APJAI
Asian Pacific Journal of
Allergy and Immunology

ORIGINAL ARTICLE

Phenotype characterization and biomarker evaluation in moderate to severe type 2-high asthma

Sahoko Imoto,[1,2] Maho Suzukawa,[1] Yuma Fukutomi,[3] Nobuyuki Kobayashi,[4] Masami Taniguchi,[3,5] Takahide Nagase,[2] Ken Ohta[1,6]

Abstract

Background: There are two major pathological phenotypes of asthma, type 2 (T2)-high and T2-low asthma, which are important in determining treatment strategies. However, the characteristics and phenotypes of T2-high asthma have not yet been fully identified.

Objective: This study aimed to identify the clinical characteristics and phenotypes of patients with T2-high asthma.

Methods: This study used data from a nationwide asthma cohort study in Japan, NHOM Asthma Study. T2-high asthma was defined as a blood eosinophils count ≥ 300 /μL and/or fractional exhaled nitric oxide level ≥ 25 ppb, and the clinical characteristics and biomarkers were compared between T2-high and T2-low asthma. Furthermore, T2-high asthma was phenotyped via hierarchical cluster analysis using Ward's method.

Results: Patients with T2-high asthma were older, less likely to be female, had longer asthma duration, had lower pulmonary function, and had more comorbidities, including sinusitis and SAS. Patients with T2-high asthma showed higher serum thymus and activation-regulated chemokine and urinary leukotriene E4 levels and lower serum ST2 levels than those with T2-low asthma. There were four phenotypes among patients with T2-high asthma: Cluster 1 (youngest, early-onset, and atopic), Cluster 2 (long duration, eosinophilic, and low lung function), Cluster 3 (elderly, female-dominant, and late-onset), and Cluster 4 (elderly, late-onset, and asthma-COPD overlap-dominant).

Conclusion: Patients with T2-high asthma have distinct characteristics and four distinct phenotypes, in which eosinophil-dominant Cluster 2 is the most severe phenotype. The present findings may be useful in precision medicine for asthma treatment in the future.

Key words: asthma, phenotype, type 2 inflammation, cluster analysis, biomarker

Citation:
Imoto, S., Suzukawa, M., Fukutomi, Y., Kobayashi, N., Taniguchi, M., Nagase, T., Ohta, K. Phenotype characterization and biomarker evaluation in moderate to severe type 2-high asthma. *Asian Pac J Allergy Immunol*. https://doi.org/10.12932/ap-021222-1510

Affiliations:
[1] National Hospital Organization Tokyo National Hospital, Tokyo, Japan
[2] Department of Respiratory Medicine, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan
[3] Clinical Research Center, National Hospital Organization Sagamihara National Hospital, Kanagawa, Japan
[4] Fureai Machida Hospital, Tokyo, Japan
[5] Shonan Kamakura General Hospital, Kanagawa, Japan
[6] Japan Anti-Tuberculosis Association, Fukujuji Hospital, Tokyo, Japan

Corresponding author:
Maho Suzukawa
Clinical Research Center,
National Hospital Organization Tokyo National Hospital,
3-1-1 Takeoka, Kiyose-City, Tokyo 204-8585, Japan
E-mail: fueta-tky@outlook.jp

Abbreviations:

ACO	asthma and COPD overlap
ACQ	asthma control questionnaire
AQLQ	asthma quality of life questionnaire
ASK-20	adherence starts with knowledge 20
CI	confidence interval
FeNO	fractional exhaled nitric oxide
FEV_1	forced expiratory volume in the first second
GERD	gastroesophageal reflux disease
GINA	Global Initiative for Asthma
IFN	interferon
IL	interleukin
LTE4	leukotriene E4

MCP-1	monocyte Chemotactic protein 1
MIP-1	macrophage inflammatory protein 1
MMP	matrix metalloproteinase
OCS	oral corticosteroid
OR	odds ratio
PDGF	platelet-derived growth factor
PSL	prednisolone
RANTES	Regulated on activation, normal T cell expressed and secreted
SAS	sleep apnea syndrome
ST2	suppression of tumorigenicity 2
TARC	Thymus and Activation-Regulated Chemokine
TIMP	tissue inhibitor of metalloproteinase
Type 2	T2

