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ORIGINAL ARTICLE

Impact of allergen sensitization on phenotypes of T2-low asthma: A post-hoc analysis of a nationwide cohort study, NHOM Asthma

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Abstract

Background: Asthma is a heterogeneous disease influenced by genetic and environmental factors. Type 2 (T2)-high asthma has been extensively studied; however, the pathophysiological mechanisms of T2-low asthma remain unclear.

Objective: The present study aimed to determine the clinical indices contributing to asthma exacerbation and identify the phenotypes of T2-low asthma.

Methods: We used data from the NHOM Asthma Study (N = 1925), a nationwide asthma cohort study conducted in Japan. T2-low asthma was defined by eosinophils < 150/μL and fractional exhaled nitric oxide levels < 25 ppb. The clinical indices associated with asthma exacerbation were identified using univariate and multivariate analyses. Hierarchical cluster analysis was performed to classify the phenotypes of T2-low asthma.

Results: Multivariate analysis revealed that younger age and comorbid allergic diseases contributed to the exacerbation of T2-low asthma. Four phenotypes were identified: Cluster 1 (n = 19, 7.8%, smoking-related T2-low asthma with preserved pulmonary function), Cluster 2 (n = 18, 7.4%, smoking-related T2-low asthma with low pulmonary function), Cluster 3 (n = 99, 40.7%, elderly, female-dominant, late-onset T2-low asthma), and Cluster 4 (n = 107, 44.0%, younger, female-dominant, comorbid with allergic disease T2-low asthma). Clusters 2 and 4 were prone to asthma exacerbation, indicating distinct allergen sensitization.

Conclusions: These findings indicate that antigen-specific IgE profiles may reflect the phenotypic heterogeneity of T2-low asthma and could serve as potential biomarkers for identifying subgroups at increased risk of exacerbations.

Key words: asthma, phenotype, allergen sensitization, cluster analysis, biomarker

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Abbreviations:

ACQ	Asthma Control Questionnaire
FeNO	fractional exhaled nitric oxide
FEV_1	forced expiratory volume in the first second
IL	interleukin
MMP	matrix metalloproteinase
OCS	oral corticosteroid
ST2	suppression of tumorigenicity 2
T2	Type 2

Introduction

Asthma is a heterogeneous group of diseases influenced by genetic and environmental factors. The use of biomarkers to classify airway inflammatory phenotypes remains controversial. In clinical practice, treatment strategies are determined based on the presence or absence of type 2 (T2) inflammation, classifying asthma into T2-high and T2-low phenotypes. T2-high asthma is driven by adaptive and innate immunity. Th2 cells and ILC2 produce Th2 cytokines, including interleukin (IL)-4, IL-5, and IL-13, resulting in immunoglobulin E (IgE) production, mucus secretion, airway hyperresponsiveness, airway remodeling, and eosinophilic inflammation. Hence, T2-high asthma includes allergic and/or eosinophilic asthma. In contrast to those of T2-high asthma, the mechanisms underlying T2-low asthma remain poorly understood. Neutrophilic or paucigranulocytic airway inflammation associated with factors such as smoking, air pollution, and obesity may be involved in the pathophysiology of T2-low asthma. Neutrophilic airway inflammation, resulting from the IL-17 pathway, is related to increased asthma severity and steroid resistance. T2-low asthma often exhibits features of mixed granulocytic asthma, characterized by concurrent airway eosinophilia and neutrophilia,[1,2] which makes understanding its pathogenesis challenging.

We previously performed a cluster analysis of patients with asthma who, despite receiving treatment, exhibited elevated blood eosinophil counts or fractional exhaled nitric oxide (FeNO) levels, indicative of T2-high inflammation, and identified four distinct phenotypes.[3] Given that many types of inflammatory cells are found in patients with T2-high asthma, we hypothesized that T2-low asthma has different phenotypes. In addition, clinically available indices and noninvasive biomarkers for pathophysiology remain necessary for managing T2-low asthma.

This study aimed to identify the clinical indices contributing to the worsening and phenotypes of T2-low asthma using data from the NHOM Asthma Study.[4] These findings contribute to the understanding of the clinical characteristics of T2-low asthma, leading to the optimization of treatment strategies.

Methods

Study design

This study was a post-hoc analysis of the NHOM Asthma Study,[4] a nationwide asthma cohort study approved by the ethics committees of the participating hospitals and registered in the University Hospital Medical Information Network Clinical Trials Registry (UMIN-CTR; 000027776). The NHOM Asthma Study was a prospective, multicenter, observational cohort study in which the details of the study design have been described previously. In brief, 1925 patients with asthma from 27 national hospitals in Japan were treated according to their physicians' standard practices to survey real-world clinical practice for asthma in Japan. The numbers of asthma exacerbations and hospitalizations during the one-year observation period following participant enrollment were also investigated. Biomarker analysis was performed using serum samples from participants receiving Global Initiative for Asthma (GINA) 4 or 5 therapies. This study was approved by the Institutional Ethical Review Board of the National Hospital Organization of Tokyo National Hospital (No. 220026; June 29, 2022). Informed consent for the secondary use of the data was obtained from the patients enrolled in the NHOM Asthma Study.

