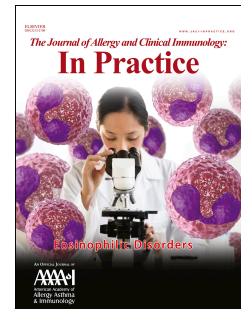


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Clinical phenotypes of patients hospitalized for an asthma exacerbation: prognostic implications

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ABSTRACT

Background: Hospitalization due to acute asthma exacerbation (AE) is a highly detrimental situation requiring critical management to prevent further deterioration, including mechanical ventilation, intensive care unit (ICU) admission, and death. However, patients hospitalized for AEs are highly heterogeneous and remain largely unexplored.

Objective: Identify clinical and inflammatory phenotypes of AE requiring hospitalization associated with in-hospital outcomes.

Methods: We performed a hierarchical cluster analysis of 825 consecutively recruited patients hospitalized for AEs. Logistic regressions were conducted to quantify the independent associations of the identified phenotypes with in-hospital outcomes. Decision tree analysis was developed to predict cluster assignment.

Results: We identified three clusters of patients, which had significantly different characteristics associated with in-hospital adverse outcomes. Cluster 1 (n=526, 63.8%) was a late-onset phenotype, cluster 2 (n=97, 11.8%) was an early-onset phenotype, and cluster 3 (n=202, 24.5%) was a phenotype with fewer eosinophils and more comorbidities. Clusters 2 and 3 had an elevated risk of death (RR_{adj} 18.10 and 19.17, respectively) and mechanical ventilation (RR_{adj} 2.56 and 5.71, respectively) than did cluster 1. Individuals in cluster 3 had an extended length of hospital stay (11 days), increased hospitalization direct costs (13,481.57 Chinese Yuan[CNY]), and a higher risk of ICU admission (RR_{adj} 2.14) than individuals in clusters 1 and 2. The decision tree assigned 90.8% of the participants correctly.

Conclusions: We identified three phenotypes with differential clinical and inflammatory characteristics associated with in-hospital adverse outcomes. These new phenotypes might have important and clinically relevant implications for the management of patients hospitalized for AEs.

Highlights box:

What is already known about this topic?

Existing studies have identified several distinct phenotypes of stable asthma, but further understanding of the heterogeneity of asthma exacerbation (AE) associated with in-hospital outcomes is necessary, which may inform individualized management in clinical practice.

What does this article add to our knowledge?

Patients with AE, immediately upon hospital admission, were grouped into three phenotypes with differential clinical and inflammatory characteristics, which were clinically relevant to disease progression and in-hospital adverse outcomes.

How does this study impact current management guidelines?

Our results indicated that the identified phenotypes and decision tree diagram have important and clinically relevant implications for the clinical practice and individualized management of patients hospitalized for AEs.

Key words: asthma, exacerbations, phenotypes, cluster analysis

Abbreviations list

AEs: asthma exacerbations

ALB: albumin

ANOVA: analysis of variance

BMI: body mass index

CCI: Charlson comorbidity index

CHOL: cholesterol

CNY: Chinese Yuan

COPD: chronic obstructive pulmonary disease

CRP: C-reactive protein

FEV₁: forced expiratory volume in 1 second

GINA: Global Initiative for Asthma

- 117 GLB: globulin
118 HDL-C: high density lipoprotein cholesterol
119 ICU: intensive care unit
120 IRB: Institutional Review Board
121 IL: interleukin
122 IQR: interquartile range
123 LDL-C: low-density lipoprotein cholesterol
124 LOS: length of stay
125 LSD: Least—Significant Difference
126 N/E: a ratio of blood neutrophils/eosinophils
127 OCS: oral corticosteroids
128 PaCO₂: partial pressure of carbon dioxide
129 PaO₂: partial pressure of oxygen
130 PEF: peak expiratory flow
131 PCT: procalcitonin
132 pMDI, pressure metered dose inhaler
133 Q1: first quartile
134 Q3: third quartile
135 RR: relative ratio
136 RR_{adj}: relative ratio after adjusting for confounders
137 SE: standard error
138 SABA: short-acting β_2 agonists
139 SAMA: short-acting muscarinic antagonists
140 SARP: Severe Asthma Research Program
141 sIgE: specific IgE
142 SPT: skin prick tests
143 TP: total protein
144 TG: triglyceride
145
146

INTRODUCTION

Assessment of asthma should incorporate measures of both current control and long-term or future risk, including asthma exacerbation (AE). In a real-world setting, exacerbations are recognized as acute episodes that cause loss of control and prompt a need for change in treatment^{1, 2}. There have been important advances in asthma management, including the publication of guidelines incorporating evidence-based recommendations for the assessment and management of AEs^{3, 4}. However, AEs remain a serious treatment challenge. They are a frequent cause of hospitalization or emergency room admission presentation⁵. Thus, AEs impose considerable suffering and patient burden and constitute a major and an additional burden on health care resources⁵. In 2016, the Global Burden of Disease collaboration estimated that there were approximately 420,000 deaths globally due to asthma⁶. Hence, AEs, particularly severe episodes, constitute a substantial ongoing cause for concern, and understanding of acute exacerbations in asthma is a major priority.

Asthma is a heterogeneous airway disease. In recent years, there has been an increasing interest in the heterogeneity and phenotyping of asthma using cluster analysis in different asthma populations⁷. Cross-sectional cluster studies identified subsets of patients sharing clinical characteristics, and several studies involving cluster analysis of stable asthma have been published. For example, Haldar *et al.* first identified three clusters in patients with asthma⁸. Moore *et al.* found that baseline and maximum achievable lung function and age at onset of the disease explained approximately 80% of the discrepancies between five clusters identified in the Severe Asthma Research Program (SARP) study⁹. Furthermore, Bourdin *et al.* conducted a prospective longitudinal cohort study from the SARP using cluster analysis¹⁰. These studies have significantly advanced knowledge of stable asthma and have important implications for clinical practice.

