yes (1)
17	Blood sample	WBC count	
18		Neutrophil %	
19		Eosinophil %	
20		Monocyte %	
21		Lymphocyte %	
22		Basophil %	
23	Asthma symptoms	Breathlessness (wk)	None (0); <1 time (1); ≥ 1 time (2)
24		Breathlessness, during sleeping	None (0); <1 time (1); ≥ 1 time (2)
25		Sneezing and nasal congestion	None (0); Yes (1)
26	Medication use	ICS	None (0); low to moderate dose (1); high dose (2)
27		OCS	None (0); Yes (1)
28		Number of controllers	<2 controllers (0); ≥ 2 controllers (1)
29		Antihistamine	None (0); Yes (1)

TABLE E3. Blood granulocyte patterns of subjects in female and male clusters

Characteristic	Females			<i>P</i> value	Males			<i>P</i> value
	Cluster 1: Late-onset, normal lung function (N = 174)	Cluster 2: Young adults, eosinophilic atopic (N = 117)	Cluster 3: Obese, neutrophilic (N = 130)		Cluster 4: Late-onset, normal lung function (N = 112)	Cluster 5: Current smokers, neutrophilic atopic (N = 116)	Cluster 6: Ex-smokers, eosinophilic (N = 71)	
Blood granulocyte patterns, n (%) [*]								
Mixed granulocyte	5 (2.9)	15 (12.8)	11 (8.5)	<.0001	7 (6.2)	13 (11.2)	8 (11.3)	<.0001
Neutrophil-predominant	35 (20.1)	22 (18.8)	40 (30.8)		14 (12.5)	38 (32.8)	13 (18.3)	
Eosinophil-predominant	21 (12.1)	31 (26.5)	18 (13.8)		19 (17.0)	20 (17.2)	29 (40.8)	
Paucigranulocytic	113 (64.9)	49 (41.9)	61 (46.9)		72 (64.3)	45 (38.8)	21 (29.6)	
Blood granulocyte patterns, n (%) [†]				<.0001				<.0001
Mixed granulocyte	24 (13.8)	35 (29.9)	34 (26.2)		21 (18.8)	36 (31.0)	28 (39.4)	
Neutrophil-predominant	51 (29.3)	22 (18.8)	38 (29.2)		23 (20.5)	38 (32.8)	10 (14.1)	
Eosinophil-predominant	36 (20.7)	41 (35.0)	22 (16.9)		31 (27.7)	25 (21.6)	25 (35.2)	
Paucigranulocytic	63 (36.2)	19 (16.2)	36 (27.7)		37 (33.0)	17 (14.7)	8 (11.3)	
Blood granulocyte patterns, n (%) [‡]				<.0001				<.0001
Mixed granulocyte	3 (1.7)	11 (9.4)	10 (7.7)		5 (4.5)	7 (6.0)	6 (8.5)	
Neutrophil-predominant	28 (16.1)	17 (14.5)	37 (28.5)		10 (8.9)	34 (29.3)	12 (16.9)	
Eosinophil-predominant	21 (12.1)	32 (27.4)	18 (13.8)		18 (16.1)	22 (19.0)	27 (38.0)	
Paucigranulocytic	122 (70.1)	57 (48.7)	65 (50.0)		79 (70.5)	53 (45.7)	26 (36.6)	

Eos, Eosinophils; *Neu*, neutrophils.

^{*}Four blood granulocyte patterns were defined by blood cell counts with ≥ 250 EOS/mm³ and blood cell counts with ≥ 5000 NEU/mm³ as cutoff points.

[†]Four blood granulocyte patterns were classified by 50th percentile (median) of eosinophil (≥ 150 EOS/mm³) and neutrophil (≥ 4020 NEU/mm³) levels in blood.

[‡]Four blood granulocyte patterns were classified by 75th percentile of eosinophil (≥ 270 EOS/mm³) and neutrophil (≥ 5330 NEU/mm³) levels in blood.