Assessments and variables

All the data were retrieved from the NHOM Asthma Study.[4] Clinical data were collected from medical charts by the physician in charge. The latest data on respiratory function tests, blood examinations, and treatment regimens were collected within one year of enrolment. Patients completed questionnaires, including the Asthma Control Questionnaire (ACQ)-6, to obtain demographic data, medical history, and comorbidities. Asthma exacerbation was defined as a condition that required systemic steroid therapy for more than three days. The primary endpoint of this study was to identify the factors contributing to exacerbation in T2-low asthma. The secondary endpoint was the identification of the clinical phenotypes in patients with T2-low asthma.

Definition of T2-low asthma and exclusion criteria

As mentioned above, based on the understanding that the number of blood eosinophils and FeNO are the most practical and useful biomarkers for determining T2 inflammation in the airway,[5] blood eosinophils and FeNO were used as indicators of T2 inflammation. T2-low asthma was defined as a blood eosinophils count < 150/μL and FeNO level < 25 ppb. Patients with missing blood eosinophil count or FeNO level data were excluded from the analysis. Furthermore, patients receiving oral corticosteroids (OCS) or biologic therapies were excluded to account for the possibility that blood eosinophil counts and FeNO levels were suppressed during the treatment course.

Statistical analysis

Univariate analysis was performed using Student's *t*-test for continuous variables and the chi-square test for non-continuous variables. Multivariate logistic regression analysis was performed to adjust for confounding factors and clinical indicators associated with the exacerbation of T2-low asthma.

A hierarchical cluster analysis using Ward's method created a dendrogram and determined the number of clusters. Age, age at asthma onset, forced expiratory volume in the first second (FEV_1) % predicted, smoking index, and sex were selected for cluster analysis. Age, age at asthma onset, forced expiratory volume in the first second (FEV_1) % predicted, smoking index, and sex were selected for cluster analysis. These variables were chosen because our analysis focused on key historical and clinical parameters readily accessible to general practitioners that reflect underlying patient characteristics. Biomarkers were excluded from analyses due to their susceptibility to treatment-related variability. To compare differences between clusters, we used analysis of variance, Kruskal–Wallis test, and chi-square test for parametric continuous, nonparametric continuous, and categorical variables, respectively.

When the 95% confidence interval of the relative risk of a given factor did not include 1, the value was considered significant (significance level, 0.05). All statistical analyses were performed using JMP pro17 (SAS Institute Inc., Cary, NC, USA). Statistical significance was set at $p < 0.05$.

Results

Characteristics of T2-low asthma compared to T2-high asthma

Of the 1925 enrolled patients with asthma, 689 with missing data on blood eosinophil counts or FeNO levels were excluded. Furthermore, after 125 patients who were receiving OCS or biologics were excluded, data from 1111 patients were analyzed (**Figure 1**).

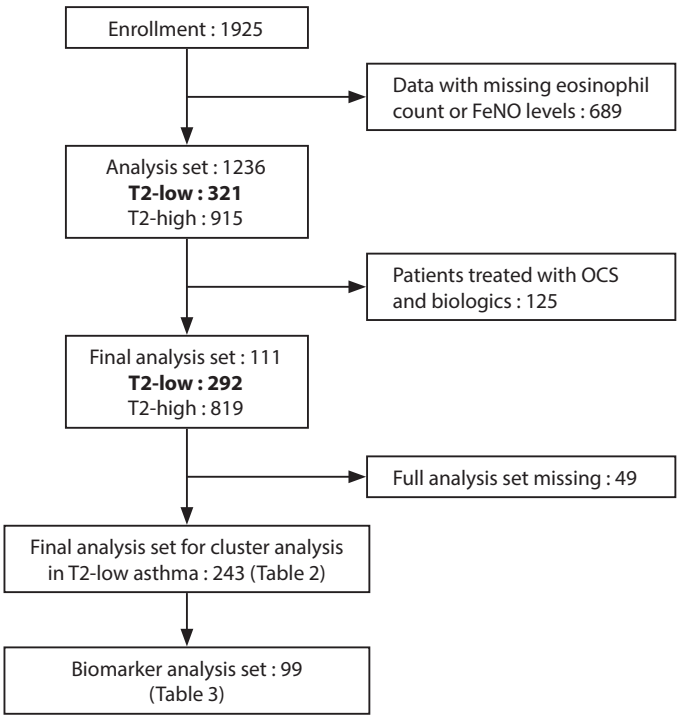

Figure 1. Flow diagram for the study.