Introduction

Asthma is a common respiratory disease characterized by airway hyper-responsiveness, chronic airway inflammation, and airway remodeling. Complex and heterogeneous inflammatory processes are involved in the pathogenesis of asthma, resulting in various phenotypes. Studies have reported on the immunological mechanisms underlying airway inflammation in which effector T cells and innate immune cells, including innate lymphoid cells (ILCs), are intricately involved.[1]

In asthma, two major phenotypes of airway inflammation have been advocated: "Type 2 (T2)-high" and "T2-low" asthma. T2-high asthma is driven by both adaptive immunities involving Th2-helper T cells and ILC2. Both Th2 cells and ILC2 produce Th2 cytokines, such as interleukin (IL)-4, IL-5, and IL-13. IL-4 and IL-13 play various roles in asthma pathogenesis, including B-cell class switch to produce IgE, mucus production, goblet cell hyperplasia, airway smooth muscle contractility, proliferation, and subepithelial fibrosis.[2] IL-5 is an important mediator for eosinophil differentiation, activation, and survival.[2] Hence, T2-high asthma is recognized to include allergic and/or eosinophilic asthma. On the other hand, T2-low asthma has been recognized to include neutrophilic and pauci-granulocytic airway inflammation caused by smoking, air pollutants, and obesity; however, much remains to be discovered.[3] In clinical practice, such classification of T2-high and T2-low asthma would enable us to perform personalized management.

In Japan, patients with severe asthma account for 7-10%, and more than 80% of severe asthma patients are reported to have T2-high asthma.[4] With the recent advances in the development of biologics for asthma therapy, the management of severe asthma, especially T2-high asthma, has remarkably improved. In T2-high asthma, biomarkers such as eosinophil count, IgE, fractional exhaled nitric oxide (FeNO), and periostin are used for classification and predicting the efficacy of biological drugs. However, a limited treatment option for T2-low severe asthma remains a major clinical challenge.

To identify the clinical characteristics and phenotypes in T2-high asthma, the present study used data from a nationwide asthma cohort study in Japan (NHOM Asthma Study) and analyzed differences in clinical indices and biomarkers compared to T2-low asthma. In addition, we further classified T2-high asthma by cluster analysis to investigate the pathogenesis in detail.

Methods

Study design

The present study was a post hoc analysis of the NHOM Asthma Study,[5] 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). NHOM-Asthma was a prospective, multicenter observational cohort study in which details of the study design were described previously.[5] In brief, 1925 enrolled asthma patients from 27 national hospitals in Japan were treated based on their physicians' standard practices to survey the real-world clinical practice for asthma in Japan, and biomarker analysis was performed using the serum and urine samples from the participants receiving GINA 4 or 5 therapy. This study was approved by the Institutional Ethical Review Board of the National Hospital Organization Tokyo National Hospital (No. 220026, June 29, 2022). Informed consent about the secondary use of data was obtained from the enrolled patients in NHOM-Asthma Study.

Assessments and variables

All data were retrieved from the NHOM Asthma Study.[5] In brief, the physician in charge collected the clinical data from the medical charts. The latest data on respiratory function test, blood examination, and treatment regimen were collected within a year of enrolment. The patients completed a questionnaire to obtain demographic data, medical history, comorbidities, asthma control questionnaire (ACQ)-6, asthma quality of life questionnaire (AQLQ), and adherence starts with knowledge 20 (ASK-20). In this post hoc analysis, the primary endpoints were the clinical characteristics and biomarkers of T2-high and T2-low asthma. The secondary endpoint was the clinical phenotype of the patients with T2-high asthma.

Definition of T2 inflammation and exclusion criteria

Based on the understanding that the number of blood eosinophils and FeNO are the most practical and helpful biomarkers for determining T2 inflammation in the airway,[6] blood eosinophils and FeNO were used to define T2 inflammation in the present study. Patients with missing data on blood eosinophil counts or FeNO levels were excluded from the analysis.

Statistical analysis

Univariate analysis was performed using the Student's *t*-test for continuous variables, and a chi-square test was used for non-continuous variables. Multivariable logistic regression analysis was performed to adjust for confounding factors and embossed biomarkers involved in T2-high or T2-low asthma. When the 95% confidence interval of the relative risk of a given factor does not include 1, the value is significant (significance level, 0.05).

