APJAI
Asian Pacific Journal of
Allergy and Immunology

ORIGINAL ARTICLE

Type 2 high asthma phenotype in children: A multidimensional clustering analysis

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Abstract

Background: Asthma is a heterogeneous disease with diverse and often poorly defined phenotypes, particularly in children.

Objective: We aimed to classify childhood asthma phenotypes using unsupervised cluster analysis based on type 2 (T2) biomarkers and to evaluate their clinical characteristics and outcomes.

Methods: We retrospectively analyzed 614 pediatric patients. Hierarchical clustering was performed using four variables: age, absolute eosinophil count (AEC), eosinophil cationic protein (ECP), and total immunoglobulin E (IgE). Clinical characteristics and 2-year outcomes were compared across clusters. The effect of age was examined using analysis of covariance and age-tertile subgroup analyses.

Results: Three distinct clusters were identified. Cluster 2, the T2-high asthma group (n = 157; median age, 8.0 years), was characterized by male predominance (69.4%), the highest levels of T2 biomarkers (AEC, ECP, IgE, and FeNO; all $P < 0.001$), airway hyperresponsiveness (bronchodilator response, $P = 0.024$; PC20, $P < 0.001$), and reduced lung function (forced expiratory volume in 1 second, $P = 0.002$). By contrast, Cluster 1 (n = 252; median age, 4.0 years) showed the highest exacerbation and steroid use rates but relatively low T2 biomarker levels. Cluster 3 (n = 205; median age, 12.0 years) had moderate T2 levels and the lowest exacerbation burden. After adjusting for age, Cluster 2 maintained 4- to 6-fold higher T2 biomarker levels compared with the other clusters (all $P < 0.001$). These cluster-specific differences were not observed in the age-tertile subgroup analysis.

Conclusion: The identified school-age T2-high cluster in childhood asthma exhibits distinct immunologic and clinical phenotypes, characterized by increased airway hyperresponsiveness, atopic features, and reduced lung function.

Key words: Asthma, Children, Cluster, Exacerbation, Type 2 inflammation

Citation:
Bae, S., Lee, H. (0000). Type 2 high asthma phenotype in children: A multidimensional clustering analysis. *Asian Pac J Allergy Immunol*, 00(0), 000-000. https://doi.org/10.12932/ap-151025-2163

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Introduction

Asthma, a heterogeneous disorder with diverse underlying mechanisms, can be classified into various phenotypes based on clusters of demographic, clinical, and immunopathological characteristics, facilitating more precise treatment approaches for severe cases.[1] With advances in research on asthma endotypes, targeting type 2 (T2) cytokines with novel biologics has emerged as a new treatment paradigm, with applications increasingly expanding to pediatric populations.[2]

Despite these advancements, prior clustering studies have addressed heterogeneity using both phenotypes and endotypes. Early-onset atopic asthma has been consistently identified as a distinct phenotype, though its clinical and functional profiles vary among studies.[3,4] For instance, one study defined early-onset atopic asthma based on airflow limitation and reported that the subgroup with marked limitation had greater healthcare utilization

and required higher doses of inhaled corticosteroids (ICS).[3] Another cohort study demonstrated that children with highly atopic asthma experienced significant airway obstruction and frequent exacerbations.[5] Conversely, other research has described early-onset atopic asthma as showing preserved pulmonary function.[4]

In pediatric populations, the use of T2 biomarkers to define phenotypes has been inconsistent. Several studies have reported varying definitions and thresholds for T2-high status. One study identified a phenotype with elevated total immunoglobulin E (IgE), elevated absolute eosinophil count (AEC), and multiple allergen sensitizations that exhibited frequent exacerbations despite preserved forced expiratory volume in 1 second (FEV1).[6] Conversely, some studies compared IgE levels without identifying a clear T2-high cluster.[3,4] In adults, a large-scale clustering analysis in severe asthma identified five distinct clusters, each characterized by elevation in only a subset of T2 biomarkers (IgE, AEC, or fractional exhaled nitric oxide [FeNO]), with none showing concurrent elevation in all three markers.[7]

This study was performed to identify asthma clusters in children based on T2 endotypes, with the ultimate goal of facilitating personalized management by linking immunologic features to clinical phenotypes.

Methods

Study population

A retrospective chart review was conducted of patients diagnosed with asthma according to the International Classification of Diseases, Tenth Revision (ICD-10 codes J45 and J46), at the Department of Pediatrics, Division of Allergy and Pulmonology, Seoul St. Mary's Hospital, between 1 January 2016 and 28 February 2023. A total of 614 patients with asthma without other underlying diseases were enrolled in the study. For patients under 6 years of age, those who experienced three or more recurrent episodes of typical asthma symptoms (wheezing, dyspnea, and chronic cough) per year and had either evidence of allergic sensitization on laboratory testing or a family history of allergic diseases were diagnosed with asthma according to the Global Initiative for Asthma guidelines.[1] For patients aged 6 years or older who were able to undergo pulmonary function testing (PFT), those who experienced the typical asthma symptoms described above and exhibited a significant bronchodilator response (defined as at least a 12% improvement from baseline in FEV1 on PFT) and/or a 20% decrease in FEV1 on a methacholine bronchial provocation test at ≤ 16 mg/mL (i.e., PC20) were diagnosed with asthma. The study was approved by the institutional review board of Seoul St. Mary's Hospital (No. KC23RASI0191). Because the data were anonymized, the requirement for written informed consent was waived.

Clinical data collection

We retrospectively reviewed the electronic medical records of all patients, including demographics; allergic comorbidities such as atopic dermatitis, allergic rhinitis, and food allergy; laboratory findings; and lung function. Laboratory tests included a complete blood count with differential, AEC, eosinophil cationic protein (ECP), total IgE, and allergen-specific IgE. The highest values of AEC, ECP, IgE, and FeNO measured during the 1-year period following asthma diagnosis were used. Allergen-specific IgE testing was conducted using the multiple-antigen simultaneous test with the AdvanSure AlloStation Alloview 2.0 (LG Life Science, Seoul, Korea) or the UniCAP system with the Phadia 200 (Phadia AB, Uppsala, Sweden). There were 67 missing values for allergen-specific IgE results. A result was considered positive when a level of at least 0.35 kU/L was obtained on either test. All allergen-specific antigens were classified into several groups based on antigen type (**Supplementary Table S1**). Lung function was assessed using spirometry, and predicted values were calculated using the equations from Choi et al. There were 283 missing values for pulmonary function data.