TABLE E4. Factor analysis in nonsmoking females and males: Orthogonal varimax rotation

	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5
Female					
BMI*	0.924	−0.077	−0.041	−0.001	0.003
Waistline	0.893	−0.060	−0.086	0.118	−0.060
Body fat	0.872	0.064	−0.006	0.000	−0.036
Blood lymphocytes (%)	0.068	0.969	0.088	0.003	−0.034
Blood neutrophils (%)*	−0.022	−0.949	−0.073	−0.020	−0.188
WBC	0.158	−0.438	0.037	−0.201	−0.135
Pre-FVC (% predicted)*	−0.034	0.036	0.965	0.038	−0.064
Pre-FEV ₁ (% predicted)*	−0.083	0.069	0.960	−0.016	0.041
Asthma-onset age*	0.026	0.068	0.051	0.900	−0.051
Age*	0.110	0.095	−0.034	0.896	0.014
Blood eosinophils (%)*	−0.098	0.120	−0.098	−0.022	0.822
Blood basophils (%)	−0.078	0.186	0.118	0.276	0.652
Total IgE*	0.081	−0.017	−0.018	−0.290	0.515
Male					
BMI*	0.931	−0.032	0.087	−0.064	0.095
Waistline	0.888	0.075	−0.065	0.165	0.031
Body fat	0.836	−0.060	−0.149	0.039	−0.136
Blood lymphocytes (%)	0.110	−0.919	0.042	−0.173	0.022
Blood neutrophils (%)*	−0.035	0.918	−0.014	0.110	−0.274
WBC	0.121	0.735	−0.100	−0.270	−0.046
Pre-FVC (% predicted)*	−0.010	−0.042	0.954	−0.032	−0.026
Pre-FEV ₁ (% predicted)*	−0.106	−0.086	0.935	0.052	−0.141
Age*	0.000	0.067	−0.100	0.894	0.010
Asthma-onset age*	0.135	−0.035	0.118	0.868	0.065
Blood eosinophils (%)*	−0.140	−0.187	−0.110	0.089	0.791
Total IgE*	0.314	0.155	−0.045	−0.254	0.727
Blood basophils (%)	−0.062	−0.218	−0.026	0.161	0.456

WBC, White blood cell.

Bold indicates features that most distinguish one cluster from others.

*Final variables were selected for the cluster analysis.

TABLE E5. Demographic characteristics of subjects in nonsmoking female and male clusters