As reported in the previous study,[3] which identified and analyzed T2-high asthma in the same population, 26% of patients with asthma were identified as having T2-low asthma based on the cutoff of blood eosinophils < 150/μL and FeNO < 25 ppb. Patients with T2-low asthma were younger, were predominantly female, and had an earlier asthma onset, shorter disease duration, less frequent comorbid sinusitis, higher pulmonary function, and a greater prevalence of gastroesophageal reflux disease and mental health disorders.

Identification of the factors contributing to exacerbations in T2-low asthma

To identify exacerbation-contributing factors in patients with T2-low asthma, we first performed a univariate analysis of each clinical variable. As shown in **Table 1**, age, age at asthma onset, allergic comorbidities, and ACQ-6 score were independently associated with exacerbations of T2-low asthma (age: 0.034 [0.0052-0.22], $p < 0.001$; age at asthma onset: 0.061 [0.0082-0.46], $p < 0.01$; allergic comorbidities: 3.9 [1.60-9.45], $p < 0.01$; and ACQ-6 score: 8.1 [1.14-57.99], $p < 0.05$).

Confounders, such as the variables with p values < 0.05 in the univariate analysis, were adjusted for by including them as covariates in the multivariate analysis. Younger age and allergic comorbidities were statistically significant independent factors associated with exacerbations of T2-low asthma (age: 0.053 [0.0046-0.62], $p < 0.05$; age at asthma onset: 0.66 [0.045-9.68], $p = 0.76$; allergic comorbidities: 3.5 [1.19-10.11], $p < 0.05$; ACQ-6 score: 5.8 [0.57-59.07], $p = 0.13$).

Cluster analysis of T2-low asthma

To identify the phenotypes of patients with T2-low asthma, we subjected 243 patients with no missing variables to cluster analysis (**Figure 1**). They were distributed into four distinct clusters (**Table 2**), the characteristics of which are summarized in **Figure 2**.

Table 1. Univariate and multivariate linear regression analysis of factors influencing asthma exacerbations.

	Univariate analysis			Multivariate analysis		
	Odds ratio	95%CI	P value	Odds ratio	95%CI	*P* value
Subjects						
Age	0.034	0.0052-0.22	< 0.001	0.053	0.0046-0.62	< 0.05
Sex, female	1.5	0.62-3.77	0.35			
BMI	1.3	0.21-8.59	0.76			
Age of asthma onset	0.061	0.0082-0.46	< 0.01	0.66	0.045-9.68	0.76
Asthma duration	0.28	0.026-2.91	0.26			

Table 1. (Continued)

	Univariate analysis			Multivariate analysis		
	Odds ratio	95%CI	P value	Odds ratio	95%CI	*P* value
Comorbidities						
Allergic disease*	3.9	1.60-9.45	< 0.01	3.5	1.19-10.11	< 0.05
Allergic rhinitis	3.2	1.29-8.08	< 0.05			
Allergic conjunctivitis	3.9	1.33-11.49	< 0.05			
Atopic dermatitis	1.4	0.42-4.61	0.60			
Food allergies	7.6	1.43-39.79	< 0.01			
Drug allergies	5.3	1.46-19.09	< 0.01			
Urticaria	3.5	1.02-12.03	< 0.05			
Sinusitis	1.4	0.58-3.21	0.47			
COPD	0.98	0.17-5.67	0.98			
GERD	0.60	0.21-1.67	0.31			
SAS	0.33	0.038-2.89	0.26			
Laboratory findings						
FeNO	0.40	0.073-2.15	0.28			
$\%FEV_1$	0.39	0.030-5.07	0.47			
Neutrophil	0.70	0.078-6.27	0.75			
Eosinophil	1.1	0.29-3.88	0.92			
IgE	3.5	0.25-50.66	0.35			
Atopy‡	1.8	0.76-4.22	0.17			
The questionnaires score						
ACQ-6	8.1	1.14-57.99	< 0.05	5.8	0.57-59.07	0.13

*Including allergic rhinitis, allergic conjunctivitis, atopic dermatitis, food allergies, drug allergies, and urticaria
‡Specific IgE responsiveness to perennial inhaled allergen
CI, Confidence interval; BMI, body mass index; FeNO, fractional exhaled nitric oxide; FEV_1, forced expiratory volume in the first second; $\%FEV_1$, percent-predicted FEV_1; ACQ, asthma control questionnaire

Table 2. Clusters in T2-low asthma.