Hierarchical cluster analysis using Ward's method was used to create a dendrogram and determine the number of clusters. Variables for cluster modeling were selected based on their contribution to the characterization of asthma phenotypes. Seven continuous variables (age, BMI, age at asthma onset, eosinophil counts, forced expiratory volume in the first second [FEV_1] % predicted, smoking index, and ACQ-6) and two categorical variables (sex and atopy) were selected for cluster analysis. To compare differences between clusters, analysis of variance and Kruskal-Wallis and chi-square tests were used for parametric continuous, nonparametric continuous, and categorical variables, respectively. All statistical analyses were performed using JMP pro16 (SAS Institute Inc., Cary, NC, USA). Statistical significance was set at $p < 0.05$.

Results

The proportions of T2-high and T2-low asthma

First, the proportions of T2-high and T2-low asthma were calculated based on the differential cutoffs for blood eosinophil numbers with FeNO ≥ 25 ppb. When eosinophils ≥ 150/μL was used as the cutoff, as much as 74.0% of the total patients with asthma were defined as having asthma with T2 inflammation. However, 64.4% and 61.5% of the total patients still had T2 inflammation when eosinophils ≥ 250/μL and ≥ 300/μL, respectively, were used as the cutoff values. In T2-high asthma defined by eosinophils ≥ 300/μL and/or FeNO ≥ 25 ppb, the proportion of patients with eosinophil ≥ 300/μL and/or FeNO ≥ 25 ppb is shown in **Figure 1**, left frame. Patients with T2-high asthma who fulfilled both eosinophils ≥ 300/μL and FeNO ≥ 25 ppb accounted for 35.3%, while T2-high asthma with only eosinophils ≥ 300/μL accounted for 15.3%, and only FeNO ≥ 25 ppb accounted for 49.4% among T2-high asthma. Definitions of eosinophils ≥ 300/μL and FeNO ≥ 25 ppb were used for T2-high asthma for the following analysis.

Characteristics of T2-high and T2-low asthma

Of the 1925 asthmatics enrolled, 689 patients with missing data on blood eosinophil counts or FeNO levels were excluded, and data from 1236 patients were analyzed (**Figure 2**). The clinical characteristics of T2-high and T2-low asthma were compared (**Table 1**). Patients with T2-high asthma were significantly older and had a higher proportion of males as compared to T2-low asthma. Patients with T2-high asthma had significantly older asthma onset but longer asthma duration. Regarding comorbidities, patients with T2-high asthma had significantly more sinusitis and sleep apnea syndrome (SAS) and fewer heart disease, and mental disorders. Blood examinations revealed that the T2-high asthma group had significantly higher eosinophil counts and total IgE levels than the T2-low asthma group. The positivity of specific IgE for molds was significantly higher among patients with T2-high asthma. Lung function, as assessed by FEV_1 % predicted, and FEV_1/FVC, was significantly deteriorated in patients with T2-high asthma. The questionnaires revealed that ACQ-6 and AQLQ were equivalent between T2-high and T2-low asthma, as were the number of unscheduled visits, exacerbations requiring systemic steroid, and admissions. For the treatment of asthma, the percentage of each GINA treatment step was similar in both asthma phenotypes. Furthermore, **Supplementary Table 1** also highlights that T2-high asthma requiring OCS or biologics was characterized by a longer asthma duration, lower pulmonary function and poorer asthma control than other T2-high asthma,