Maintenance treatment was classified into steps according to the Global Initiative for Asthma guideline based on the dosage of ICS in use.[1] Significant asthma exacerbations were assessed over a 2-year period following asthma diagnosis. An exacerbation event was defined as an emergency room visit or hospital admission due to asthma exacerbation, characterized by worsening symptoms such as dyspnea, cough, wheezing, or chest tightness during the follow-up period. Each exacerbation was classified as either viral or non-viral based on respiratory virus polymerase chain reaction results, with particular attention to rhinovirus. Asthma exacerbation rates were calculated as the number of exacerbations per person-year, representing the annual rate of exacerbations experienced by an individual.

Statistical analysis

Cluster variables included age and T2 biomarkers. The T2 biomarkers analyzed were AEC, ECP, IgE, and FeNO, with iterative approaches used to identify the optimal combination of variables (**Figure 1**). The final variables selected for analysis were age, AEC, ECP, and IgE (**Figure 1B**). Additionally, to determine whether these findings were influenced by age, participants were stratified into age tertiles for further comparison; however, the distinct T2-high group observed in **Figure 1B** was not consistently identified in this analysis (**Figure 1D–F**). Missing data included 35 values for AEC, 156 for ECP, and 110 for IgE, all of which were imputed using the random forest method. All variables were standardized before clustering to ensure equal weighting. Cluster analysis was conducted using a hierarchical clustering method with Ward's linkage.

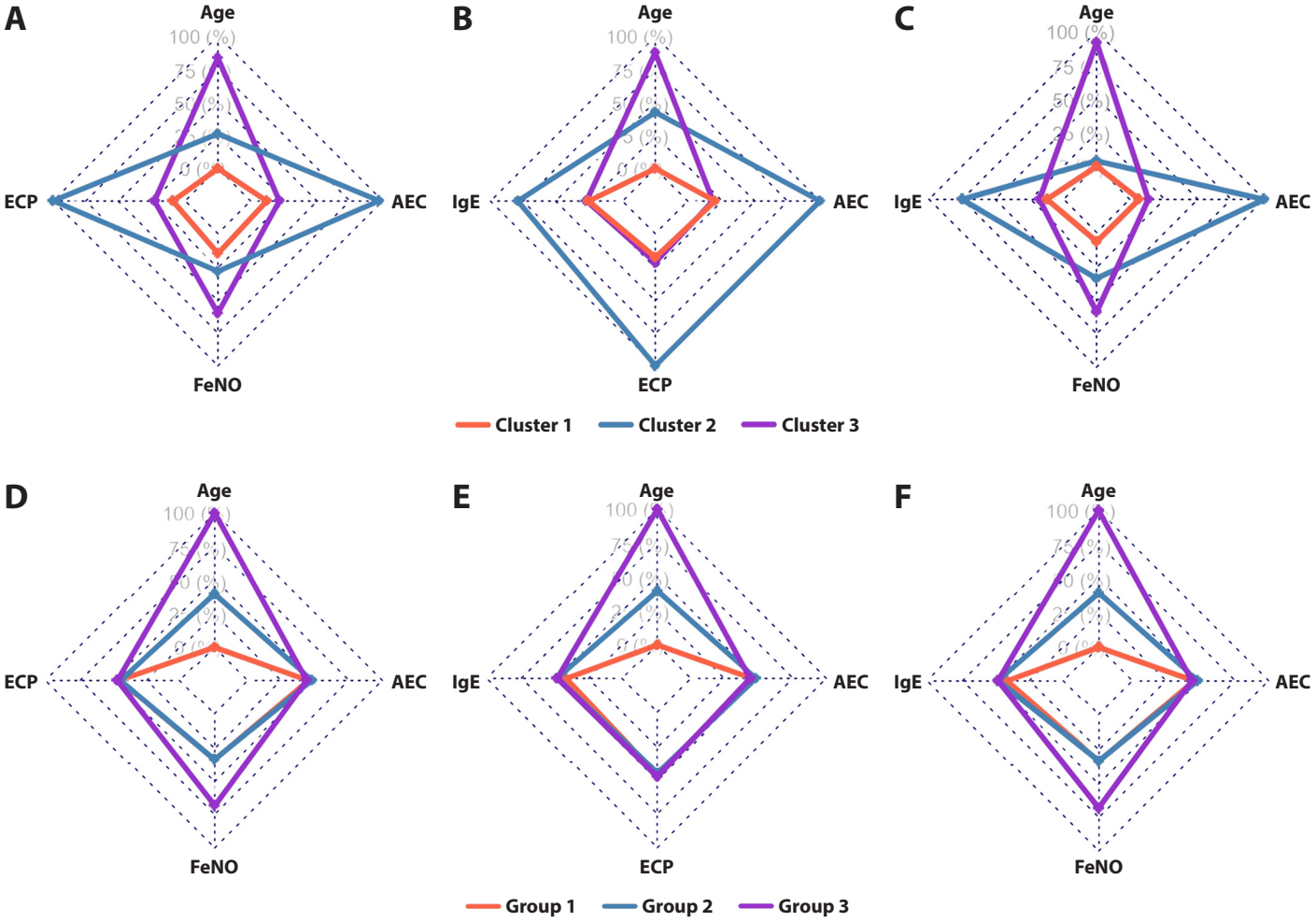

Figure 1. Schematic visualization of clustering analysis using different combinations of T2 biomarkers.
Radar plots comparing standardized T2 biomarker levels across clusters using different combinations: (A) Age, ECP, AEC, and FeNO; (B) Age, IgE, AEC, and ECP (final selected combination for analysis); (C) Age, IgE, AEC, and FeNO. Cluster 2 (blue) consistently shows the highest T2 biomarker levels. Panels (D-F) show age tertile-based groups for comparison.
ECP, eosinophil cationic protein; FeNO, fractional exhaled nitric oxide; IgE, immunoglobulin E; AEC, absolute eosinophil count.