	Cluster 1 (n = 19)	Cluster 2 (n = 18)	Cluster 3 (n = 99)	Cluster 4 (n = 107)	Significance (*P* value)
Summary	Smoking-related T2-low asthma with preserved pulmonary function	Smoking-related T2-low asthma with low pulmonary function	Elderly, female-dominant, late-onset, T2-low asthma	Younger, female-dominant, Complicated with allergic disease, T2-low asthma	
Age at enrolment, years	67.9 ± 8.1	70.8 ± 10.5	68.4 ± 9.8	43.2 ± 12.7	< 0.0001
Sex, % female	21.1	22.2	81.8	80.4	< 0.0001
BMI, kg/m^2	24.5 ± 3.7	24.9 ± 4.1	24.7 ± 4.7	23.9 ± 4.7	0.19
Age of asthma onset, years	51.8 ± 18.8	45.3 ± 23.3	55.6 ± 14.3	24.0 ± 15.1	< 0.0001
Asthma duration, years	13.4 ± 12.8	24.2 ± 22.0	11.8 ± 11.9	13.3 ± 14.1	0.26

Table 2. (Continued)

	Cluster 1 (n = 19)	Cluster 2 (n = 18)	Cluster 3 (n = 99)	Cluster 4 (n = 107)	Significance (*P* value)
Course of asthma, %					< 0.0001
Carried over from childhood asthma	5.3	5.6	0.0	9.5	
Recurred from childhood asthma	5.3	5.6	2.0	24.8	
Adult onset	89.5	88.9	98.0	65.7	
Family history of asthma, %	40.7	52.0	31.7	36.9	0.31
Smoking status, index	50.2 ± 15.7	19.2 ± 11.1	1.8 ± 4.9	3.0 ± 6.5	< 0.0001
Current smoker, %	26.3	11.1	0.0	4.7	
Former smoker, %	73.7	77.8	21.2	27.1	
Never smoker, %	0.0	11.1	78.8	68.2	
Pet keeping, %	21.1	27.8	22.5	30.0	0.62
cats/ dogs, %	22.2	21.1	19.2	29.0	0.42
Others, %	0.0	0.0	1.0	3.7	0.42
Comorbidities, %					
Sinusitis	21.1	33.3	33.7	38.3	0.52
Allergic disease*	21.1	27.8	39.4	55.1	<0.01
COPD	26.7	17.7	2.1	1.9	<0.001
GERD	21.1	11.1	25.3	24.3	0.61
SAS	10.5	5.6	4.0	5.6	0.72
Hypertension	31.6	38.9	38.4	15.9	<0.01
Diabetes mellitus	21.1	22.2	10.1	0.93	<0.001
Heart disease	5.3	16.7	7.1	5.6	0.39
Osteoporosis	0.0	22.2	14.1	3.7	<0.01
Mental disorder	33.3	7.4	12.1	23.9	<0.05
Laboratory findings					
WBC count, /μL	7063.2 ± 2479.7	6466.7 ± 2285.5	5634.6 ± 1580.3	6012.6 ± 1744.9	< 0.05
Blood eosinophils, /μL	66.7 ± 47.8	60.0 ± 49.7	73.8 ± 42.4	75.0 ± 41.7	0.51
Blood neutrophils, /μL	4926.4 ± 2210.4	4809.7 ± 1699.8	3724.8 ± 1494.8	3788.5 ± 1395.7	0.13
Total IgE, IU/mL	240.2 ± 450.5	400.6 ± 914.6	168.4 ± 321.2	274.7 ± 858.0	0.36
Atopy‡, %	56.3	76.9	48.1	70.6	< 0.05
Specific IgE (dust mites), %	36.8	33.3	27.6	60.4	< 0.0001
Specific IgE (molds), %	21.1	40.0	13.4	12.5	< 0.05
Specific IgE (cats/dogs), %	10.5	6.7	4.3	19.8	< 0.01
Specific IgE (pollen), %	42.1	46.7	51.0	55.1	0.71
Specific IgE (insects), %	33.3	33.3	18.1	41.8	< 0.05
FEV_1, mL	2423.7 ± 543.6	1540.0 ± 503.2	2085.5 ± 570.8	2724.9 ± 658.7	< 0.0001
FEV_1 % pred, %	107.0 ± 13.1	65.3 ± 14.9	113.0 ± 17.5	101.6 ± 13.6	< 0.0001
FEV_1/FVC, %	74.8 ± 7.1	61.0 ± 10.3	76.9 ± 7.7	82.8 ± 8.2	< 0.0001
FeNO, ppb	13.7 ± 6.2	15.4 ± 5.1	16.3 ± 5.3	13.7 ± 5.2	< 0.01

Table 2. (Continued)

	Cluster 1 (n = 19)	Cluster 2 (n = 18)	Cluster 3 (n = 99)	Cluster 4 (n = 107)	Significance (*P* value)
The questionnaires score					
ACQ	0.72 ± 0.79	0.92 ± 0.97	0.63 ± 0.68	0.73 ± 0.88	0.64
Treatment					
ICS, %					0.13
Low	50.0	0.0	60.0	38.5	
Medium	50.0	0.0	33.3	50.0	
High	0.0	100.0	6.7	11.5	
LABA, %	89.5	94.4	85.9	77.6	0.17
LTRA, %	52.6	55.6	56.6	53.3	0.97
Theophylline, %	26.3	27.8	27.3	23.4	0.92
LAMA, %	15.8	16.7	6.1	10.3	0.33
Thermoplasty, %	0.0	0.0	0.0	1.9	0.46
No of exacerbation	0.50 ± 0.85	1.9 ± 4.55	0.31 ± 0.79	0.91 ± 1.2	< 0.05
Exacerbation, %	30.0	25.0	19.4	55.9	< 0.05
Recurrent exacerbations (≥ 2/year), %	33.3	75.0	18.5	35.0	0.24
No. of admission, %	0.0 ± 0.0	0.25 ± 0.71	0.028 ± 0.17	0.11 ± 0.41	0.46
Admission, %	0.0	12.5	2.8	8.8	0.48