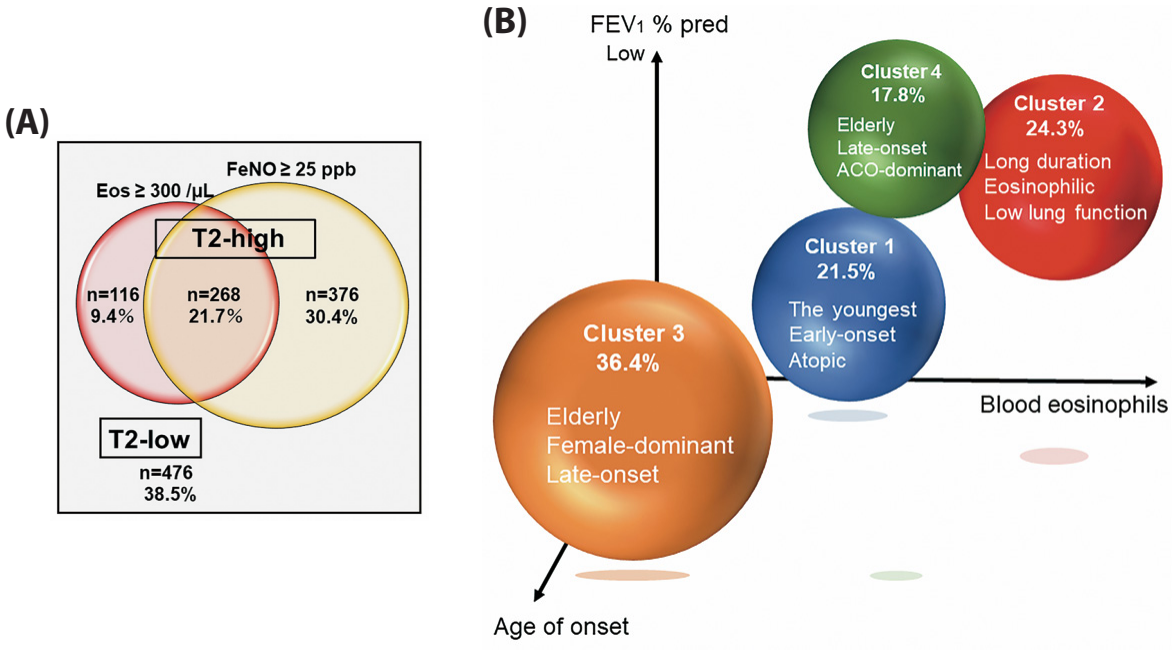

Figure 1. The proportions of T2-high and T2-low asthma (A) and the graphical representation of the characteristics of the four clusters in T2-high asthma with blood eosinophils, age of asthma onset, and FEV_1 % pred (B).

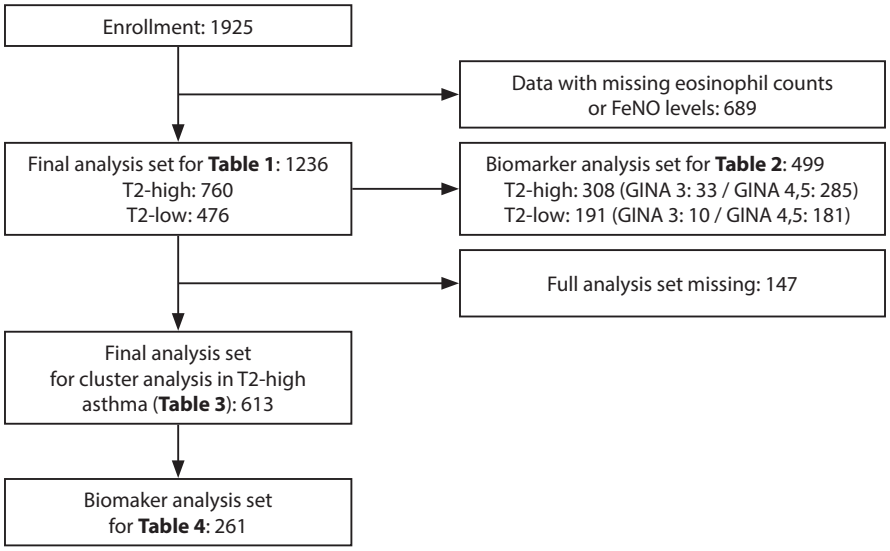

Figure 2. Flow diagram for the study.

Table 1. Baseline characteristics.