Although several clusters and risk factors associated with AEs have been identified by cluster analysis, there are few studies that have utilized cluster analysis

to identify the phenotypic profiles of patients hospitalized for AEs¹¹. Sekiya *et al.* examined the clinical characteristics and heterogeneity of patients with severe or life-threatening AE by cluster analysis using responses to structured questionnaires, without including objective indicators (e.g., inflammatory biomarkers) and exploring important in-hospital outcomes (e.g., death, mechanical ventilation)¹². Tanaka *et al.* performed a secondary analysis of Sekiya *et al.* 's data, using cluster analysis, to explore the patterns of factors preceding AEs in a real-world setting^{11, 12}. Recently, Qiu *et al.* obtained parameters from patients recovering from an AE during hospitalization and identified five phenotypes¹³.

To the best of our knowledge, few studies have grouped patients with an AE into different phenotypes immediately at hospital admission, and then validated those identified phenotypes associated with disease progression and risk of in-hospital adverse outcomes assessed for index hospitalization. Investigation of the heterogeneity of AE would help identify patients at increased risk of serious asthma-related adverse outcomes during hospitalization such as the need for mechanical ventilation, intensive care unit (ICU) admission, prolonged length of stay (LOS), death, and economic burden. Furthermore, recognizing heterogeneity among patients with AEs is essential to guide management and determine priorities for critical care, particularly with respect to potentially modifiable elements or “treatable traits” that impact adverse asthma-related outcomes^{14, 15}.

We hypothesized that groups of patients with asthma hospitalized for AEs would be defined based on sociodemographic, clinical, and inflammatory characteristics, and categorized based on their differences (or similarities) into clusters. The identified clusters would be associated with in-hospital outcomes assessed for index hospitalization, which may have potential implications for individualized management in clinical practice.

METHODS

Study design and patients

Adult participants (≥ 18 years) with a diagnosis of asthma and an unplanned hospitalization for an acute AE were prospectively and consecutively recruited from the inpatient department at the West China Hospital Center, Sichuan University, from January 2009 to April 2014. Asthma was determined by physicians based on diagnostic features of a history of both variable respiratory symptoms and variable airflow obstruction, which had been demonstrated in a stable phase before admission. Variable airflow obstruction was confirmed by evidence of either airway hyperresponsiveness or bronchodilator responsiveness with a more than 12% and 200 mL increase in forced expiratory volume in one second (FEV₁) from the baseline³. An initial cross-sectional assessment was conducted among consecutively enrolled patients to identify clusters or ‘phenotypes’ based on clinical and demographic features. These participants were prospectively observed until discharge or death, which was designed to explore these identified clusters in relation to asthma-related events assessed for index hospitalization. Treatments received by patients during the study were determined by their treating physicians. The study was reviewed and approved by the Institutional Review Board at West China Hospital, Sichuan University (Chengdu, China) (No. 425). All participants provided written informed consent prior to participation. Additional details on study design and patients are provided in the Online Repository Text.

Data collection and clinical assessments at baseline

The patients underwent a standardized assessment within 48 hours after admission that collected information regarding demographic characteristics, body mass index (BMI), smoking status, duration of asthma, and prehospital controller medications in the stable phase. The severity of the AE was classified in accordance with the proposed algorithm for management of AEs based on the Global Initiative for Asthma (GINA) guidelines³ (Figure E1, Table E4). Atopic status was identified

by either positive skin prick tests (SPT) or specific IgE (sIgE) level >0.35 kU/L in serum before admission when the participants were in the stable phase¹⁶. Systemic corticosteroid use in our study was defined and classified into two categories: 1) maintenance oral corticosteroids (OCS) (prehospital controller medication in the stable phase of asthma), or 2) rescue systemic corticosteroids (OCS or intravenous corticosteroid use for exacerbation within 7 days before hospital admission). Additional details on data collection and clinical assessments are provided in the Online Repository Text and Table E1.

Inflammatory biomarkers and laboratory testing

Arterial blood gas analysis, blood counts, immunology and inflammation tests, biochemistry, and sputum culture were performed immediately upon hospital admission. More details on the methods for these measurements are provided in the Online Repository Text.

Management of asthma exacerbations in hospitalized patients

As this was a “real-world” study, management was determined by the patients’ consulting physicians based on the GINA recommendations³ (Figure E1) . All included participants underwent a brief history, physical examination, and systematic assessments as recommended by the GINA guidelines³ (Table E3 and E4) . More details about the management of AEs are described in the Online Repository Text.

Primary and secondary in-hospital outcomes

To explore the associations of the identified clusters with the outcomes that occurred within the in-hospital period, all participants were followed up until discharge or death. The primary and secondary outcomes were restricted to in-hospital events. In particular, the primary outcomes were death, hospital LOS, and total direct hospitalization costs. The secondary outcomes included systemic

corticosteroid exposure, noninvasive and invasive mechanical ventilation, ICU admission, and length of ICU stay.

Cluster and statistical analyses

Additional details on the process of variable selection and statistical analysis are provided in the Online Repository Text. In total, 150 distinct variables were collected (Figure 1). Variables with missing data for >10% of participants (n=26) were excluded. For the remaining 124 variables, missing data were imputed using the multiple imputation method (multilevel and generalized linear regression)¹³. An exploratory factor analysis¹⁷, for continuous variables, or a multiple correspondence analysis¹⁸, for categorical variables, was performed on the remaining 42 variables to identify those variables that should be included in the cluster analysis. Finally, the following 11 variables were included in the cluster analysis: age, sex, age at asthma onset, atopic status, pack years (the number of cigarettes smoked per day×years of smoking), prehospital rescue OCS use for exacerbation, partial pressure of oxygen (PaCO₂; mmHg), neutrophils and blood eosinophils (×10⁹/L), and c-reactive protein (CRP; mg/L) and serum albumin (ALB; g/L).