The optimal number of clusters was determined based on the dendrogram and validated using silhouette analysis and the gap statistic. To evaluate differences among clusters, the Kruskal–Wallis test was applied for continuous variables and the chi-square test for categorical variables, with Bonferroni correction for multiple comparisons. Analysis of covariance (ANCOVA) was performed for each biomarker, with cluster as the main factor and age as a covariate, to assess cluster differences independent of age effects. Adjusted means and 95% confidence intervals were calculated, and F-statistics were used to test for significant differences among clusters after controlling for age.

All analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC, USA) and R software version 4.3.1 (R Foundation for Statistical Computing, Vienna, Austria).

Results

Among 614 patients, hierarchical clustering identified three distinct clusters with significant differences in age, T2 biomarkers (AEC, ECP, IgE, and FeNO), atopic features, and clinical outcomes during the 2-year follow-up (**Table 1**).

Cluster characteristics

Cluster 1 (n = 252, median age 4.0 years) represented the youngest group, with frequent viral-induced exacerbations. These children had the lowest rate of allergen sensitization (70%) but the highest exacerbation rates (30.952 ± 2.478/100 patient-years [PY], $P < 0.001$), particularly viral exacerbations (18.651 ± 1.924/100 PY), including rhinoviral episodes (12.103 ± 1.550/100 PY). Systemic steroid and ICS use was highest in this cluster (**Table 2**).

Table 1. Characteristics of 614 subjects and their comparisons between Clusters.

	Total (n = 614)	Cluster 1 (n = 252)	Cluster 2 (n = 157)	Cluster 3 (n = 205)	*P*
Age (y), median [IQR]	7.0 [4.0; 11.0]	4.0 [3.0; 6.0]	8.0 [5.0; 11.0]	12.0 [9.0; 15.0]	< 0.001†‡§
Sex (Male, %)	380 (61.9)	160 (63.5)	109 (69.4)	111 (54.1)	0.01‡
AEC (/mm³), median [IQR]	290.0 [130.0; 540.0]	210.0 [90.0; 360.0]	750.0 [600.0; 1050.0]	200.0 [110.0; 300.0]	< 0.001†‡
IgE (IU/mL), median [IQR]	216.5 [50.2; 588.3]	122.4 [29.0; 321.3]	749.9 [350.8; 1728.5]	167.2 [53.1; 396.1]	< 0.001†‡
ECP (ng/mL),median [IQR]	59.3 [31.5; 95.1]	43.4[20.5; 65.0]	127.0 [102.0; 176.0]	49.0[28.6; 69.4]	< 0.001†‡
FeNO (ppb), median [IQR]	17.8 [13.0; 26.0]	15.1 [11.9; 18.9]	25.2 [16.7; 37.0]	19.0 [12.7; 30.3]	< 0.001†‡§
Comorbidities (%)					
Food allergy	107 (17.4)	45 (17.9)	35 (22.3)	27 (13.2)	0.074
Atopic dermatitis	171 (27.9)	66 (26.2)	60 (38.2)	45 (22.0)	0.002‡
Allergic rhinitis	322 (52.4)	104 (41.3)	85 (54.1)	133 (64.9)	< 0.001§
Family history (%)					
Asthma	98 (16.0)	45 (17.9)	24 (15.3)	29 (14.1)	0.54
Allergic rhinitis	258 (42.0)	121 (48.0)	58 (36.9)	79 (38.5)	0.041
Allergen-specific IgE positivity (%)					
All allergens	446 (81.5)	161 (70.0)	130 (97.0)	155 (84.7)	< 0.001†‡§
Indoor allergens	380 (69.6)	121 (52.8)	118 (88.1)	141 (77.0)	< 0.001†§
Indoor (HDM) allergens	321 (58.8)	87 (38.0)	110 (82.1)	124 (67.8)	< 0.001†§
Pollen allergens	194 (35.5)	65 (28.3)	67 (50.0)	62 (33.9)	< 0.001†‡
Pollen (tree) allergens	164 (30.0)	56 (24.3)	59 (44.0)	49 (26.8)	< 0.001†‡
Pollen (grass) allergens	118 (21.6)	35 (15.2)	46 (34.3)	37 (20.2)	< 0.001†‡
Pollen (weed) allergens	134 (24.5)	42 (18.3)	52 (38.8)	40 (21.9)	< 0.001†‡
Food allergens	261 (47.7)	100 (43.5)	88 (65.7)	73 (39.9)	< 0.001†‡
Class 1 food allergens	198 (36.2)	92 (40.0)	65 (48.5)	41 (22.4)	< 0.001†‡
Class 2 food allergens	123 (22.5)	25 (10.9)	54 (40.3)	44 (24.0)	< 0.001‡§
Pulmonary function test, median [IQR]					
FVC (%), median [IQR]	96.0 [86.0; 107.0]	94.5 [86.0; 107.0]	93.0 [84.0; 104.0]	97.5 [88.0; 107.0]	0.137
FEV1 (%), median [IQR]	88.0 [78.0; 97.0]	89.5 [83.0; 100.0]	84.0 [75.0; 93.0]	89.0 [78.5; 98.0]	0.002†
BDR (%), median [IQR]	8.0 [2.0; 22.5]	5.5 [0.0; 24.5]	12.0 [5.0; 27.0]	6.5 [2.0; 21.0]	0.024
PC20, median [IQR]	25.0 [7.2; 25.0]	21.9 [4.8; 25.0]	7.8 [1.9; 25.0]	25.0 [16.5; 25.0]	< 0.001‡

Note: Values are presented as median [inter-quartile range]. Allergen-specific IgE positivity was defined when class 1 or more was confirmed in the immunoassay capture test (UniCAP) or multiple allergen simultaneous test (MAST). Each specific allergen was grouped as shown in **Table S1**. *P* values indicate the significance level of the Kruskal-Wallis test for between-group comparisons. Bonferroni correction was applied for multiple comparisons. †Cluster 1 versus 2, ‡Cluster 2 versus 3, §Cluster 1 versus 3

Abbreviations: AEC, absolute eosinophil count; IQR, inter-quartile range; IgE, immunoglobulin E; ECP, eosinophil cationic protein; FeNO, fractional exhaled nitric oxide; HDM, house dust mite; FVC, forced vital capacity; FEV1, forced expiratory volume in one second; BDR, bronchodilator responsiveness; PC20, provocative dose of methacholine that results in a 20% fall in FEV1.