Variables	T2-high (n = 760)	T2-low (n = 476)	*P* value
Age at enrolment, years	62.63 ± 0.52†	58.84 ± 0.76	< 0.0001
Sex, % female	55.9	68.9	< 0.0001
BMI, kg/m^2	24.07 ± 0.18	24.06 ± 0.20	0.96
Age of asthma onset, years	44.54 ± 0.81	41.19 ± 1.10	< 0.05
Asthma duration, years	15.82 ± 0.55	13.90 ± 0.69	< 0.05
Smoking status, pack-year	10.42 ± 0.71	10.07 ± 1.06	0.77
Comorbidities, %			
Sinusitis	44.57	36.68	< 0.01
Allergic disease*	63.68	63.87	0.95
COPD	6.34	6.82	0.75
GERD	16.19	20.13	0.082
SAS	8.71	5.41	< 0.05
Heart disease	5.03	8.01	< 0.05
Mental disorder	9.18	17.08	< 0.0001
Laboratory findings			
Blood eosinophils, /μL	365.70 ± 12.85	116.00 ± 3.65	< 0.0001
Total IgE, IU/mL	545.30 ± 46.63	305.30 ± 34.62	< 0.001
Atopy‡, %	60.39	58.61	0.53
Specific IgE (dust mites), %	48.44	44.91	0.24
Specific IgE (molds), %	40.52	19.87	< 0.0001
Specific IgE (cats/dogs), %	17.66	14.61	0.18
Specific IgE (pollen), %	59.94	55.51	0.14
Specific IgE (insects), %	31.90	30.38	0.62
FEV_1 % pred, %	91.87 ± 0.82	100.80 ± 1.03	< 0.0001
FEV_1/FVC, %	70.48 ± 0.47	75.95 ± 0.55	< 0.0001
FeNO, ppb	51.12 ± 1.47	14.80 ± 0.25	< 0.0001
The questionnaires score			
ACQ-6	0.76 ± 0.03	0.69 ± 0.028	0.13
AQLQ total score	5.92 ± 0.033	5.89 ± 0.036	0.60
GINA treatment steps, %			0.74
1	5.92	5.25	
2	6.71	5.46	
3	22.76	22.06	
4	34.47	37.82	
5	30.13	29.41	
No of unscheduled visits	0.42 ± 0.066	0.44 ± 0.086	0.48
No of exacerbations requiring systemic steroid	0.94 ± 0.14	0.92 ± 0.16	0.93
No of admissions	0.088 ± 0.026	0.080 ± 0.029	0.67

†Numeric data are expressed as mean ± SD.
*Including allergic rhinitis, allergic conjunctivitis, atopic dermatitis, pollinosis, food allergies, drug allergies, anaphylaxis, and urticaria
‡Specific IgE responsiveness to common inhaled allergen
ACQ, asthma control questionnaire; AQLQ, asthma quality of life questionnaire; FEV_1, forced expiratory volume in the first second; FeNO, fractional exhaled nitric oxide; GERD, gastroesophageal reflux disease; GINA, Global Initiative for Asthma; SAS, sleep apnea syndrome

which is a feature of asthma specific to the T2-high phenotype. The positivity for mold-specific IgE was significantly higher in T2-high asthma requiring OCS or biologics compared to other T2-high asthma, which may further support their involvement (**Supplementary Table 1**).

Serum and urine biomarker levels of T2-high and T2-low moderate-to-severe asthma

In the NHOM Asthma Study, serum and urine samples were collected from patients receiving GINA treatment steps 4 or 5 from the physicians' report, but patients with GINA 3 therapy were mixed as shown in **Figure 2**.

As shown in **Table 2**, the levels of serum IL-5, thymus and activation-regulated chemokine (TARC), tissue inhibitor of metalloproteinase 1 (TIMP-1), and urinary leukotriene E4 (LTE4) were significantly higher in T2-high asthma compared to T2-low asthma (IL-5: 3.20 ± 0.21 pg/mL vs. 2.45 ± 0.11 pg/mL, $p < 0.01$; TARC: 0.85 ± 0.056 ng/mL vs. 0.67 ± 0.036 ng/mL, $p < 0.05$; TIMP-1: 138.14 ± 1.86 ng/mL vs. 132.15 ± 1.74 ng/mL, $p < 0.05$; Urinary LTE4: 203.90 ± 31.01 pg/mg creatinine vs. 110.50 ± 7.60 pg/mg creatinine, $p < 0.05$) while no cytokine was higher in T2-low asthma. Notably, serum IL-4, IL-13, and periostin levels, the major players in T2 inflammation, were equivalent between the T2-high and T2-low asthma groups.

Next, the users of oral corticosteroids (OCS) and biologics were excluded because these drugs may affect cytokine levels; to adjust for confounders such as age, sex, BMI, smoking history, and comorbidities, multivariable logistic regression models were also constructed (**Table 2**). TARC and urinary LTE4 were independently associated with T2-high asthma (TARC: 1.733 [1.130-2.657], $p < 0.05$; urinary LTE4: 1.756 [1.288-2.394], $p < 0.001$). In contrast, suppression of tumorigenicity 2 (ST2) was found to be an independent biomarker for T2-low asthma (ST2: 0.945 [0.895-0.998], $p < 0.05$). The emphasis on IL-4 and IL-5 as biomarkers when analyzed for the most severe form of T2-high asthma, which requires the use of OCS or biologics, suggests that they may not be highly sensitive indicators (**Supplementary Table 2**).