Cluster analysis was conducted by applying a two-step process. First, Ward's minimum-variance hierarchical clustering method was performed using an agglomerative approach and Ward's linkage¹⁹. Selected variables to construct the cluster model were standardized using z-scores. In recognition that clustering is dependent on user-defined inputs, we used an iterative approach over a range of clusters (3 clusters up to a maximum of 5 clusters) to define the optimal number of clusters to characterize the data. Calinski and Harabasz's pseudo-F index was calculated and maximized to obtain the optimum number of clusters¹⁸⁻²⁰. Then, K-means analysis was carried out using the prespecified number of clusters²¹. A decision tree algorithm was applied to all variables used in the cluster analysis to predict the cluster for each subject, and misclassification rates were calculated^{22, 23}.

Categorical variables are summarized as frequencies and proportions. Continuous variables were tested for Gaussian distribution (Shapiro-Wilk test) and are expressed as mean and standard deviation or median and interquartile range (IQR). The difference between clusters for each variable was evaluated using analysis of variance (ANOVA) or Kruskal–Wallis test for continuous variables and the Chi-square test or Fisher’s exact test for categorical variables, as appropriate. Unless otherwise specified, when we found differences among clusters, the Bonferroni correction was used to explore whether the medians of the clusters differed significantly from each other.

Subgroup analyses for primary and secondary in-hospital outcomes were performed, excluding the participants who did not receive the treatment algorithm indicated by the asthma guidelines. Furthermore, we used logistic regression models to evaluate the correlation between identified asthma phenotypes and in-hospital adverse outcomes before and after adjusting for BMI and Charlson comorbidity index (CCI). In addition, logistic regression models were performed to identify important risk factors related to adverse in-hospital outcomes, which were adjusted for age, sex, prehospital maintenance or rescue corticosteroid use, smoking, atopic status, BMI, and CCI.

Two-tailed P values <0.05 were considered statistically significant. Statistical analysis was performed using SPSS (Version 23.0, IBM Corp., Armonk, NY, USA).

RESULTS

Subject characteristics

Between January 2009 and April 2014, 874 participants were consecutively admitted to the hospital, of whom 825 were included in the cross-sectional study after excluding those for whom the asthma diagnosis was incorrect ($n=23$), or who were transferred to ICU ($n=5$), died ($n=1$), were discharged from the hospital within 48 hours of admission ($n=6$), or had incomplete clinical data ($n=14$) (Figure 1). All recruited patients were followed up until death or discharge, regardless of whether

they received treatment in accordance with the GINA and Chinese asthma guideline³.
²⁴. Some patients had contraindications to the use of systemic corticosteroids, such
 as epilepsy (n=1, 0.12%), active peptic ulcer (n=2, 0.24%), or gastrointestinal
 bleeding (n=2, 0.24%), but all of these patients received nebulized corticosteroids
 plus short-acting β_2 agonists (SABA) and/or short-acting muscarinic antagonists
 (SAMA) for acute asthma. The demographic and clinical characteristics of the
 included participants are shown in Table E2.

Cluster profile

According to Calinski and Harabasz's pseudo-F index, three was the optimum
 number of clusters²⁰. Thus, three distinct phenotypic clusters were identified (Table 1).
 Using stepwise discriminant analysis, the influential variables (i.e., the important
 predictors in clustering) for cluster assignment were early-onset asthma (F=17671.192,
 $P<0.001$), prehospital rescue OCS use for exacerbation (F=542.143, $P<0.001$), CRP
 level (F=56.466, $P<0.001$), blood neutrophils (F=36.078, $P<0.001$), partial pressure of
 oxygen (PaO₂; F=10.852, $P<0.001$), age (F=9.039, $P<0.001$), and blood eosinophils
 (F=7.690, $P<0.001$). The other variables, including pack years (F=1.886, $P=0.152$),
 sex (F=1.600, $P=0.203$), and atopic status (F=0.200, $P=0.818$) did not contribute
 significantly to cluster assignment. The three clusters with distinct characteristics
 were identified as follows:

Cluster 1: Late-onset asthma

Cluster 1 was characterized by late-onset asthma (n=526) and had the greatest
 number of patients (63.8%) (Table 1). Participants in this cluster had a higher blood
 eosinophil count (0.11 [0.01, 0.32] $\times 10^9/L$) than those in cluster 3 (0.02 [0, 0.20]
 $\times 10^9/L$; $P<0.001$) (Table 2).

Cluster 2: Early-onset asthma

This cluster had the fewest number of patients (n=97, 11.8%) (Table 1). A
 greater proportion of patients in cluster 2, than in clusters 1 and 3, had early-onset
 asthma (100% vs. 0% vs. 1.0%, $P<0.001$) and a family history of asthma (20.6% vs.
 9.9% vs. 10.4%, $P=0.008$). The participants in this cluster had the longest duration of

asthma (40.0 [30.0 50.0] years, $P<0.001$), and more eosinophils ($0.10 [0.01, 0.28] \times 10^9/L$) and basophils ($0.02 [0.01, 0.03] \times 10^9/L$) than those in cluster 3 ($0.02 [0, 0.20] \times 10^9/L$; $0.02 [0.01, 0.02] \times 10^9/L$, respectively) ($P<0.017$) (Table 2). Furthermore, they had increased levels of CRP ($11.10 [1.26, 32.60]$ mg/L) and interleukin(IL)-6 ($9.44 [7.08, 21.38]$ ng/L) than did those in cluster 1 ($2.83 [0.50, 11.88]$ mg/L and $3.68 [1.74, 8.66]$ ng/L, respectively) (all $P<0.017$) (Table 2).