*Including allergic rhinitis, allergic conjunctivitis, atopic dermatitis, food allergies, drug allergies, and urticaria
‡Specific IgE responsiveness to perennial inhaled allergen
BMI, body mass index; WBC, white blood cells; FeNO, fractional exhaled nitric oxide; FEV_1, forced expiratory volume in the first second; $\%FEV_1$, percent-predicted FEV_1; ICS, inhaled corticosteroid; LABA, long-acting β_2 agonist; LAMA, long-acting muscarinic antagonists; LTRA, leukotriene receptor antagonist; ACQ, asthma control questionnaire.

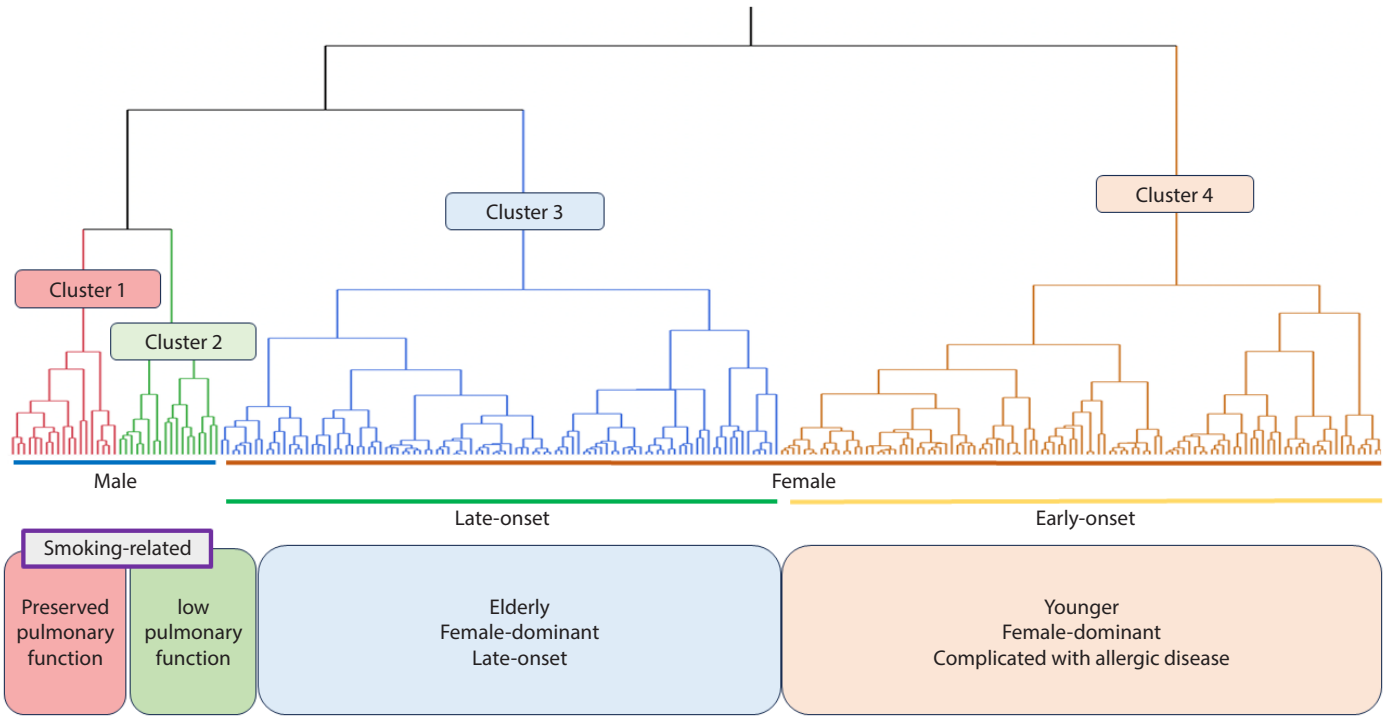

Figure 2. A summary of the dendrogram with four clusters in T2-low asthma.

Cluster 1: Smoking-related T2-low asthma with preserved pulmonary function

Nineteen patients (7.8%) were grouped into Cluster 1. This cluster was male-dominant, had a markedly high smoking status (50.2 ± 15.7 pack-year), and had a high prevalence of comorbidities with chronic obstructive pulmonary disease (COPD), diabetes, and mental disorders. Pulmonary function, including FEV_1% predicted and FEV_1/FVC, was well-preserved despite a high smoking index.