Table 2. Comparison of biomarkers between T2-high and T2-low moderate to severe asthma and multivariable logistic regression analysis of biomarkers for T2-high asthma.

Variables	Univariate analysis			Multivariable logistic regression analysis		
	T2-high (n = 308)	T2-low (n = 191)	*P* value	OR	95%CI	*P* value
Eotaxin [pg/mL]	177.50 ± 7.93†	182.20 ± 8.73	0.70	0.859	0.704-1.047	0.13
IFN-γ [pg/mL]	103.90 ± 34.62	67.27 ± 13.20	0.42	1.035	0.955-1.122	0.40
IL-1RA [ng/mL]	1.29 ± 0.027	1.28 ± 0.038	0.92	0.935	0.578-1.512	0.78
IL-2 [pg/mL]	129.60 ± 16.71	123.10 ± 18.05	0.80	1.006	0.932-1.085	0.88
IL-4 [pg/mL]	47.14 ± 3.84	50.29 ± 5.00	0.62	0.798	0.556-1.147	0.22
IL-5 [pg/mL]	3.20 ± 0.21	2.45 ± 0.11	< 0.01	1.093	0.940-1.271	0.25
IL-6 [pg/mL]	3.11 ± 0.20	2.97 ± 0.19	0.63	1.023	0.954-1.097	0.53
IL-7 [pg/mL]	17.26 ± 0.52	17.35 ± 0.61	0.91	0.998	0.974-1.022	0.85
IL-8 [pg/mL]	13.74 ± 0.87	13.64 ± 0.83	0.94	0.622	0.143-2.699	0.53
IP-10 [pg/mL]	18.43 ± 0.53	17.67 ± 0.71	0.38	1.051	0.121-9.161	0.96
IL-12p70 [pg/mL]	212.30 ± 24.84	189.80 ± 28.45	0.56	1.019	0.967-1.075	0.48
IL-13 [pg/mL]	227.40 ± 12.16	242.60 ± 31.29	0.64	1.090	0.852-1.394	0.49
IL-17A [pg/mL]	5.22 ± 0.37	5.59 ± 0.51	0.53	0.976	0.946-1.008	0.14
IL-18 [pg/mL]	185.60 ± 5.57	198.50 ± 7.01	0.15	0.907	0.745-1.103	0.33
IL-25 [pg/mL]	116.80 ± 6.57	132.80 ± 26.31	0.55	1.073	0.858-1.343	0.53
IL-33 [pg/mL]	26.71 ± 7.03	22.86 ± 4.49	0.69	1.090	0.852-1.394	0.49
Leptin [ng/mL]	29.13 ± 1.50	30.29 ± 1.63	0.61	1.004	0.992-1.016	0.56
MCP-1 [pg/mL]	398.20 ± 12.27	377.30 ± 9.82	0.23	1.050	0.903-1.221	0.53
MIP-1α [pg/mL]	94.07 ± 5.43	94.26 ± 5.04	0.98	0.951	0.751-1.205	0.68
MIP-1β [pg/mL]	250.40 ± 11.83	250.00 ± 17.66	0.98	0.983	0.900-1.075	0.71

Table 2. (Continued)