Table 2. Two-year outcomes across Clusters, including medication use and acute exacerbations.

	Total (n = 614)	Cluster 1 (n = 252)	Cluster 2 (n = 157)	Cluster 3 (n = 205)	*P*
Medication use					
OCS (mg/kg/year prednisolone), median [IQR]	2.0 [0.0;7.9]	5.9 [0.0; 11.9]	1.5 [0.0; 6.9]	0.0 [0.0; 4.0]	< 0.001†§
ICS (mcg/year), median [IQR]	30000.0 [2000.0; 82500.0]	41250.0 [6000.0; 95250.0]	19200.0 [0.0; 76800.0]	28800.0 [0.0; 62100.0]	0.01
Highest maintenance step ≥ 4 (%)	84 (13.7)	28 (11.1)	26 (16.6)	30 (14.6)	0.29
Acute exacerbation events (Event /100 patient-year)					
Total exacerbation	19.300 ± 1.254	30.952 ± 2.478	14.650 ± 2.160	8.537 ± 1.443	< 0.001†§
Viral exacerbation	9.935 ± 0.899	18.651 ± 1.924	6.369 ± 1.424	1.951 ± 0.690	< 0.001†‡§
Nonviral exacerbation	1.221 ± 0.315	0.992 ± 0.444	0.955 ± 0.552	1.707 ± 0.645	> 0.999
Rhinoviral exacerbation	6.922 ± 0.751	12.103 ± 1.550	5.096 ± 1.274	1.951 ± 0.690	< 0.001†§

Note: Values are presented as median [inter-quartile range]. P values indicate the significance level of the Kruskal-Wallis test for between-group comparisons. Bonferroni correction was applied for multiple comparisons. †Cluster 1 versus 2, ‡Cluster 2 versus 3, §Cluster 1 versus 3
Abbreviations: OCS, oral corticosteroid; IQR, inter-quartile range; ICS, inhaled corticosteroid.

Cluster 2 (n = 157, median age 8.0 years) exhibited a T2-high phenotype with male predominance (69.4%, *P* = 0.01) and the highest prevalence of atopic dermatitis (38.2%, *P* = 0.002). This cluster demonstrated peak T2 biomarker levels: AEC (750.0 [600.0; 1050.0]/mm3), ECP (127.0 [102.0; 176.0] ng/mL), total IgE (749.9 [350.8; 1728.5] IU/mL), and FeNO (25.2 [16.7; 37.0] ppb; all *P* < 0.001). Notably, the prevalence of allergen sensitization was 97% (defined as at least one positive skin prick test), with a predominant pattern of multiple allergen sensitizations. Pulmonary function test results revealed significant airway hyperresponsiveness, with low PC20 (7.8 [1.9; 25.0] mg/mL, *P* < 0.001), high bronchodilator responsiveness (12.0 [5.0; 27.0]%, *P* = 0.024), and reduced FEV1 (84.0 [75.0; 93.0]%, *P* = 0.002).

Cluster 3 (n = 205, median age 12.0 years) represented the oldest group, with predominantly allergic rhinitis (64.9%, *P* < 0.001) and elevated FeNO (19.0 [12.7; 30.3] ppb), while other atopic features were less pronounced. This cluster had the lowest exacerbation rates (8.537 ± 1.443/100 PY, *P* < 0.001) and minimal systemic steroid use.

Age-adjusted analysis

ANCOVA controlling for age confirmed that differences in T2 biomarkers among clusters remained highly significant (**Table 3**). Cluster 2 maintained 4- to 6-fold higher levels compared with the other clusters: AEC (F = 283.2, *P* < 0.001), ECP (F = 231.6, *P* < 0.001), total IgE (F = 73.2, *P* < 0.001), and FeNO (F = 9.1, *P* < 0.01).

Table 3. Age-adjusted analysis of T2 Biomarkers across clusters. (ANCOVA Results)

Biomarker	Age Effect (β)	Cluster 1	Cluster 2	Cluster 3	F-value	*P*-value
AEC (/mm³)	-0.0058				F(2,575) = 283.2	< 0.001
Age-adjusted mean (95% CI)		0.22 (0.19-0.24)	0.91 (0.82-1.00)	0.25 (0.23-0.27)		
ECP (ng/mL)	0.0198				F(2,454) = 231.6	< 0.001
Age-adjusted mean (95% CI)		42.26 (37.95-46.57)	138.63 (126.46-150.80)	47.35 (42.49-52.21)		
Total IgE (IU/mL)	-0.9265				F(2,500) = 73.2	< 0.001
Age-adjusted mean (95% CI)		229.34 (184.40-274.28)	1469.12 (1112.80-1825.44)	258.28 (212.43-304.12)		
FeNO (ppb)	2.2146				F(2,316) = 9.1	< 0.01
Age-adjusted mean (95% CI)		22.04 (20.35-23.73)	34.53 (27.40-41.66)	21.39 (18.13-24.64)		

Age-adjusted means (95% confidence intervals) from ANCOVA (Analysis of Covariance) controlling for age.
Abbreviations: AEC, absolute eosinophil count; CI, confidence interval; ECP, eosinophil cationic protein; IgE, immunoglobulin E; FeNO, fractional exhaled nitric oxide