Cluster 2: Smoking-related T2-low asthma with low pulmonary function

Eighteen patients with asthma (7.4%) were grouped into Cluster 2. Similar to Cluster 1, this cluster had a high prevalence of comorbidities such as COPD, diabetes, and mental disorders. Cluster 2 was the oldest and had the most prevalent comorbidity of osteoporosis among the four clusters. Pulmonary function was assessed using the FEV_1% predicted, and FEV_1/FVC was the worst. Furthermore, compared with the exacerbation rate, the number of exacerbation episodes requiring systemic steroids was the highest among the four clusters, indicating the presence of patients who experienced repeated exacerbations, although this elevation in numbers was not statistically significant.

Cluster 3: Elderly, female-dominant, late-onset T2-low asthma

Cluster 3 comprised 99 patients with asthma and was the second largest cluster (40.7%). This cluster was female-dominant and had the latest onset of asthma. Pulmonary function, evaluated using FEV_1% predicted and FEV_1/FVC, was preserved, and the rate and number of asthma exacerbations were the lowest, indicating that it was the mildest asthma group among the four clusters. Notably, the mild group had the lowest positivity rate for perennial allergens, corresponding to IgE specific to dust mites, molds, and insects.

Cluster 4: Younger, female-dominant, complicated with allergic disease T2-low asthma

Cluster 4 was the largest (n = 107, 44.0%) among patients with T2-low asthma. This cluster was the youngest and most female-dominant early-onset cluster. Among patients in this cluster, 24.8% had a recurrence of their childhood asthma. Notably, the most common comorbidity was allergic disease, and the percentage of patients with IgE specific to dust mites, cats/dogs, and insect antigens was the highest among the four clusters, despite no significant differences in total IgE levels and pet-keeping rates. Furthermore, Cluster 4 exhibited the highest exacerbation rate and the second highest number of exacerbations, followed by Cluster 2.

Biomarkers

Table 3 summarizes the serum biomarkers across the four clusters. Clusters 1 and 2, which were characterized by smoking-related asthma, showed elevated serum levels of IL-1RA, matrix metalloproteinase (MMP)-3, suppression of tumorigenicity 2 (ST2)/IL1R4, and YKL-40/CHI3L. Notably, Cluster 2 exhibited the highest serum periostin levels among the four clusters.

Clusters 3 and 4 exhibited elevated leptin levels. Despite the high incidence of allergic comorbidities, we found no elevation in serum periostin or thymus and activation-regulated chemokine levels, which are biomarkers of T2 inflammation.

Table 3. Biomarkers among T2-low asthma clusters.

	Cluster 1 (n = 10)	Cluster 2 (n = 9)	Cluster 3 (n = 39)	Cluster 4 (n = 41)	Significance (*P* value)
Summary	Smoking-related T2-low asthma with preserved pulmonary function	Smoking-related T2-low asthma with low pulmonary function	Elderly, female-dominant, late-onset, T2-low asthma	Younger, female-dominant, Complicated with allergic disease, T2-low asthma	
IL-1RA [ng/mL]	1.3 ± 0.34	1.7 ± 0.42	1.1 ± 0.37	1.3 ± 0.53	< 0.01
IL-2 [pg/mL]	269.0 ± 565.8	46.9 ± 52.2	91.1 ± 163.4	170.3 ± 334.2	0.23
IL-4 [pg/mL]	69.5 ± 84.0	51.3 ± 36.3	42.7 ± 68.8	53.7 ± 47.5	0.21
IL-5 [pg/mL]	2.9 ± 2.0	2.5 ± 1.1	2.4 ± 1.3	2.3 ± 0.80	0.86
IL-6 [pg/mL]	3.1 ± 1.9	4.1 ± 4.4	2.9 ± 2.1	3.0 ± 3.0	0.60
IL-8/CXCL8 [pg/mL]	14.3 ± 4.4	18.4 ± 24.3	12.7 ± 7.9	14.0 ± 13.9	0.93
IL-13 [pg/mL]	286.0 ± 358.2	289.5 ± 227.9	187.3 ± 160.5	259.0 ± 267.7	0.32
IL-33 [pg/mL]	84.7 ± 222.4	11.0 ± 13.3	15.7 ± 25.2	17.8 ± 24.9	0.57
Leptin [ng/mL]	18.4 ± 10.9	23.6 ± 22.6	35.9 ± 24.4	35.6 ± 22.1	< 0.05
MMP3 [ng/mL]	38.4 ± 28.0	39.3 ± 28.0	18.9 ± 11.3	24.1 ± 21.1	< 0.01
Periostin [ng/mL]	372.9 ± 228.9	533.5 ± 285.8	388.8 ± 169.8	312.1 ± 155.5	< 0.01

Table 3. (Continued)