Variables	Univariate analysis			Multivariable logistic regression analysis		
	T2-high (n = 308)	T2-low (n = 191)	*P* value	OR	95%CI	*P* value
MMP-1 [ng/mL]	4.80 ± 0.23	4.98 ± 0.24	0.60	0.974	0.923-1.027	0.33
MMP-2 [ng/mL]	271.31 ± 4.33	263.05 ± 5.02	0.22	1.001	0.998-1.004	0.49
MMP-3 [ng/mL]	27.19 ± 1.07	26.18 ± 1.50	0.58	0.993	0.980-1.007	0.34
MMP-8 [ng/mL]	1.37 ± 0.058	1.32 ± 0.078	0.60	1.139	0.914-1.419	0.25
MMP-12 [pg/mL]	51.87 ± 4.40	45.38 ± 4.68	0.33	1.077	0.810-1.431	0.61
Periostin [ng/mL]	372.56 ± 10.76	359.63 ± 13.16	0.45	1.000	0.999-1.001	0.78
PDGF-BB [ng/mL]	8.29 ± 0.20	7.91 ± 0.22	0.22	1.052	0.983-1.127	0.14
RANTES [ng/mL]	34.31 ± 0.87	33.66 ± 1.14	0.65	1.010	0.996-1.025	0.16
ST2 [ng/mL]	8.30 ± 0.27	8.85 ± 0.31	0.20	0.945	0.895-0.998	< 0.05
TARC [ng/mL]	0.85 ± 0.056	0.67 ± 0.036	< 0.05	1.733	1.130-2.657	< 0.05
TIMP-1 [ng/mL]	138.14 ± 1.86	132.15 ± 1.74	< 0.05	1.006	0.999-1.014	0.11
TGF-β [ng/mL]	18.97 ± 0.38	19.06 ± 0.41	0.88	1.018	0.981-1.057	0.34
TNF-α [pg/mL]	6.45 ± 0.64	6.17 ± 0.73	0.78	1.007	0.984-1.030	0.55
YKL-40 [ng/mL]	77.59 ± 5.32	80.62 ± 10.67	0.78	0.999	0.997-1.001	0.44
Urinary LTE4 [pg/mg creatinine]	203.90 ± 31.01	110.50 ± 7.60	< 0.05	1.756	1.288-2.394	< 0.001

†Numeric data expressed as geometric mean ± geometric SD.
CI, confidence interval; IFN, interferon; IL, interleukin; LTE4, leukotriene E4; MCP-1, monocyte Chemotactic protein 1; MIP-1, macrophage inflammatory protein 1; MMP, matrix metalloproteinase; OR, odds ratio; PDGF, platelet-derived growth factor; RANTES, Regulated on activation, normal T cell expressed and secreted; ST2, suppression of tumorigenicity 2; TARC, Thymus and Activation-Regulated Chemokine; TIMP, tissue inhibitor of metalloproteinase

Cluster analysis of T2-high asthma

To identify phenotypes among patients with T2-high asthma in the present study, 613 patients with T2-high asthma and no missing variables were subjected to cluster analysis as shown in **Figure 2**. They were distributed in four distinct clusters, the characteristics of which are summarized in **Figure 1**, right frame and **Table 3**.

Cluster 1: The youngest, early-onset, atopic T2-high asthma

A total of 132 patients (21.5%) were grouped in Cluster 1, which included the youngest patients with the earliest asthma onset among the four clusters in T2-high asthma. The patients in Cluster 1 were mostly atopic and had frequent comorbidities of allergic diseases, such as allergic rhinitis, allergic conjunctivitis, atopic dermatitis, pollinosis, food allergies, and drug allergies. Positivity for specific IgE against dust mites, cats/dogs, and pollen was highest in this cluster. Pulmonary functions, including FEV_1 % predicted and FEV_1/FVC, were well-preserved in this cluster. For asthma therapy, patients with GINA treatment step 1 had the highest representation. The ACQ-6 and AQLQ scores were the lowest and highest, respectively.

Cluster 2: Long duration, eosinophilic T2-high asthma

Cluster 2 included 149 patients (24.3%). This cluster had the longest asthma duration of 22.24 ± 1.14 years and was characterized with the highest number of blood eosinophils of 515.94 ± 28.69 /μL. Among the four clusters, this cluster had the most prevalent comorbidity of sinusitis and aspirin-induced asthma. FeNO levels were the highest, and patients with GINA treatment step 5, especially OCS users, were the most frequently included in this cluster. Pulmonary function in terms of FEV_1 % predicted and FEV_1/FVC was the second lowest among the four clusters. Cluster 2 had the worst asthma control as assessed by the highest ACQ-6 score and the lowest AQLQ score, the most frequent admissions, as well as the highest proportion of patients with GINA treatment step 5.

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

Cluster 3 formed the largest cluster (n = 223, 36.4%) among patients with T2-high asthma. This cluster was the oldest, female-dominant, and had the latest onset of asthma. Osteoporosis was the most frequent among the four clusters. Interestingly, this cluster had the lowest blood eosinophil count and total IgE levels. In addition, this cluster had the highest ASK-20 scores among the four clusters.