Characteristic	Females				Males			
	Cluster 1: Late-onset, normal lung function (N = 126)	Cluster 2: Young adults, atopy (N = 132)	Cluster 3: Obese, neutrophilic (N = 143)	P value	Cluster 4: Late-onset, normal lung function (N = 86)	Cluster 5: Young adults, eosinophilic atopic (N = 47)	Cluster 6: Mixed granulocyte, poor lung function (N = 30)	P value
Age (y)	61.21 ± 8.199	39.76 ± 10.05	60.44 ± 10.22	<.0001	61.17 ± 10.633	37.49 ± 9.523	61.57 ± 14.292	<.0001
Age of asthma onset (y)	57.94 ± 10.39	31.68 ± 10.62	52.17 ± 16.02	<.0001	54.92 ± 13.825	28.91 ± 13.395	44.13 ± 23.196	<.0001
Onset <16 y, n (%)	0	12 (9.1)	8 (5.6)	.003	3 (3.5)	12 (25.5)	6 (20.0)	.001
Asthma duration (y)	3.28 ± 7.43	8.08 ± 11.91	8.27 ± 13.64	<.0001	6.26 ± 12.74	8.57 ± 12.685	17.43 ± 21.489	.002
Obese indicators								
BMI	24.83 ± 4.00	24.05 ± 4.20	27.22 ± 5.11	<.0001	25.11 ± 3.24	25.89 ± 4.45	24.18 ± 2.93	.123
BMI ≥ 30 kg/m ² , n (%)	14 (11.1)	11 (8.3)	34 (23.8)	<.0001	6 (7.0)	6 (12.8)	1 (3.3)	.291
Body fat, n (%)	35.98 ± 6.56	33.91 ± 7.46	37.87 ± 8.08	<.0001	23.99 ± 6.17	23.89 ± 5.64	23.80 ± 6.39	.988
Waistline (cm)	83.56 ± 11.47	79.97 ± 11.19	89.13 ± 11.74	<.0001	88.45 ± 8.72	87.81 ± 10.91	90.22 ± 6.91	.520
WHR	0.86 ± 0.08	0.83 ± 0.11	0.89 ± 0.10	<.0001	0.92 ± 0.059	0.89 ± 0.063	0.92 ± 0.055	.005
Lung function								
Pre-FEV ₁ , % predicted	101.09 ± 12.68	89.23 ± 11.58	71.69 ± 14.47	<.0001	96.61 ± 10.17	87.25 ± 11.29	59.03 ± 10.28	<.0001
Pre-FVC, % predicted	106.40 ± 11.35	97.26 ± 10.84	79.88 ± 12.78	<.0001	99.89 ± 10.43	92.43 ± 11.21	71.63 ± 11.41	<.0001
FEV ₁ /FVC ratio	0.79 ± 0.06	0.79 ± 0.08	0.75 ± 0.10	<.0001	0.78 ± 0.08	0.80 ± 0.10	0.66 ± 0.10	<.0001
Atopy status								
Total IgE (IU/mL)*	105.3 ± 259.5	235.5 ± 402.3	170.4 ± 282.3	.006	121.2 ± 167.5	280.7 ± 640.7	358.4 ± 439.1	.002
Comorbidities, n (%)								
Allergic rhinitis	39 (31.0)	63 (47.7)	35 (24.5)	<.0001	35 (40.7)	21 (44.7)	7 (23.3)	.147
Atopic dermatitis	3 (2.4)	11 (8.3)	3 (2.1)	.017	1 (1.2)	4 (8.5)	1 (3.3)	.098
Blood sample inflammatory cells								
WBC count	6.41 ± 1.93	8.25 ± 5.89	7.60 ± 3.23	.001	6.81 ± 2.77	6.96 ± 2.063	8.058 ± 3.93	.115
Neutrophil (%)	55.55 ± 9.58	62.97 ± 10.26	62.58 ± 9.73	<.0001	62.74 ± 11.67	54.46 ± 7.49	64.37 ± 11.49	<.0001
Eosinophil (%)*	2.65 ± 2.36	3.18 ± 2.90	2.84 ± 3.22	.720	3.01 ± 3.56	3.53 ± 2.40	4.34 ± 4.24	.180
Monocyte (%)	5.65 ± 1.79	5.12 ± 1.53	5.20 ± 1.56	.019	5.85 ± 2.23	5.87 ± 2.12	6.09 ± 1.82	.863
Lymphocyte (%)	35.59 ± 9.32	28.06 ± 8.91	28.80 ± 9.33	<.0001	27.72 ± 9.72	35.28 ± 7.55	24.69 ± 10.05	<.0001
Basophil (%)*	0.49 ± 0.37	0.41 ± 0.26	0.42 ± 0.35	.445	0.51 ± 0.82	0.50 ± 0.33	0.48 ± 0.36	.971
Blood granulocyte patterns, n (%)†				<.0001				.021
Mixed granulocyte	2 (1.6)	15 (11.4)	13 (9.1)		1 (1.2)	5 (10.6)	4 (13.3)	
Neutrophil-predominant	15 (11.9)	36 (27.3)	39 (27.3)		19 (22.1)	5 (10.6)	8 (26.7)	
Eosinophil-predominant	18 (14.3)	28 (21.2)	21 (14.7)		17 (19.8)	11 (23.4)	9 (30.0)	
Paucigranulocytic	91 (72.2)	53 (40.2)	70 (49.0)		49 (57.0)	26 (55.3)	9 (30.0)	
Blood granulocyte patterns, n (%)‡				<.0001				<.0001
Mixed granulocyte	18 (14.3)	25 (18.9)	36 (25.2)		11 (12.8)	5 (18.5)	11 (40.7)	
Neutrophil-predominant	21 (16.7)	51 (38.6)	49 (34.3)		35 (40.7)	5 (10.6)	8 (26.7)	
Eosinophil-predominant	46 (36.5)	42 (31.8)	36 (25.2)		25 (29.1)	22 (46.8)	8 (26.7)	
Paucigranulocytic	41 (32.5)	14 (10.6)	22 (15.4)		15 (17.4)	15 (31.9)	3 (10.0)	
Blood granulocyte patterns, n (%)§								

Mixed granulocyte				<.0001			.041
Neutrophil-predominant	1 (0.8)	13 (9.8)	12 (8.4)		1 (1.2)	4 (8.5)	4 (13.3)
Eosinophil-predominant	13 (10.3)	33 (25.0)	35 (24.5)		17 (19.8)	4 (8.5)	6 (20.0)
Paucigranulocytic	19 (15.1)	30 (22.7)	22 (15.4)		17 (19.8)	12 (25.5)	9 (30.0)

BMI, Body mass index; *FEV₁*, forced expiratory volume in 1 second; *FVC*, forced vital capacity; *IgE*, immunoglobulin E; *WBC*, white blood cell; *WHR*, waist to hip ratio.