Cluster 3: Phenotype with fewer eosinophils and more comorbidities

Cluster 3 included 202 (24.5%) patients and was characterized by older age, a high CCI, relatively more intense maintenance medications for anti-asthma drug use (inhaled corticosteroid [ICS]/long- acting β_2 agonist [LABA], anticholinergic drugs, and theophylline) in the stable phase, and severe or life-threatening exacerbations (55.5%) (Table 1). Furthermore, compared with cluster 1, cluster 3 had a greater proportion of patients who used prehospital maintenance OCS (6.4%) and rescue OCS (74.3%) ($P<0.001$). In addition, patients in cluster 3 had elevated neutrophils ($7.28 [4.20, 10.99] \times 10^9/L$), CRP ($12.95 [2.10, 46.48]$ mg/L), and IL-6 levels ($9.36 [4.33, 17.89]$ ng/L), but a lower blood eosinophil count ($0.02 [0, 0.20] \times 10^9/L$), than those in cluster 1 (all $P<0.017$). Intriguingly, cluster 3 had distinctly increased high density lipoprotein cholesterol (HDL-C) levels ($1.56 [1.26, 1.91]$ mmol/L) when compared with cluster 1 ($1.43 [1.13, 1.74]$ mmol/L) ($P=0.001$). We found that there was a greater proportion of patients with a positive bacterial sputum culture in cluster 3 (39.1%), than in cluster 1 (28.3%) and cluster 2 (22.7%) ($P=0.004$). Additionally, a higher proportion of patients with a positive fungus sputum culture was identified in cluster 3 (20.3%) than in cluster 1 (10.3%) ($P<0.001$). In our study, more patients in cluster 3 (14.4%) were found to have high levels of procalcitonin (PCT) (≥ 0.05 ng/mL) than in cluster 1 (6.5%) ($P<0.005$) (Table 2).

In-hospital outcomes of prespecified clusters

Patients in clusters 2 and 3 had an increased risk of in-hospital death than patients in cluster 1 (3.1% vs. 4.0% vs. 0.2%, $P<0.001$) (Table 3, Figure 2). Furthermore, there was an extended length of hospital stay for patients in cluster 3

than those in clusters 1 and 2 (11 [8, 15] vs. 10 [7, 13] vs. 9 [6, 13] days, $P=0.003$). Compared with patients in clusters 1 (9402.33 [6328.90, 13649.68] Chinese Yuan [CNY]) and 2 (9016.16 [4881.80, 15003.16] CNY), patients in cluster 3 (13481.57 [7740.75, 26123.88] CNY) had higher direct costs for hospitalization ($P<0.001$) (Table 3, Figure 2).

Patients in clusters 1 and 3 received a higher cumulative dosage of systemic corticosteroids within the first 7 days than those in cluster 2 (320 [280.0, 480.0] vs. 332.0 [280.0, 480.0] vs. 280.0 [280.0, 386.7] mg, $P=0.003$) (Figure E2). The pattern of daily systemic corticosteroid dose over time was assessed using log-transformed means of daily systemic corticosteroid use after admission in each cluster, and showed an underlying quadratic trend ($P<0.001$), indicating that a significant drop in systemic steroid dosage was observed on day 4 after admission in all clusters (Figure E2). Moreover, the incidence of requirement for mechanical ventilation was higher in clusters 2 and 3 than in cluster 1 (11.3% vs. 23.8% vs. 4.9%, $P<0.001$). In addition, there was a greater proportion of patients in cluster 3 than in clusters 1 and 2 who experienced noninvasive ventilation (18.3% vs. 4.0% vs. 7.2%, $P<0.001$) and ICU admission (18.8% vs. 9.9% vs. 8.2%, $P=0.002$). This resulted in a distinctly extended stay in the ICU for individuals in cluster 3 than in the other clusters (cluster 3: 80.0 [19.3, 209.3] vs. cluster 1: 21.0 [11.0, 61.5] vs. cluster 2: 36.5 [5.3, 97.5] hours, $P<0.05$) (Table 3, Figure 2). When the patients who did not receive the treatment in accordance with the asthma guidelines were excluded, the subgroup analysis indicated that the primary and secondary in-hospital outcomes across these three clusters remained the same (Table E5).

Logistic regression analyses

We further utilized multivariable logistic regression models to analyze the risk of death, ICU admission, and mechanical ventilation among the identified clusters (Figure E3). After adjusting for BMI and CCI, similar results were obtained (Figure 3). Compared with cluster 1, cluster 2 (Relative ratio_{adj} [RR_{adj}] [95% CI], 18.10 [1.85, 176.66]) and cluster 3 (19.17 [2.37, 155.33]) were at a significantly increased risk of

in-hospital death. Moreover, the patients in cluster 2 (RR_{adj} [95% CI], 2.56 [1.21, 5.38]) and cluster 3 (5.71 [3.42, 9.54]) had a significantly increased risk of mechanical ventilation during hospitalization. Furthermore, cluster 3 had a higher risk of ICU admission (RR_{adj} [95% CI], 2.14 [1.35, 3.38]), noninvasive ventilation (4.91 [2.77, 8.69]), and invasive ventilation (6.40 [3.13, 13.08]) than did cluster 1.

We further explored the risk factors of adverse in-hospital outcomes related to AEs (Online Repository Text, Tables E6 and E7).

Decision tree analysis

We developed a tree diagram to predict cluster assignment of patients (Figure 4). Using only two variables, prehospital rescue use of OCS for exacerbation and age of onset (cut at age 12), 90.8% of patients could be assigned to the correct cluster. Accuracy of classification in the discriminant analysis was not improved if all 11 variables used in cluster analysis were included.

DISCUSSION

To the best of our knowledge, this is the first study that used cluster analysis to group patients with an AE into different phenotypes immediately at hospital admission, and further validated the identified phenotypes associated with disease progression and in-hospital adverse outcomes assessed for index hospitalization. Here, we identified three phenotypes in patients with AEs in a real-world setting using cluster analysis: “late-onset,” “early-onset,” and a phenotype of “fewer eosinophils and increased comorbidities.” Of these three, the “fewer eosinophils and increased comorbidities” phenotype was significantly associated with worse in-hospital outcomes.