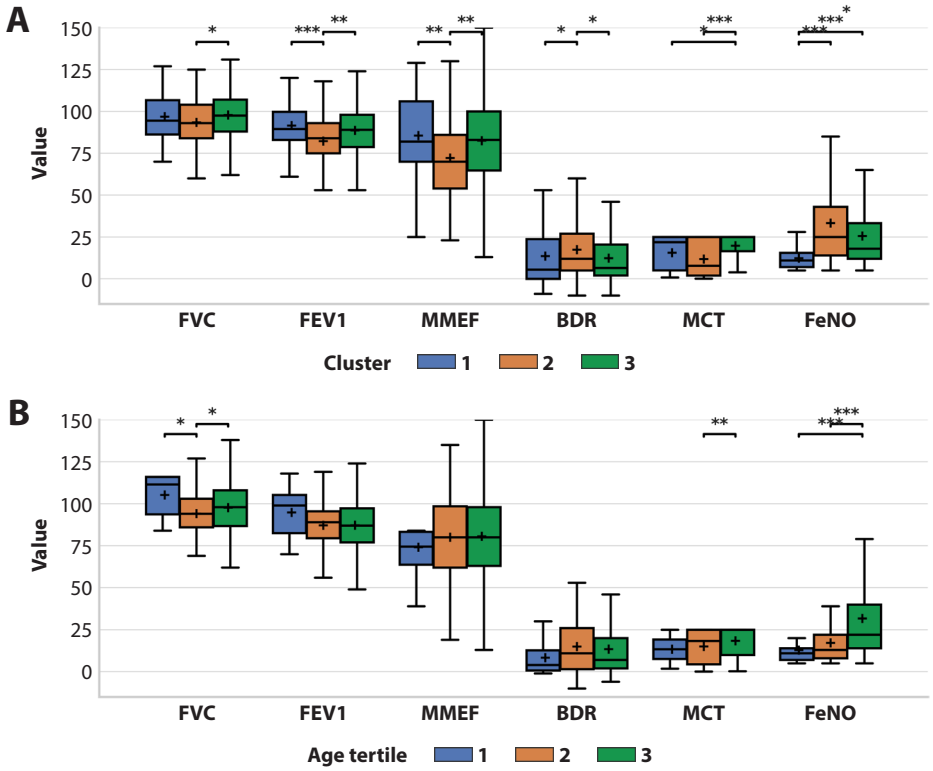

Figure 2. Pulmonary function and airway inflammation across clusters and age groups.
Box plots comparing pulmonary function parameters and airway inflammation markers across (A) clusters and (B) age tertile groups. Statistical significance: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

FVC, forced vital capacity (% predicted); FEV1, forced expiratory volume in one second (% predicted); MMEF, maximal mid-expiratory flow (% predicted); BDR, bronchodilator responsiveness (%); MCT, methacholine challenge test PC20 (mg/mL, displayed as inverse log scale); FeNO, fractional exhaled nitric oxide (ppb).

Allergen sensitization patterns

Cluster 2 demonstrated consistently higher sensitization rates across all allergen categories compared with the other clusters, with extensive allergen sensitization (97.0% vs 70.0% and 84.7%, $P < 0.001$) (**Figure 3A**). Indoor allergen sensitization was most pronounced (88.1% vs 52.8% and 77.0%, $P < 0.001$), primarily driven by house dust mites (82.1% vs 38.0% and 67.8%, $P < 0.001$). Pollen allergen sensitization was also highest in Cluster 2 (50.0% vs 28.3% and 33.9%, $P < 0.001$), including tree pollens (44.0% vs 24.3% and 26.8%) and grass pollens (34.3% vs 15.2% and 20.2%).

Class 2 food allergens (fruits/vegetables) showed the highest sensitization in Cluster 2 (40.3% vs 10.9% and 24.0%, $P < 0.001$), while Class 1 food allergens also demonstrated the highest rates in Cluster 2 (48.5%) compared with Clusters 3 (22.4%) and 1 (40.0%) ($P < 0.001$).

Age tertile–based analysis showed gradual increases in indoor allergen sensitization with age and decreases in Class 1 food allergen sensitization. However, no single age group exhibited the extensive sensitization profile observed in Cluster 2.

Age tertile-based subgroup analysis

Age tertile–based analysis (median ages: 3.0, 7.0, and 13.0 years) showed that AEC and ECP levels did not differ significantly among age groups, whereas total IgE and FeNO were higher in the oldest group, consistent with age-related reference ranges (**Supplementary Table S2**). Importantly, no single age group demonstrated the extensive allergen sensitization pattern observed in Cluster 2, supporting cluster-based grouping over age-alone categorization for identifying distinct asthma phenotypes. By contrast, asthma exacerbations, as well as the use of systemic and inhaled corticosteroids, were significantly higher in the youngest group (Group 1; 37.391 ± 2.851/100 PY, $P < 0.001$), aligning with the findings for Cluster 1 (**Supplementary Table S3**).