Vales presented as mean $\pm$ SD for continuous variables and n (%) for categorical variables.

*IgE levels were logarithmically transformed before statistical testing.

†Four blood granulocyte patterns were defined by blood cell counts of ≥ 250 EOS/mm³ and blood cell counts of ≥ 5000 NEU/mm³ as cut- off points.

‡Four blood granulocyte patterns were classified by 50th percentile (median) of eosinophil and neutrophil levels in blood.

§Four blood granulocyte patterns were classified by 75th percentile of eosinophils and neutrophil levels in blood.

TABLE E6. Asthma symptoms and medication of subjects in nonsmoking female and male clusters

Characteristic	Females			P value	Males			P value
	Cluster 1: Late-onset, normal lung function (N = 126)	Cluster 2: Young adults, atopy (N = 132)	Cluster 3: Obese, neutrophilic (N = 143)		Cluster 4: Late-onset, normal lung function (N = 86)	Cluster 5: Young adults, eosinophilic atopic (N = 47)	Cluster 6: Mixed granulocyte, poor lung function (N = 30)	
Frequency of asthma symptoms, past year								
Breathlessness, n (%)				.021				.480
No	45 (35.7)	33 (25.0)	34 (23.8)		27 (31.4)	13 (27.7)	7 (23.3)	
<1 time/wk	49 (38.9)	67 (50.8)	84 (58.7)		38 (44.2)	23 (48.9)	19 (63.3)	
≥1 time/wk	32 (25.4)	32 (24.2)	25 (17.5)		21 (24.4)	11 (23.4)	4 (13.3)	
Breathlessness, during sleeping, n (%)				.155				.288
No	72 (57.1)	61 (46.2)	72 (50.7)		64 (74.4)	33 (70.2)	19 (63.3)	
<1 time/wk	23 (18.3)	36 (27.3)	42 (29.6)		12 (14.0)	7 (14.9)	9 (30)	
≥1 time/wk	31 (24.6)	35 (26.5)	28 (19.7)		10 (11.6)	7 (14.9)	2 (6.7)	
Sneezing and nasal congestion, n (%)				.156				.218
No	65 (51.6)	52 (39.7)	67 (46.9)		38 (44.2)	17 (36.2)	8 (26.7)	
Yes	61 (48.4)	79 (60.3)	76 (53.1)		48 (55.8)	30 (63.8)	22 (73.3)	
ICS, n (%)				.058				.634
None	31 (24.6)	40 (30.3)	27 (18.9)		19 (22.1)	6 (12.8)	7 (23.2)	
Low-to-moderate-dose ICS	49 (38.9)	61 (46.2)	71 (49.7)		37 (43.0)	21 (44.7)	14 (46.7)	
High-dose ICS	46 (36.5)	31 (23.5)	45 (31.5)		30 (34.9)	20 (42.6)	9 (30.0)	
OCS, n (%)	16 (12.7)	24 (18.2)	23 (16.2)	.474	10 (11.6)	7 (14.9)	4 (13.3)	.863
Controllers, n (%)								
≥2 controllers	30 (23.8)	23 (17.4)	41 (28.7)	.088	25 (15.3)	12 (25.5)	11 (36.7)	.575
Antihistamine, n (%)	50 (39.7)	71 (53.8)	49 (34.5)	.004	26 (30.2)	20 (42.6)	5 (16.7)	.055

All values presented as n (%) for categorical variables.

The high-dose ICS is equivalent to >800 µg/d for budesonide and >500 µg/d for fluticasone.

Controllers include ICSs, OCSs, theophylline, and leukotrienes.

TABLE E7. Asthma control–related outcomes in nonsmoking female and male clusters

Characteristic	Females			<i>P</i> value	Males			<i>P</i> value
	Cluster 1: Late-onset, normal lung function (N = 126)	Cluster 2: Young adults, atopy (N = 132)	Cluster 3: Obese, Neutrophilic (N = 143)		Cluster 4: Late-onset, normal lung function (N = 86)	Cluster 5: Young adults, eosinophilic atopic (N = 47)	Cluster 6: Mixed granulocyte, poor lung function (N = 30)	
Asthma exacerbations, n (%)	7 (5.6)	17 (12.9)	16 (11.2)	.122	4 (4.7)	3 (6.4)	4 (3.6)	.262
TAQLQ	49.78 ± 17.18	54.20 ± 16.60	49.41 ± 17.52	.040	44.69 ± 14.16	45.55 ± 13.52	47.47 ± 15.15	.331
ACT	21.15 ± 3.13	20.56 ± 3.46	21.43 ± 2.82	.066	21.77 ± 2.37	21.16 ± 2.04	21.80 ± 3.16	.534

Asthma exacerbations were defined as at least 1 hospitalization or emergency department visit during the past year.