Although Qiu *et al.* recently studied the heterogeneity of AEs requiring hospitalization, that study was significantly different from our current study¹³. The patients in Qiu *et al.*’s study were assessed from days 3 to 10 after hospital admission, when patients were no longer in the acute phase of an AE, and had moved to the recovery phase. Further, they excluded patients with “real-life” comorbidities such as

asthma-COPD overlap. Our study had relatively lenient inclusion and exclusion criteria, which included patients with comorbidities, as well as patients who eventually died, were admitted to the ICU, or who underwent mechanical ventilation. Therefore, the population in our study was more representative, as were the clinical data included in the analysis, in reflecting AEs in real-life settings. Our results identified three important clinical phenotypes of exacerbations, of which, the phenotype with fewer eosinophils and increased comorbidities had higher treatment requirements and the worst outcomes. Patients with this phenotype required more systemic glucocorticoids and mechanical ventilation, ICU admission, and had an extended hospital LOS, more direct hospitalization costs, and increased mortality. As this study was based on a real-world environment, patients with asthma-COPD overlap were not excluded. The participants in cluster 3 had some features resembling COPD, such as being older and having less eosinophilic inflammation and more comorbidities. However, the differences in outcomes between the phenotypes could not be completely attributed to COPD due to the lack of difference in the distribution of asthma-COPD overlap among the three clusters (cluster 1: 22.4% vs. cluster 2: 23.7% vs. cluster 3: 26.2%, $P=0.555$). As a novel addition to our cluster analyses, we developed a decision tree diagram using only two variables (prehospital rescue OCS for exacerbation and age of onset) and found that 90.8% of patients could be assigned to the correct cluster, indicating the implementation of these clusters in clinical practice. Our findings have important and clinically relevant implications for the management of patients hospitalized for AEs, such as detecting high-risk patients hospitalized for AE, judging prognosis, and identifying treatable traits.

We conducted this study in a real-world setting to provide information representative of patients in clinical practice. All patients with AEs were consecutively recruited and included patients with comorbid conditions. Our subgroup analysis indicated that the primary and secondary in-hospital outcomes remained the same, when it was performed excluding the patients who did not receive the treatment algorithm indicated by asthma guidelines³. It should be noted that the patients excluded for this analysis were a very small proportion of the

overall study population; nonetheless, documented data on such patients are valuable for informing practice. In order to establish a comprehensive dataset and ensure adequate information that truly represents the characteristics in a real-world setting, we captured data on sociodemographics, medical history, preexisting conditions, and conditions at admission (as many as 150 variables were recorded initially), as well as multidimensional information to evaluate in-hospital outcomes. Collecting such data allowed us to be able to adjust for confounding factors that may have impacted the exposure or outcomes of interest. Furthermore, to ensure the reliability of our real-world study, the patients in our study were diagnosed, treated, and managed by qualified consulting physicians who received standardized clinical training in our hospital. Together, these strategies improve the clinical relevance and generalization of our results to clinical practice.

A major consideration for us was how many variables were selected for the cluster analysis. If too many or too few variables are used, it is difficult to describe and interpret the profiles of clusters in a meaningful way. Some studies selected variables for cluster analysis in a subjective manner and were not transparent about their selection process. For example, a study of patients with asthma and depressive symptoms only included variables in the cluster analysis that were significantly different between patients with asthma who had depressive symptoms and those without depressive symptoms, which may have introduced some degree of subjectivity or selection bias²⁵. Another study that explored how low-income patients with asthma were distributed across a cluster analysis reported that they only applied multicollinearity and similarity as criteria to determine the inclusion of variables²⁶. In the current study, dimension-reduction techniques such as exploratory factor analysis and multiple correspondence analysis were utilized to reduce the number of variables entered into the analysis. In addition, the number of variables selected for clustering (n=11) was considered to be feasible for others seeking to reproduce these results²⁷. Moreover, some systemic inflammation biomarkers that were included in the cluster analysis, such as blood neutrophils, eosinophils, CRP, and albumin, have previously been reported to be associated with asthma²⁸⁻³¹.

Generally, peripheral blood eosinophil count has potential utility as a biomarker of eosinophilic airway inflammation and may predict a favorable response to corticosteroids³². Cluster 1 comprised patients with higher blood eosinophil counts and had a better prognosis. Although the patients in cluster 2 exhibited similar blood eosinophilia counts as cluster 1, patients in cluster 2 had a worse prognosis, indicating that other factors modified in-hospital outcomes. We investigated this and found that the patients in cluster 2 exhibited blood neutrophilia, which indicated insensitivity to corticosteroids. Second, the patients in cluster 2 exhibited relatively remarkable systemic inflammation, reflected in IL-6 and CRP levels. The association between failure to respond to corticosteroids and systemic inflammation has been demonstrated in other diseases. For example, Wine *et al.* reported that the steroid-sparing ability of anti-IL-6 antibodies in juvenile rheumatoid arthritis and elevated levels of IL-6 strongly predicted corticosteroid failure in acute severe ulcerative colitis³³. These results are important and suggest that the steroid responsiveness of blood eosinophilia can be modified by co-existing systemic neutrophilic inflammation, and may help explain the association between resistance to corticosteroids and systemic inflammation in asthma.

Clinical researchers have proposed the deconstruction of asthma into individual traits that can be measured and modified (i.e., “treatable traits”)¹⁴. Increased eosinophilic inflammation in peripheral blood samples in asthma is associated with favorable responses to corticosteroids and an increased risk of exacerbations following corticosteroid withdrawal³⁴⁻³⁷. These findings suggest that a high blood eosinophil count represents a common “treatable trait” for asthma (treated with OCS). Therefore, identifying eosinophilic and neutrophilic inflammation and distinguishing eosinophilic from non-eosinophilic asthma may be useful for the prevention and treatment of AE. Patients in cluster 3 with fewer blood eosinophils may benefit more from targeting systemic neutrophilic inflammation and comorbidity rather than systemic eosinophilic inflammation. Neutrophilic inflammation, as a prominent feature of AE, has been linked to steroid resistance in individuals with asthma, which may explain why the patients in cluster 3, while

having been administered the highest amount of systemic corticosteroid during hospitalization, had the worst in-hospital prognosis³⁸.