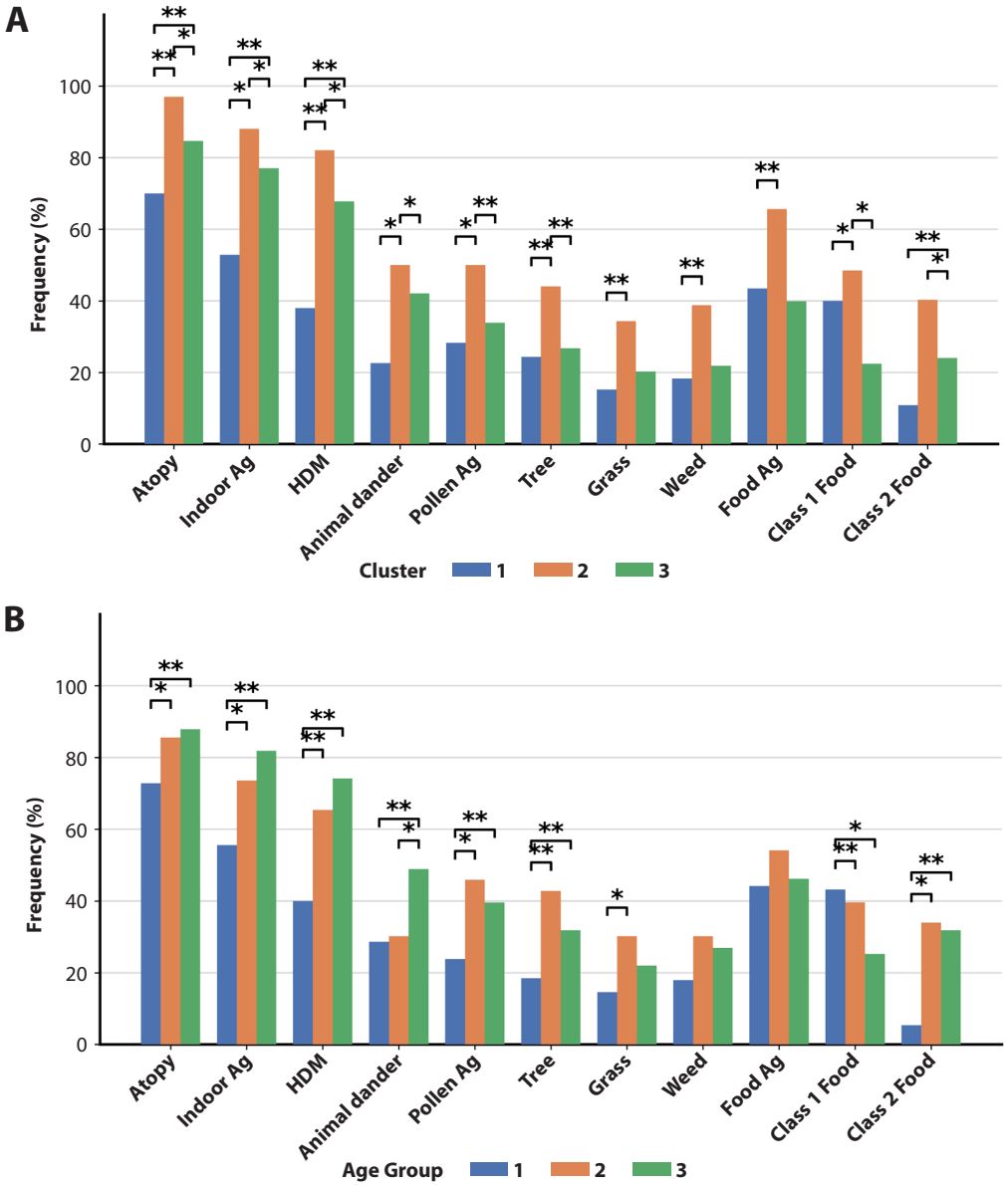

Figure 3. Allergen sensitization patterns across clusters and age groups.
Comparison of allergen-specific IgE sensitization frequencies across (A) clusters and (B) age tertile groups. Statistical significance: *P < 0.05, **P < 0.01, ***P < 0.001.

Atopy, any allergen sensitization; Ag, allergens; HDM, house dust mite allergens; Class 1 Food, basic food allergens (milk, egg, wheat, etc.); Class 2 Food, cross-reactive food allergens (fruits, vegetables).

Discussion

T2 inflammation is a key driver of asthma and a major target for biologic therapies. In this study, Cluster 2 exhibited distinct T2-high asthma features, characterized by elevated T2 biomarkers and extensive allergen sensitization. These findings suggest that T2-high asthma emerges as a distinct phenotype during school age, with male predominance and atopic comorbidities, and that airway hyperresponsiveness and reduced lung function may reflect early T2-driven pathologic remodeling.[8,9] Epithelial damage can trigger IL-33 release, promoting airway remodeling through IL-13–mediated cascades.[9] Notably, these functional deficits distinguish our cohort from previous findings. In the Severe Asthma Research Program, which included patients older than 12 years, atopic asthma clusters generally exhibited preserved lung function, with chronic airflow obstruction primarily associated with longer disease duration.[10] Similarly, the Korean Childhood Asthma Study identified a male-dominant atopic asthma cluster resembling the demographic profile of our Cluster 2, yet that group also demonstrated relatively preserved pulmonary function.[4] The significant airway hyperresponsiveness and reduced lung function observed in our Cluster 2, in contrast to these cohorts, highlight a distinct phenotype that may experience T2-driven airway remodeling even at school age. Despite markedly elevated T2 biomarkers, Cluster 2 maintained relatively controlled exacerbation rates while receiving the lowest ICS doses among all clusters during follow-up, suggesting stable clinical outcomes under routine corticosteroid-based management.

This observation aligns with prior studies showing that eosinophilic, T2-high asthma can remain clinically well controlled even in the presence of a high inflammatory burden.[11,12]

The relationship between viral infections and T2 inflammation in pediatric asthma remains complex. Previous studies have shown that patients with T2-high asthma are more susceptible to exacerbations, raising questions about the role of T2 immune–related cells and cytokines in virus-induced asthma exacerbations.[13] In T2-high asthma, antiviral defense mechanisms involving IFN-γ and CD8+ T cells appear to be impaired.[14] In particular, rhinovirus can induce airway damage and exacerbate asthma symptoms by suppressing type I/III IFN production and inducing IL-33 release from airway epithelial cells, a key driver of T2 inflammation linked to asthma development.[15-19] IL-33–mediated responses are significantly elevated in individuals with preexisting asthma compared with naïve controls.[18]

Interestingly, preschool-aged children in Cluster 1 had the highest rates of viral exacerbations despite low T2 markers and preserved PFTs, whereas Cluster 2 showed T2-high inflammation but fewer viral exacerbations. While some studies suggest that T2 inflammation may increase viral susceptibility, our findings indicate that age-related factors play a dominant role in shaping exacerbation patterns in early childhood.[13,14] Using an unsupervised clustering approach across a broad pediatric age range, preschool children with viral-dominant wheezing were naturally grouped into Cluster 1, distinct from the T2-high phenotype observed predominantly in the school-aged group (Cluster 2). Cluster 1 likely represents a preschool viral wheeze phenotype, which has been reported in prior studies to often improve as children transition to school age.[20,21] This pattern supports the inclusion of a heterogeneous pediatric age population, demonstrating that viral-associated exacerbations are more prominent in early childhood, whereas T2-driven features become more apparent with increasing age. Nonetheless, current wheezing status remains the most robust clinical indicator for symptom-based childhood asthma phenotyping, highlighting the need for long-term follow-up to track asthma trajectories from early wheezing.[22]