ACT was used for assessing asthma control during the past 4 wk; TAQLQ was used for assessing quality of life of asthma patients during the past 2 wk.

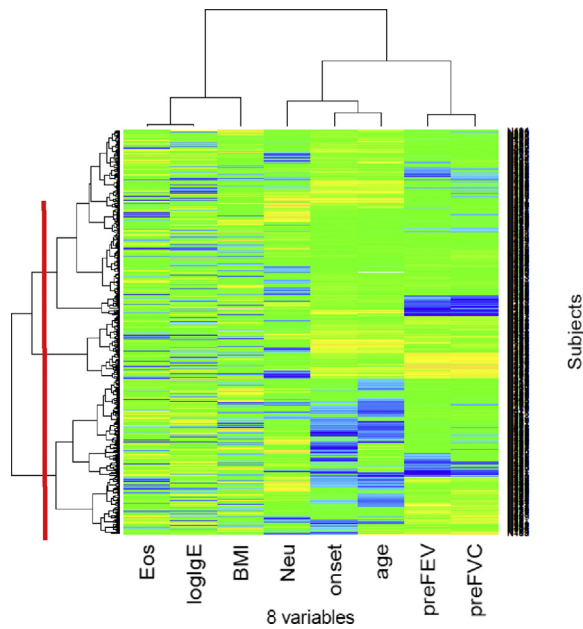


FIGURE E1. Cluster heatmap with dendrogram in female asthmatic patients. *Eos*, Eosinophils; *Neu*, neutrophils.

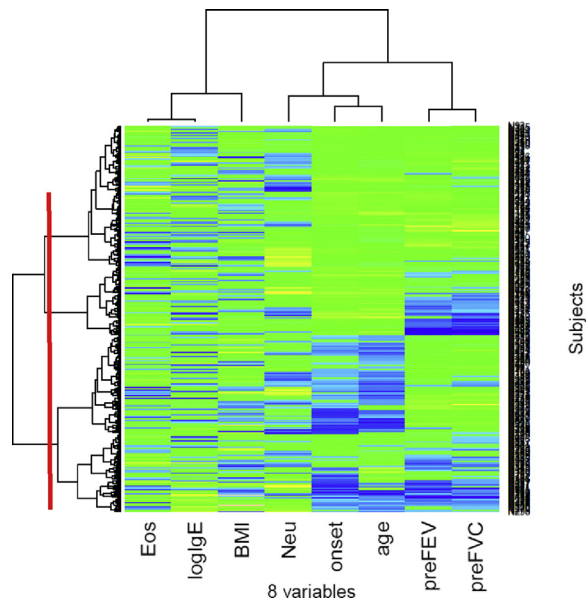
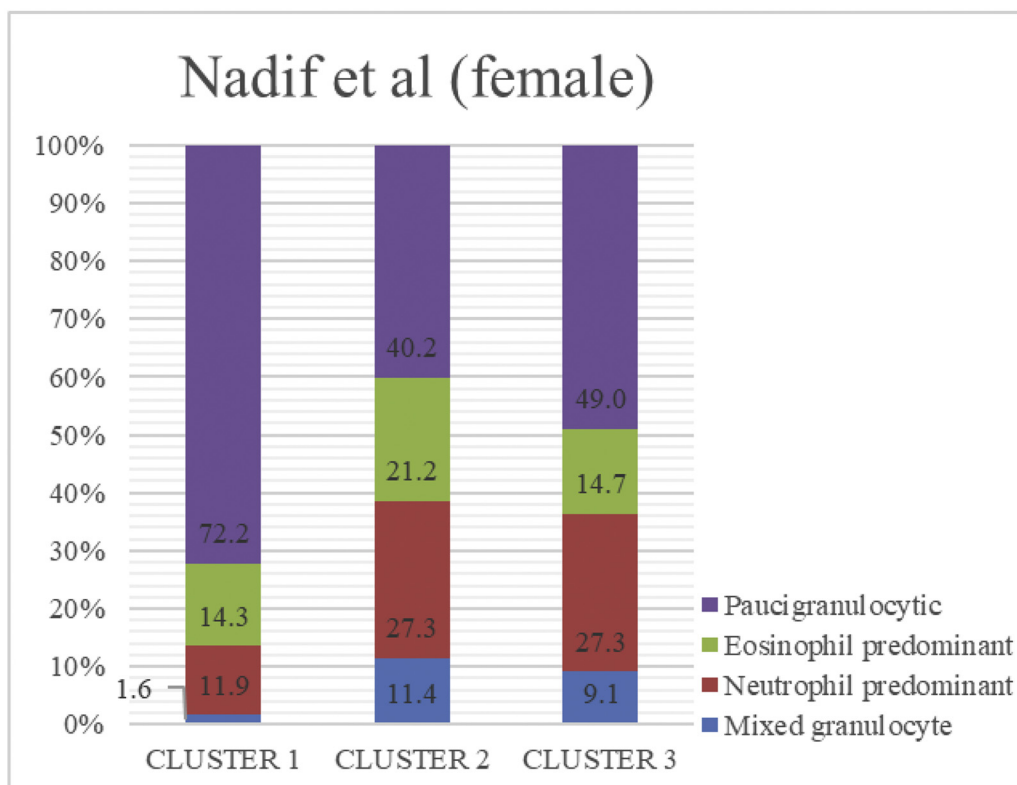