It has been demonstrated that glucocorticoids are capable of downregulating the number of eosinophils by inducing eosinophil apoptosis in asthmatic patients³⁹⁻⁴². However, glucocorticoids exert a protective effect on human neutrophil survival by delaying apoptosis⁴³. These findings, combined with a higher proportion of prehospital OCS for maintenance and/or rescue use and a lower level of peripheral blood eosinophils in our patients in cluster 3, suggest that prehospital OCS use may be a factor in reducing blood eosinophils in cluster 3. However, patients in cluster 3 had higher levels of IL-6 and CRP, which would not be explained by prehospital OCS use because glucocorticoids reduce the expression of IL-6 and CRP⁴⁴. These results suggest that the increased blood neutrophils in cluster 3 that were disproportional to the decreased blood eosinophils, could be partly explained by the presence of non-type 2 inflammation in patients in cluster 3. Other strategies, such as targeting systemic neutrophilic inflammation and comorbidity, may improve the prognosis of patients in cluster 3 and should be further explored.

Treatable traits have been demonstrated to be able to predict the future risk of asthma-related outcomes^{45, 46}. In our study, blood neutrophils independently predicted an increased risk of in-hospital death, ICU admission, and mechanical ventilation. These results suggest that fewer eosinophils or more neutrophils as treatable traits may be associated with adverse in-hospital prognosis. In addition, we identified several different treatable traits among the three clusters, each of which is clinically relevant and modifiable, such as allergic status, medication adherence, comorbidity evaluated by the CCI, non-eosinophilic inflammation, and respiratory infection. Our analyses have provided important new knowledge in terms of identifying treatable traits of AE, at least in terms of adverse in-hospital outcomes. Identifying treatable traits that have a large impact on asthma outcomes informs the design of intervention studies that aim to improve in-hospital outcomes. Although the design of randomized controlled trials that aim to test the identified treatable

traits is complex, further prospective treatment studies to determine whether any traits are associated with an improved outcome are necessary.

This study can inform and change management and treatment of AE by aiding in identifying high-risk patients, targeting high-risk factors and treatable traits, and providing future research directions. First, patients in cluster 3 had the worst in-hospital prognosis. Therefore, in clinical practice, we need to pay more attention to patients with the characteristics identified in cluster 3. Second, some clinical characteristics that differed between the three clusters can be treated as treatable traits or important inflammatory biomarkers, which may help improve patient outcomes. Third, the decision tree analysis from this study can help to identify clusters accurately and improve the clinical feasibility of our study. Fourth, this study also provides important information to guide future research on AE⁴⁷. For example, patients with AEs in cluster 3 could be included in randomized controlled trials that explore the relationships of “treatable traits” with adverse in-hospital outcomes.

Although we performed a prospective observational study in a real-world setting to identify and validate phenotypes in patients hospitalized for AEs and found a phenotype characterized by fewer eosinophils and more comorbidities that requires greater attention, there were some limitations that need to be addressed. First, we used blood eosinophil count as a substitute for sputum eosinophils to predict the eosinophilic phenotype. However, the accuracy of this approach has been confirmed⁴⁸. Second, subjectivity of variable selection cannot be totally eliminated in cluster analysis, although a broad range of variables and objective statistical methods were applied. Third, measurements of airway obstruction and airway inflammation phenotype by sputum induction would be helpful for phenotyping and clustering, but is unavailable, especially for patients with severe or life-threatening AE, in clinical practice. Moreover, the cause of exacerbation, such as viral positivity, was not identified. Fourth, there may be recall bias in determining late- and early-onset asthma based on age at asthma onset, although several studies have asserted that self-reported age at asthma onset is of high fidelity^{49, 50}. Finally,

identifying phenotypes of patients from a single center without validation in another population may limit the generalizability of the results. However, to some extent, findings from a real-world study are characterized by good external validity and generalizability⁵¹.

CONCLUSION

To the best of our knowledge, this is the first study to identify clusters in patients with AEs in a real-world setting, and conduct a prospective observational study to assess the importance of these prespecified clusters. As a result, we identified a late-onset phenotype, an early-onset phenotype, and a phenotype with fewer eosinophils and more comorbidities, the latter being significantly associated with worse in-hospital outcomes, including administration of systemic glucocorticoids, mechanical ventilation, ICU admission, longer hospital LOS, more direct hospitalization charges, and death. These findings have important implications for the individualized assessment and management of patients hospitalized for AEs in clinical practice.

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CONFLICT of INTEREST STATEMENT

On behalf of all authors, the corresponding author states that there is no conflict of

interest.

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Tables

Table 1. Demographic and clinical characteristics of the included patients with acute asthma grouped by cluster analysis.