Cluster 2's extensive allergen sensitization profile supports an intrinsic T2-high predisposition rather than cumulative exposure because no age group achieved this broad sensitization spectrum. While Class 1 food allergens showed the expected age-related decline, reflecting natural resolution, Class 2 food allergens demonstrated a typical T2-high pattern, with peak sensitization in Cluster 2. Class 2 sensitization may serve as a marker of the T2-high phenotype for early identification and targeted therapies. The pronounced indoor allergen sensitization in Cluster 2, particularly to house dust mites, aligns with recent studies demonstrating the central role of perennial allergens in maintaining chronic T2 inflammation.[23] House dust mite allergens are recognized as major triggers of T2 bronchial inflammation through both adaptive and innate immune pathways, with house dust mite sensitization affecting up to 85% of individuals with asthma worldwide.[24] Because these patients exhibit the specific inflammatory pathways targeted by T2 biologics, identifying this distinct phenotype may help stratify patients who are more likely to respond favorably to such mechanism-based interventions.[25,26] These patterns may facilitate early recognition of T2-high asthma and guide personalized biologic therapies targeting T2 pathways.

Our study differs from previous cluster analyses in two key aspects. First, unlike prior studies that often relied on clinical parameters measured during treatment or at mixed time points, we performed clustering based on objective biomarkers obtained shortly after initial diagnosis. This approach minimizes the confounding effects of therapeutic interventions and allows for more accurate identification of intrinsic biological phenotypes. Second, we conducted a stratified analysis of clinical outcomes by specifically distinguishing viral-induced exacerbations from overall asthma exacerbations. This distinction was important for refining the characterization of the T2-high cluster. By separating viral-dominant exacerbations (characteristic of Cluster 1), we showed that the disease burden in Cluster 2 is primarily driven by intrinsic T2 inflammatory pathways rather than transient viral triggers.

Several limitations should be considered when interpreting our findings. First, the retrospective design may introduce selection bias, and the 2-year follow-up period may be insufficient to determine long-term prognosis. Accordingly, our findings should be interpreted as reflecting clinical outcomes during the study period rather than definitive long-term predictions. Second, missing data for some variables, although imputed using validated methods, may have influenced the clustering results. Further studies are needed to investigate long-term phenotypic changes and prognosis, particularly regarding the emergence of the T2-high cluster in relation to age. Nevertheless, this study provides insight into T2-high asthma phenotypes in children, taking age distribution into account and contributing to a better understanding of the role of T2 inflammation in childhood asthma. Our findings are also relevant to the progression and prognosis of adult T2-high asthma, which is well established to originate in early childhood and to be associated with more severe disease.[13,27]

Conclusion

We identified a school-age T2-high cluster characterized by increased airway hyperresponsiveness and reduced lung function. These findings demonstrate that clustering analysis based on T2 biomarkers can identify clinically meaningful asthma phenotypes that are not captured by age-based categorization alone. Further studies are needed to elucidate the immunologic development and the impact of T2 inflammation on asthma prognosis in pediatric patients.

Acknowledgement

Statistical consultation was supported by the Department of Biostatistics of the Catholic Research Coordinating Center.

Conflict of Interest

The authors declare that they have no conflict of interest to disclose.

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Supplemental material

Supplementary Table S1. Categorized allergen-specific Immunoglobulin E based on similarity.

Inhalant allergen	Indoor allergen	Indoor (house dust mite [HDM]) allergens	*Acarus siro*, *Dermatophagoides farinae* (D.f), *Dermatophagoides pteronyssinus* (D.p), house dust, Storage mite and *Tyrophagus putrescentiae*,
		Indoor (fungi and cockroaches) allergens	*Alternaria alternata*, *Aspergillus fumigatus*, *Candida albicans*, *Cladosporium herbarum*, cockroach, *Mucor racemosus*, *Penicillium notatum*, *Rhizopus nigricans* and mold mix
		Indoor (animal dander) allergens	cat, dog, guineapig, hamster, horse, rabbit and sheep
	Pollen allergen	Pollen (tree) allergens	acacia, alder, ash, birch, cypress, Japanese cedar, hazel, latex, oak white, pine, poplar, sallow willow, sycamore and tree mix
		Pollen (grass) allergens	bermuda grass, grass mix, redtop grass, reed grass, rye grass, sweet vernal grass and timothy grass
		Pollen (weed) allergens	cocklebur, dandelion, English plantain, goldenrod, Japanese hop, lamb's quarter, mugwort, oxeye daisy, pigweed, ragweed false, ragweed short, Russian thistle and weed mix
Food allergen	Class 1 food allergens		anchovy, cheddar cheese, codfish, egg white, egg yolk, mackerel, milk, salmon, tuna and wheat
	Class 2 food allergens		apple, banana, bromelain, carrot, celery, citrus, cucumber, peach, potato, kiwi, mango and tomato
	Others allergens		Brazil nut, cacao, cashewnut, chestnut, coconut, hazel nut, macadamia nut, soybean, peanut, walnut, white bean clam, crab, scallop, shrimp, squid, mussel, barley, buckwheat, garlic, honey bee, onion, maize, mushroom, pupa silk cocoon, rice, sesame, silk worm, yeast and yellow jacket.

Supplementary Table S2. Characteristics of 614 subjects based on age tertile-based subgroup analysis.