	Cluster 1 (n = 10)	Cluster 2 (n = 9)	Cluster 3 (n = 39)	Cluster 4 (n = 41)	Significance (*P* value)
ST2/IL1R4 [ng/mL]	12.8 ± 5.3	12.0 ± 7.1	8.9 ± 3.6	7.8 ± 3.6	< 0.05
TARC/CCL17 [pg/mL]	817.4 ± 511.4	772.3 ± 591.5	562.6 ± 386.5	510.4 ± 510.4	0.25
YKL40/CHI3L1 [ng/mL]	121.4 ± 179.3	108.3 ± 58.5	123.5 ± 264.8	28.4 ± 21.1	< 0.0001

†Numeric data expressed as mean ± SD.
IL, interleukin; MCP-1, monocyte Chemotactic protein 1; MIP-1, macrophage inflammatory protein 1; MMP, *matrix metalloproteinase*; ST2, suppression of tumorigenicity 2; TARC, Thymus and Activation-Regulated Chemokine

Discussion

The present study identified the factors contributing to the exacerbation and phenotypes of T2-low asthma using data from a nationwide asthma cohort study in Japan.[4] We defined T2-low asthma as blood eosinophils < 150/μL and FeNO < 25 ppb. In multivariate analysis, younger age and allergic disease comorbidities were identified as contributing factors to asthma exacerbation in T2-low asthma.

Cluster analysis identified four phenotypes: Cluster 1 (smoking-related T2-low asthma with preserved pulmonary function), Cluster 2 (smoking-related T2-low asthma with low pulmonary function), Cluster 3 (elderly, female-dominant, late-onset T2-low asthma), and Cluster 4 (younger, female-dominant, complicated with allergic disease T2-low asthma). Given that Cluster 4 represents the largest cluster and is characterized by a higher propensity for exacerbations, it is likely to have exerted a substantial influence on the outcomes of the multivariate analysis. However, cluster analysis also identified Cluster 2 as a phenotype characterized by a tendency for frequent recurrent exacerbations. Specific IgE positivity in response to molds was highest in Cluster 2, and to mites, cats/dogs, and insects in Cluster 4, suggesting that antigen-specific IgE profiles vary according to the phenotypic characteristics of patients with T2-low asthma.

Numerous studies have suggested that allergic diseases, particularly rhinitis, are associated with the development of adult-onset asthma.[6,7] Adult-onset asthma is positively associated with the number of allergic diseases, and this association decreases with age.[8,9] However, to what extent the association between allergic multimorbidity and asthma reflects a true causal association and to what extent it is attributable to shared underlying mechanisms between asthma and other allergic diseases remains unclear.

Rhinitis influences asthma through several mechanisms, such as the release of inflammatory mediators into the airways or systemic circulation, activation of neural reflex pathways, enhanced production of bone marrow-derived progenitor cells involved in inflammation, increased exposure of the lower airways to airborne contaminants due to mouth breathing, and greater demand for the conditioning of inspired air.[10] The mechanism of inflammation may differ depending on the organ, and multiple organs should be considered in the multimorbidity approach to allergic diseases.

Next, we conducted cluster analysis and performed a detailed investigation with a particular focus on clusters characterized by frequent or recurrent exacerbations among the four identified clusters. Cluster 4 exhibited the highest exacerbation rate and was the largest cluster, indicating a potential influence on the outcomes of the multivariate analysis. This cluster was characterized by early-onset asthma and a high prevalence of comorbid allergic diseases (77.6%), along with the highest sensitization rates to perennial allergens, including dust mites, dogs, cats, and insects. Furthermore, Cluster 2 was identified as another phenotype prone to frequent exacerbations, distinguished by the highest rate of fungal sensitization among the four clusters.

Not all asthma cases are inherently associated with allergen sensitization, and many sensitized individuals do not develop asthma or other allergic diseases.[11,12] Moreover, different manifestations of atopic diseases may coexist in the same patient or emerge at different time points.[13] Therefore, when interpreting atopic asthma, allergen-specific IgE must only be considered as a reference value.

Furthermore, only 16.6% of adult atopic asthma cases are estimated to be attributable to genetic susceptibility to atopy,[14] and the genes regulating IgE production and those mediating susceptibility to asthma show little overlap.[15,16] These findings suggest that the causal role of atopy in adult-onset asthma remains uncertain and that allergen sensitization is a secondary phenomenon after asthma onset.[14]

Whether it arises from prior allergen sensitization or secondary sensitization following asthma onset, chronic airway inflammation driven by persistent allergen exposure is likely to be a key factor in asthma exacerbation. This mechanism may underlie the increased exacerbation rates observed in Clusters 2 and 4, both of which exhibited higher frequencies of allergen sensitization. In other words, antigen-specific IgE may be a useful marker for identifying phenotypes prone to asthma exacerbations among T2-low asthma patients.