Baseline characteristics	Cluster 1	Cluster 2	Cluster 3	P value
n	526	97	202	
Age, years, median (Q1,Q3)	55.0 (44.0, 65.0)	48.0 (40.5, 58.5) ^{††}	58.0 (44.0, 68.0) ^{***}	<0.001
Female, n (%)	315 (59.9)	66 (68.0)	131 (64.9)	0.202
BMI, kg/m ² , median (Q1,Q3)	22.65 (20.59, 25.71)	21.64 (19.53, 25.39)	23.95 (21.33, 25.75)	0.240
Pack years, median (Q1,Q3) ^{‡‡‡}	0 (0, 60)	0 (0, 0)	0 (0, 80)	0.185
Atopic status, n (%)	223 (42.4)	43 (44.3)	82 (40.6)	0.818
Family history of asthma, n (%)	52 (9.9)	20 (20.6) ^{††}	21 (10.4) [*]	0.008
Asthma duration, years, median (Q1,Q3)	7.0 (2.0, 20.0)	40.0 (30.0, 50.0) ^{†††}	10.0 (5.0, 20.0) ^{†††***}	<0.001
Early-onset asthma, n (%)	0 (0)	97 (100.0) ^{†††}	2 (1.0) ^{***}	<0.001
Prehospital maintenance medications				
ICS/LABA, n (%)	147 (27.9)	20 (20.6)	77 (38.1) ^{†**}	0.003
Anticholinergic drugs, n (%)	15 (2.9)	2 (2.1)	16 (7.9) ^{††}	0.004
Theophylline, n (%)	126 (24.0)	25 (25.8)	89 (44.1) ^{†††***}	<0.001
Leukotriene modifier, n (%)	47 (8.9)	7 (7.2)	21 (10.4)	0.656
Maintenance OCS, n (%)	1 (0.8)	1 (1.0)	13 (6.4) ^{†††}	<0.001
Prehospital OCS use for exacerbations, n (%) [#]	6 (1.1)	18 (18.6) ^{†††}	150 (74.3) ^{†††***}	<0.001
Asthma exacerbation severity, n (%) ^{‡‡}				
Mild to moderate	327 (62.2)	54 (55.7) ^{††}	90 (44.6) ^{†††}	<0.001
Severe	166 (31.6)	26 (26.8)	68 (33.7)	
Life-threatening	33 (6.3)	17 (17.5)	44 (21.8)	
Charlson comorbidity index (CCI), median (Q1,Q3)	1.0 (0, 3.0)	1.0 (0, 2.0)	2.0 (0, 3.0) ^{†***}	0.003
Arterial blood gas, median (Q1,Q3) [‡]				
pH	7.40 (7.37, 7.42)	7.40 (7.37, 7.43)	7.40 (7.37, 7.44)	0.876
PaCO ₂ , mmHg [*]	40.21 (35.00, 47.23)	41.30 (34.98, 50.12)	41.51 (35.56, 54.18)	0.085
PaO ₂ , mmHg	75.50 (65.35, 85.65)	71.09 (60.70, 81.48)	80.00 (65.85, 94.15)	0.118

	87.85)	97.50)	95.35)	
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BMI: body mass index; ICS, inhaled corticosteroid; LABA, long-acting β_2 agonist; OCS, oral corticosteroids; PaCO₂: partial pressure of carbon dioxide; PaO₂: partial pressure of oxygen; Q1, first quartile; Q3, third quartile; SABA, short-acting β_2 agonist.

[†] $P < 0.017$, ^{††} $P < 0.005$, ^{†††} $P < 0.001$ vs. cluster 1, with the Bonferroni correction

^{*} $P < 0.017$, ^{**} $P < 0.005$, ^{***} $P < 0.001$ vs. cluster 2, with the Bonferroni correction

[#] Rescue OCS use for exacerbation within 7 days before hospital admission

[‡] Within the first 48 h of hospitalization

^{‡‡} The severity of asthma exacerbations classified as mild to life-threatening was defined based on the GINA guidelines according to the patients' deterioration of symptoms and/or expiratory airflow (Table E4)

^{‡‡‡} Pack years: the number of cigarettes smoked per day $\times$ years of smoking

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Table 2. Clinical characteristics of the included patients with acute asthma grouped by cluster analysis.

Baseline characteristics	Cluster 1	Cluster 2	Cluster 3	P value
n	526	97	202	
Cell counts in peripheral blood [‡]				
Leukocytes, $\times 10^9/L$, median (Q1,Q3)	7.20 (5.66, 9.62)	8.49 (6.72, 11.40) ^{††}	8.92 (6.41, 12.43) ^{†††}	<0.001
Neutrophils, $\times 10^9/L$, median (Q1,Q3)	4.91 (3.50, 7.44)	6.43 (4.42, 9.53) ^{†††}	7.28 (4.20, 10.99) ^{†††}	<0.001
Neutrophils, %, median (Q1,Q3)	70.80 (59.75, 81.74)	76.35 (65.22, 85.29) ^{††}	80.03 (67.98, 90.54) ^{†††}	<0.001
Eosinophils, $\times 10^9/L$, median (Q1,Q3)	0.11 (0.01, 0.32)	0.10 (0.01, 0.28)	0.02 (0, 0.20) ^{†††**}	<0.001
Eosinophils, %, median (Q1,Q3)	1.92 (0.13, 5.19)	1.40 (0.10, 3.44)	0.26 (0, 2.80) ^{†††**}	<0.001
Eosinophils $\geq 300/\mu L$, n (%)	140 (26.6)	21 (21.6)	32 (15.8) ^{††}	0.008
Lymphocytes, $\times 10^9/L$, median (Q1,Q3)	1.38 (0.95, 1.83)	1.10 (0.77, 1.99)	1.07 (0.61, 1.84) ^{†††}	<0.001
Lymphocytes, %, median (Q1,Q3)	19.03 (12.01, 27.55)	15.99 (8.73, 23.15) ^{††}	13.24 (6.49, 23.44) ^{†††}	<0.001
Basophils, $\times 10^9/L$, median (Q1,Q3)	0.02 (0.01, 0.03)	0.02 (0.01, 0.03)	0.01 (0.01, 0.02) ^{†††*}	0.001
Basophils, %, median (Q1,Q3)	0.22 (0.11, 0.43)	0.19 (0.11, 0.33)	0.13 (0.07, 0.25) ^{†††**}	<0.001
Clinical immunology and inflammation [‡]				
CRP, mg/L, median (Q1,Q3)	2.83 (0.50, 11.88)	11.10 (1.26, 32.60) ^{†††}	12.95 (2.10, 46.48) ^{†††}	<0.001
PCT ≥ 0.05 ng/mL, n (%) [‡]	34 (6.5)	7 (7.2)	29 (14.4) ^{††}	0.003
IgE, IU/mL median (Q1,Q3)	53.16 (22.11, 157.4)	21.43 (0, 42.87)	36.66 (8.07, 69.02)	0.115
IL-6, ng/L, median (Q1,Q3)	3.68 (1.74, 8.66)	9.44 (7.08, 21.38)	9.36 (4.33, 17.89) [†]	0.002
Sputum culture [‡]				
Bacteria, n (%)	149 (28.3)	22 (22.7)	79 (39.1) ^{†**}	0.004
Fungus, n (%)	54 (10.3)	16 (16.5)	41 (20.3) ^{††}	0.001
Biochemistry, median (Q1,Q3) [‡]				