FIGURE E2. Cluster heatmap with dendrogram in male asthmatic patients. *Eos*, Eosinophils; *Neu*, neutrophils.

A



B

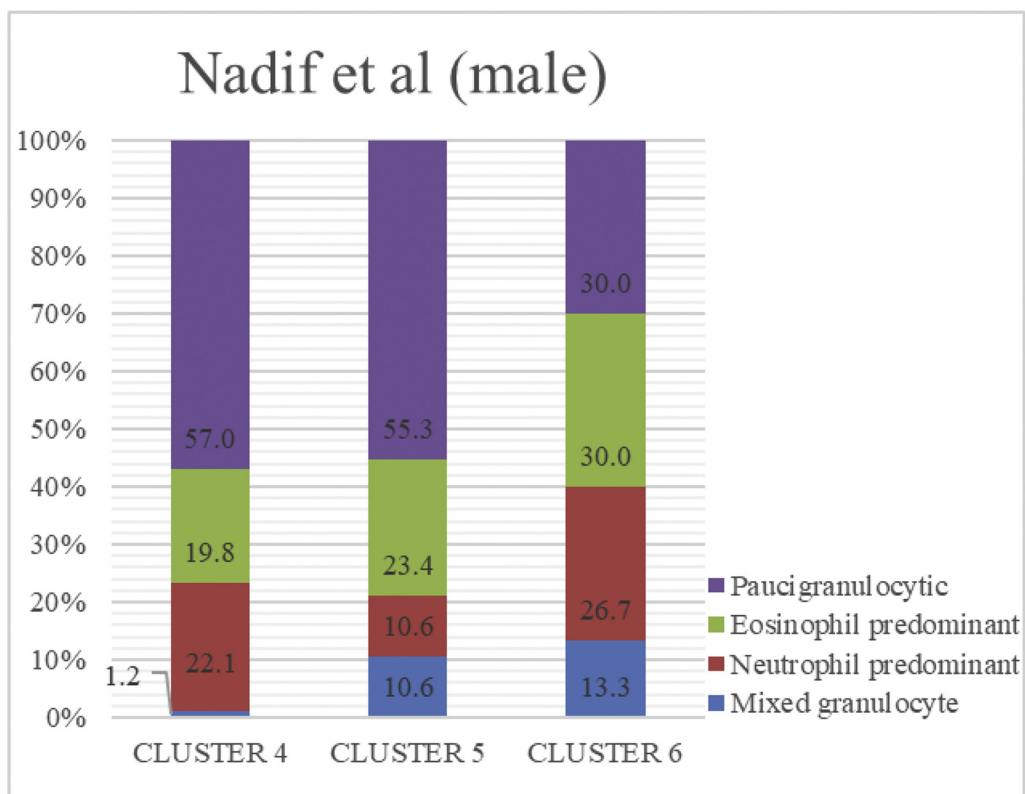


FIGURE E3. A and B, Blood granulocyte patterns in 3 female and male clusters, respectively. Subjects were assigned to 1 of 4 possible patterns based on blood eosinophil percentages ($<250 \text{ EOS/mm}^3$ or $\geq 250 \text{ EOS/mm}^3$) and blood neutrophil percentages ($<5000 \text{ NEU/mm}^3$ or $\geq 5000 \text{ NEU/mm}^3$). *Eos*, Eosinophils; *Neu*, neutrophils.