	Group 1 (n = 230)	Group 2 (n = 172)	Group 3 (n = 212)	*P*
Age (y), median [IQR]	3.0 [2.6; 4.0]	7.0 [6.0; 8.0]	13.0 [11.0; 15.0]	< 0.001†‡§
Sex (Male, %)	147 (63.9)	102 (59.3)	131 (61.8)	0.641
Absolute eosinophil count (/mm^3), median [IQR]	310.0 [120.0; 490.0]	265.0 [140.0; 559.2]	275.0 [140.0; 540.0]	0.78
IgE (IU/mL), median [IQR]	134.2 [32.9; 398.1]	285.6 [79.5; 722.7]	299.0 [78.0; 641.9]	< 0.001†§
ECP (ng/mL), median [IQR]	57.8 [27.5; 94.2]	53.1 [29.5; 86.5]	62.5 [35.8; 101.0]	0.258
FeNO (ppb), median [IQR]	17.0 [13.3; 20.3]	14.0 [10.0; 22.5]	24.0 [15.0; 40.0]	< 0.001‡§
Comorbidities (%)				
Food allergy	44 (19.1)	34 (19.8)	29 (13.7)	0.203
Atopic dermatitis	71 (30.9)	49 (28.5)	51 (24.1)	0.273
Allergic rhinitis	71 (30.9)	116 (67.4)	135 (63.7)	< 0.001†§
Family history (%)				
Asthma	40 (17.4)	30 (17.4)	28 (13.2)	0.4
Allergic rhinitis	104 (45.2)	76 (44.2)	78 (36.8)	0.159
Allergen-specific IgE positivity (%)				
All allergens	150 (72.8)	136 (85.5)	160 (87.9)	< 0.001†§
Indoor allergens	114 (55.6)	117 (73.6)	149 (81.9)	< 0.001†§
Indoor (HDM) allergens	82 (40.0)	104 (65.4)	135 (74.2)	< 0.001†§
Indoor (Fungi & Cockroaches) allergens	17 (8.3)	27 (17.0)	41 (22.5)	< 0.001§
Indoor (Animal dander) allergens	59 (28.6)	48 (30.2)	89 (48.9)	< 0.001‡§
Pollen allergens	49 (23.8)	73 (45.9)	72 (39.6)	< 0.001†§
Pollen (tree) allergens	38 (18.4)	68 (42.8)	58 (31.9)	< 0.001†§
Pollen (grass) allergens	30 (14.6)	48 (30.2)	40 (22.0)	< 0.001†
Pollen (weed) allergens	37 (18.0)	48 (30.2)	49 (26.9)	0.017
Food allergens	91 (44.2)	86 (54.1)	84 (46.2)	0.149
Class 1 food allergens	89 (43.2)	63 (39.6)	46 (25.3)	0.001‡§
Class 2 food allergens	11 (5.3)	54 (34.0)	58 (31.9)	< 0.001†§
Others Positivity	36 (17.5)	33 (20.8)	33 (18.1)	0.71
Pulmonary function test, median [IQR]				
FVC (%), median [IQR]	111.5 [93.5; 116.0]	94.0 [86.0; 103.0]	98.0 [86.5; 108.0]	0.014
FEV1, median [IQR] (%)	99.0 [80.0; 106.5]	89.0 [79.0; 96.0]	87.0 [77.0; 97.5]	0.382
BDR (%), median [IQR]	4.0 [0.5; 14.5]	11.0 [1.0; 26.0]	7.0 [2.0; 20.0]	0.535
PC20, median [IQR]	13.4 [1.9; 25.0]	18.3 [4.3; 25.0]	25.0 [9.8; 25.0]	0.034

Note: Values are presented as median [inter-quartile range]. Allergen-specific IgE positivity was defined when class 1 or more was confirmed in the immunoassay capture test (UniCAP) or multiple allergen simultaneous test (MAST). Each specific allergen was grouped as shown in **Supplementary Table 3**. *P* values indicate the significance level of the Kruskal-Wallis test for between-group comparisons. Bonferroni correction was applied for multiple comparisons. †Cluster 1 versus 2, ‡Cluster 2 versus 3, §Cluster 1 versus 3

BDR, bronchodilator responsiveness; ECP, eosinophil cationic protein; FeNO, fractional exhaled nitric oxide; FEV1, forced expiratory volume in one second; FVC, forced vital capacity; HDM, house dust mite; IgE, immunoglobulin E; IQR inter-quartile range; PC20, provocative dose of methacholine that results in a 20% fall in FEV1

Supplementary Table S3. Two-year outcomes across subgroups, including medication use and acute exacerbations.

	Group 1 (n = 230)	Group 2 (n = 172)	Group 3 (n = 212)	*P*
Medication use				
OCS (mg/kg/year prednisolone), median [IQR]	5.9 [0.0; 12.4]	2.9 [0.0; 8.0]	0.0 [0.0; 3.0]	< 0.001†§
ICS (mcg/year), median [IQR]	42600.0 [6000.0; 112000.0]	19200.0 [0.0; 57600.0]	28800.0 [0.0; 76800.0]	< 0.001†
Highest maintenance step ≥ 4 (%)	10 (4.3)	39 (22.7)	35 (16.5)	< 0.001†§
Acute exacerbation events (Event /100 patient-year)				
Total exacerbation	37.391 ± 2.851	10.174 ± 1.720	7.075 ± 1.292	< 0.001†§
Viral exacerbation	21.304 ± 2.152	5.233 ± 1.233	1.415 ± 0.578	< 0.001†§
Nonviral exacerbation	0.870 ± 0.435	1.163 ± 0.581	1.651 ± 0.624	> 0.999
Rhinoviral exacerbation	13.478 ± 1.712	4.942 ± 1.199	1.415 ± 0.578	< 0.001†§
Rhinovirus ever (%)	49 (21.3)	15 (8.7)	7 (3.3)	< 0.001†§

Note: Values are presented as median [inter-quartile range]. *P* values indicate the significance level of the Kruskal-Wallis test for between-group comparisons. Bonferroni correction was applied for multiple comparisons. †Cluster 1 versus 2, ‡Cluster 2 versus 3, §Cluster 1 versus 3
ECP, eosinophil cationic protein; HDM, house dust mite; ICS, inhaled corticosteroid; OCS, oral corticosteroid.