Fungal exposure and sensitization have been implicated in the exacerbation of asthma severity and poor disease control.[17] Several studies have demonstrated a dose-dependent association between the fungal burden and the severity and persistence of asthma symptoms.[18] Based on these findings, fungal sensitization may play a significant role in the increased frequency of exacerbations observed in Cluster 2. Furthermore, previous research has demonstrated that endotoxin levels are elevated in households with pets,

such as cats or dogs, as well as in low-income and unsanitary living conditions,[19,20] although pet-keeping rates do not differ significantly among clusters. Increased endotoxin exposure in patients with asthma is associated with decreased pulmonary function.[21] Exposure to various antigens and endotoxins may contribute to asthma exacerbation, as observed in Cluster 4. Given these findings, environmental control and lifestyle modifications are essential for maintaining a hygienic living environment, and allergen immunotherapy and omalizumab should be considered viable therapeutic options. Allergen immunotherapy ameliorates asthma symptoms, attenuates airway hyperresponsiveness, reduces risk of exacerbation, and decreases medication requirements. Allergen immunotherapy may also alter the natural history of allergic diseases by preventing the onset of asthma, limiting the development of new sensitizations, and modulating both allergen-specific and nonspecific immune responses, including enhancement of antiviral immunity.[22] Omalizumab has been shown to effectively reduce exacerbation rates even in patients with asthma characterized by low blood eosinophil counts and FeNO levels.[23] In addition to its immunomodulatory effects, omalizumab may facilitate airway epithelium repair.[24] Collectively, these findings suggest that, in allergen-sensitized patients with T2-low asthma, both allergen immunotherapy and omalizumab may confer clinical benefits through mechanisms extending beyond classical T2-mediated inflammation.

Blood eosinophil counts and FeNO levels are commonly used as biomarkers to assess T2 inflammation in the management of asthma, as recommended by the GINA guidelines.[25] Total IgE levels are influenced by non-specific factors such as smoking, tumors, medications, immune diseases, infections, and atopic diseases.[26,27] Recent studies have indicated that total IgE is not an independent biomarker for selecting biologics or predicting therapeutic responses,[28,29] suggesting its limited utility as a marker of T2 inflammation. This study offers insights into the potential role of allergen sensitization in influencing phenotypes associated with asthma exacerbation in patients with T2-low asthma, as defined by blood eosinophil counts and FeNO levels. By highlighting the relevance of allergen sensitization within the context of T2-low inflammatory profiles, these findings contribute to an improved understanding of asthma pathophysiology and phenotype characterization.

However, the phenotypic characteristics and inflammatory patterns associated with allergen sensitization remain unclear. Although clusters dominated by sensitization to dust mites exhibit the strongest association with asthma onset, information regarding their specific inflammatory profiles is limited.[30,31]

In the biomarker analysis in this study, IL-1RA, MMP3, ST2/IL1R4, and YKL-40/CHI3L, previously reported to be elevated in ACO,[32,33] were found to be increased in Clusters 1 and 2 (**Table 3**). Additionally, in Cluster 2, serum periostin levels were elevated, suggesting the coexistence of T2 inflammation and highlighting the diversity and complexity of the pathophysiology of T2-low asthma. In contrast, no specific biomarkers indicative of a distinct inflammatory profile were identified in Cluster 4.

This study had the following limitations. First, it was limited to Japan with a relatively small number of participants. Second, exclusion of patients receiving OCS or biologics may have introduced a selection bias by underrepresenting individuals with more severe asthma. A supplementary cluster analysis that included these patients revealed four clusters with comparable clinical and immunological profiles. The respective proportions of cluster 1 through 4 patients treated with OCS were 7.1, 7.4, 6.5, and 3.2% (p = 0.83). Biologics were administered in 10.0%, 7.1%, 1.1%, and 5.2%, respectively (p = 0.15), indicating no statistically significant differences across clusters. Notably, the frequency of asthma exacerbations remained highest in Cluster 4 (27.8%, 33.3%, 23.1%, and 61.5% in Clusters 1 through 4, respectively), reinforcing the robustness of the original clustering results. These findings suggest minimal potential impact of selection bias on the primary analysis results. Finally, the assessment of T2-low asthma was based on blood eosinophil counts and FeNO levels measured during routine clinical practice in patients who were already undergoing asthma treatment. Therefore, the findings suggest the presence of a "T2-low inflammatory state" rather than a definitive diagnosis of T2-low asthma. Particularly in patients undergoing ongoing treatment, the evaluation of T2-low status is inherently complex and requires multiple longitudinal measurements of biomarkers for accurate classification.[34,35] These limitations highlight the need for further investigation in future studies.

In conclusion, allergen sensitization may be involved in the development of an exacerbation-prone phenotype in adult patients with T2-low inflammation. The findings of this study suggest that assessing allergen sensitization in T2-low asthma helps evaluate the risk of exacerbations and informs the consideration of therapeutic strategies, such as environmental control, allergen immunotherapy, and treatment with omalizumab.

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Conflict of interest

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Author contributions

- Sahoko Imoto: Investigation, Data Curation, Writing – Original Draft.
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- Yuma Fukutomi: Investigation, Resources.
- Nobuyuki Kobayashi: Investigation, Resources.
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- Hiroyuki Nagase: Conceptualization, Methodology.
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