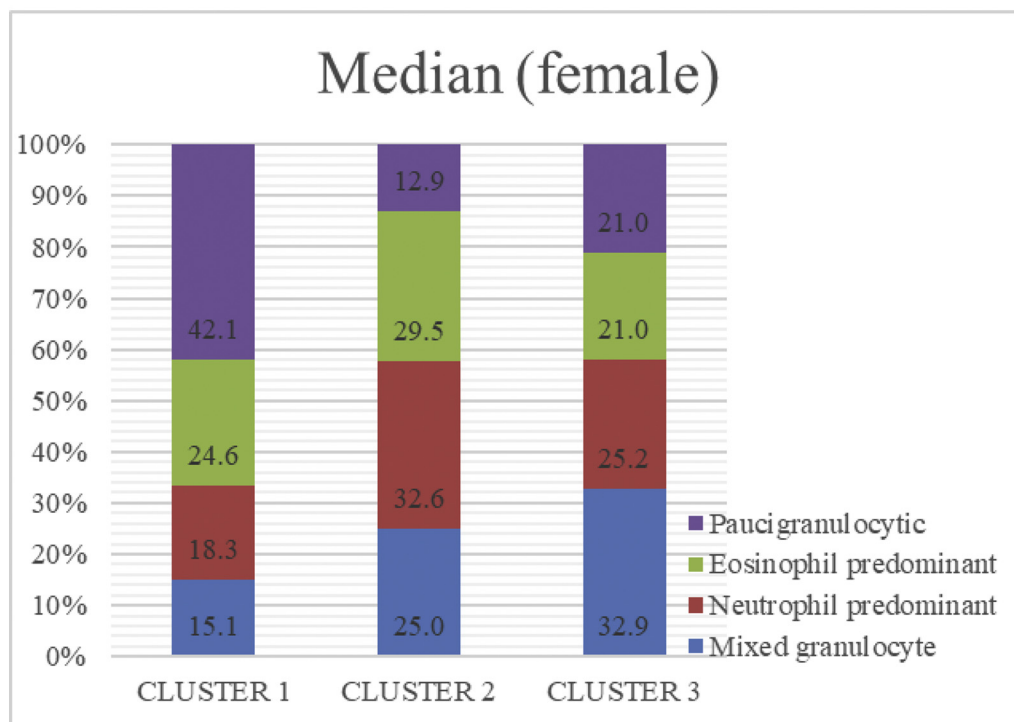
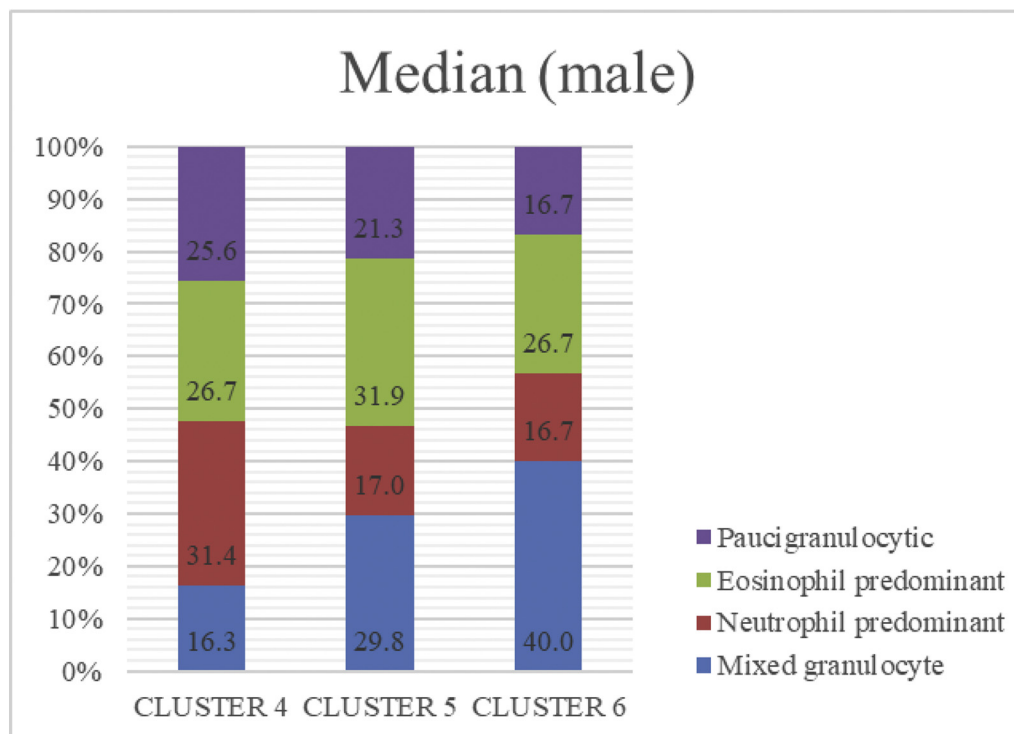
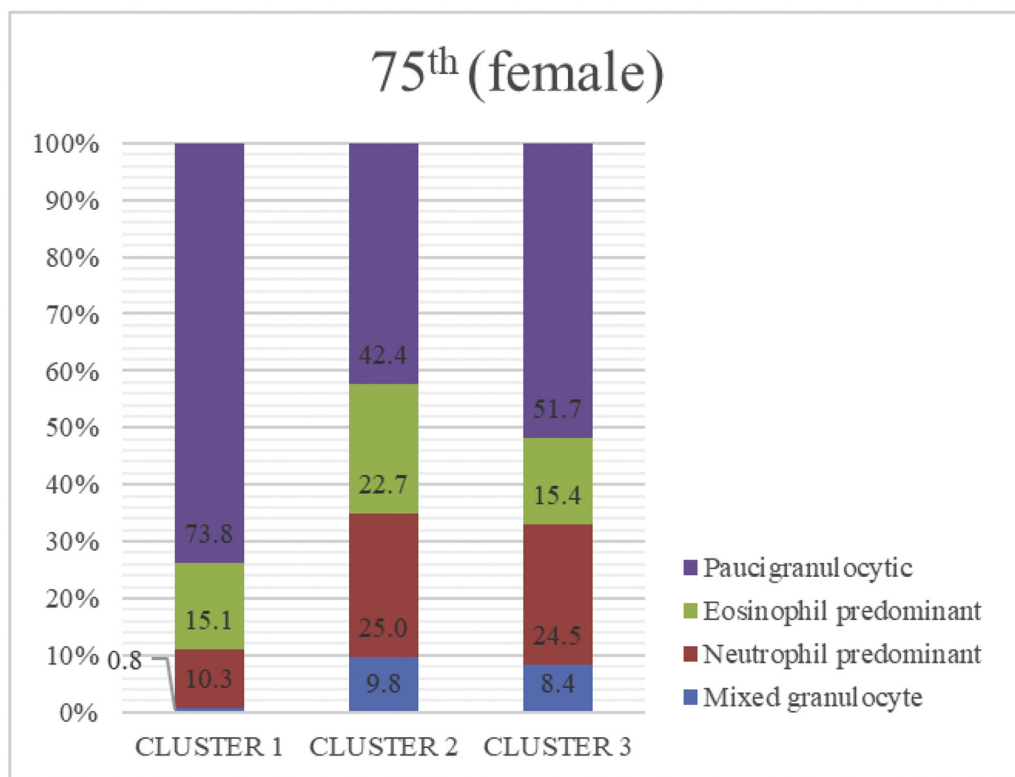
A**B**

FIGURE E4. A and B, Blood granulocyte patterns in 3 female and male clusters. Subjects were assigned to 1 of 4 possible patterns based on 50th percentile of blood eosinophil ($<150 \text{ EOS/mm}^3$ or $\geq 150 \text{ EOS/mm}^3$) and blood neutrophil ($<4020 \text{ NEU/mm}^3$ or $\geq 4020 \text{ NEU/mm}^3$). *Eos*, Eosinophils; *Neu*, neutrophils.

A



B



FIGURE E5. A and B, Blood granulocyte patterns in 3 female and male clusters. Subjects were assigned to 1 of 4 possible patterns based on 75th percentile of blood eosinophil (<270 EOS/mm³ or ≥ 270 EOS/mm³) and blood neutrophil (<5330 NEU/mm³ or ≥ 5330 NEU/mm³). *Eos*, Eosinophils; *Neu*, neutrophils.

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