TP, g/L	67.60 (63.20, 73.10)	67.10 (62.25, 74.75)	67.15 (61.65, 72.85)	0.638
ALB, g/L	41.60 (38.30, 44.50)	41.60 (37.75, 44.70)	39.95 (37.10, 43.43) ^{††}	0.016
GLB, g/L	26.20 (23.40, 29.90)	26.70 (22.30, 31.15)	26.30 (23.00, 30.98)	0.750
TG, mmol/L	1.06 (0.75, 1.51)	1.15 (0.77, 1.66)	1.13 (0.77, 1.59)	0.339
CHOL, mmol/L	4.39 (3.71, 5.09)	4.33 (3.53, 5.02)	4.44 (3.78, 5.25)	0.596
LDL-C, mmol/L	2.46 (1.94, 3.01)	2.43 (1.77, 2.85)	2.37 (1.73, 2.97)	0.272
HDL-C, mmol/L	1.43 (1.13, 1.74)	1.40 (1.10, 1.90)	1.56 (1.26, 1.91) ^{††}	0.004
ALB, albumin; CRP, c-reactive protein; CHOL; cholesterol; GLB, globulin; HDL-C, high density lipoprotein cholesterol; IL, interleukin; LDL-C, low-density lipoprotein cholesterol; PCT, procalcitonin; TP, total protein; TG, triglyceride. [†]$P < 0.017$, ^{††}$P < 0.005$, ^{†††}$P < 0.001$ vs. cluster 1, with the Bonferroni correction [*]$P < 0.017$, ^{**}$P < 0.005$, ^{***}$P < 0.001$ vs. cluster 2, with the Bonferroni correction [‡] Within the first 48 h of hospitalization				

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Table 3. Primary and secondary in-hospital outcomes of all patients grouped by cluster analysis.

Outcomes	Cluster 1	Cluster 2	Cluster 3	P value
n	526	97	202	
Primary in-hospital outcomes				
Death, n (%)	1 (0.2)	3 (3.1) [†]	8 (4.0) ^{†††}	<0.001
Hospital LOS, days	10 (7, 13)	9 (6, 13)	11 (8, 15) ^{††**}	0.003
Direct cost for hospitalization, CNY	9402.33 (6328.9, 13649.68)	9016.16 (4881.8, 15003.16)	13481.57 (7740.75, 26123.88) ^{†††} ***	<0.001
Secondary in-hospital outcomes				
Systemic corticosteroid use within the first 7 days (methylprednisolone dose equivalent, mg) [‡]	320.0 (280.0, 480.0) (n=523)	280.0 (280.0, 386.7) (n=96)	332.0 (280.0, 480.0) ^{††*} (n=201)	0.003 ^{##}
Mechanical ventilation, n (%)	26 (4.9)	11 (11.3) [†]	48 (23.8) ^{†††*}	<0.001
Noninvasive ventilation, n (%)	21 (4.0)	7 (7.2)	37 (18.3) ^{†††*}	<0.001
Invasive ventilation, n (%)	12 (2.3)	4 (4.1)	25 (12.4) ^{†††}	<0.001
ICU admission, n (%)	52 (9.9)	8 (8.2)	38 (18.8) ^{††*}	0.002
ICU stay, h [#]	21.0 (11.0, 61.5)	36.5 (5.3, 97.5)	80.0 (19.3, 209.3) [†]	0.021

CNY, Chinese Yuan; ICU, intensive care unit; LOS, length of stay.

Data are not normally distributed and presented as median (quartile 1, quartile 3).

[†] $P<0.017$, ^{††} $P<0.005$, ^{†††} $P<0.001$ vs. cluster 1, with the Bonferroni correction

^{*} $P<0.017$, ^{**} $P<0.005$, ^{***} $P<0.001$ vs. cluster 2, with the Bonferroni correction

[#]In patients with ICU admission

^{##}One-way ANOVA was used after log transformations; Least—Significant Difference (LSD) was used as post hoc tests with a 0.05 significance level

[‡]The patients without contraindication of systemic corticosteroids including active peptic ulcer or gastrointestinal bleeding. No significant difference was shown in the numbers of patients in this analysis among the 3 clusters (Fisher's exact test value=0.642. $P=1.000$).

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Figure legends

Figure 1. Flowchart of study.

Figure 2. Radar charts representing the patients' characteristics, and the primary and secondary in-hospital outcomes in cluster 1 (blue), cluster 2 (yellow), and cluster 3 (red). ALB, albumin; CRP, C-reactive protein; ICU, intensive care unit; IV, invasive ventilation; MV, mechanical ventilation; NV, noninvasive ventilation; OCS, oral corticosteroids; PaCO₂, partial pressure of carbon dioxide; prehospital OCS, prehospital OCS for exacerbation.

Figure 3. Correlations of three identified clusters with (A) mortality, (B) intensive care unit (ICU) admission, (C) mechanical ventilation, (D) noninvasive ventilation, and (E) invasive ventilation; logistic regression analysis, with cluster 1 as the reference, after adjusting for BMI and CCI. BMI, body mass index; CCI, Charlson comorbidity index; CI, confidence interval; RR_{adj}, relative ratio after adjusting for BMI and CCI.

Figure 4. Tree analysis and misclassification of predicted clinical cluster assignment. Patients were assigned to the three clusters using two variables: prehospital rescue OCS for exacerbation and age of onset (cut at age 12). Percent of patients correctly assigned to each cluster is indicated within each shape. Individual figure size is proportional to the frequency of a specific cluster. OCS, oral corticosteroids.