Table 3. Clusters in T2-high asthma.

	Cluster 1 (n = 132)	Cluster 2 (n = 149)	Cluster 3 (n = 223)	Cluster 4 (n = 109)	Significance (*P* value)
Summary	The youngest, Early-onset, Atopic, T2-high asthma	Long duration, Eosinophilic, Low lung function, T2-high asthma	Elderly Female-dominant, Late-onset, T2-high asthma	Elderly, Late-onset, ACO-dominant, T2-high asthma	
Age at enrolment, years	45.53 ± 0.91†	61.54 ± 0.86	70.10 ± 0.70	69.38 ± 1.00	< 0.0001
Sex, % female	56.06	67.11	70.85	13.76	< 0.0001
BMI, kg/m^2	24.70 ± 0.46	23.86 ± 0.43	23.81 ± 0.35	24.20 ± 0.50	0.99
Age of asthma onset, years	22.85 ± 1.39	35.15 ± 1.30	57.01 ± 1.07	54.59 ± 1.52	< 0.0001
Asthma duration, years	15.41 ± 1.22	22.24 ± 1.14	13.09 ± 0.94	13.95 ± 1.34	< 0.0001
Smoking status, pack-year	4.76 ± 8.07	4.45 ± 8.77	2.76 ± 6.24	35.93 ± 25.40	< 0.0001
Comorbidities, %					
Sinusitis	35.61	53.02	45.00	42.06	< 0.05
Allergic disease*	83.30	69.80	66.37	45.87	< 0.0001
COPD	1.56	4.23	0.48	26.21	< 0.0001
GERD	11.36	15.44	18.83	19.27	0.23
SAS	11.36	12.08	6.73	10.09	0.28
Hypertension	19.70	29.53	32.74	39.45	< 0.01
Diabetes mellitus	3.79	8.72	12.11	13.76	< 0.05
Heart disease	1.52	6.04	6.28	6.42	0.11
Osteoporosis	0.76	6.71	12.11	2.75	< 0.0001
Aspirin-induced asthma	7.87	13.48	5.83	2.88	< 0.05
Mental disorder	20.47	8.39	5.66	5.71	< 0.001
Laboratory findings					
Blood eosinophils, /μL	339.05 ± 30.48	515.94 ± 28.69	274.49 ± 23.45	366.43 ± 33.55	< 0.0001
Total IgE, IU/mL	581.22 ± 116.98	487.70 ± 109.58	427.17 ± 91.04	1047.70 ± 129.05	< 0.01
Atopy‡, %	81.06	67.79	53.36	60.55	< 0.0001
Specific IgE (dust mites), %	72.87	49.66	36.28	45.63	< 0.0001
Specific IgE (molds), %	27.69	31.69	25.35	32.04	0.49
Specific IgE (cats/ dogs), %	32.28	16.90	14.08	10.00	< 0.0001
Specific IgE (pollen), %	74.22	63.45	57.48	53.40	< 0.01
Specific IgE (insects), %	35.51	27.35	27.47	44.44	< 0.05
FEV_1 % pred, %	97.81 ± 1.69	83.35 ± 1.59	101.91 ± 1.30	76.49 ± 1.86	< 0.0001
FEV_1/FVC, %	77.76 ± 1.02	67.79 ± 0.96	72.35 ± 0.78	62.29 ± 1.12	< 0.0001
FeNO, ppb	48.14 ± 3.53	55.88 ± 3.32	49.57 ± 2.71	50.01 ± 3.88	< 0.05
The questionnaires score					
ACQ-6	0.33 ± 0.056	1.35 ± 0.053	0.47 ± 0.043	0.88 ± 0.062	< 0.0001
AQLQ total score	6.31 ± 0.073	5.24 ± 0.072	6.20 ± 0.062	5.89 ± 0.085	< 0.0001
ASK-20	69.51 ± 0.61	70.87 ± 0.60	73.74 ± 0.50	72.87 ± 0.69	< 0.0001

Table 3. (Continued)

	Cluster 1 (n = 132)	Cluster 2 (n = 149)	Cluster 3 (n = 223)	Cluster 4 (n = 109)	Significance (*P* value)
GINA treatment steps, %					< 0.0001
1	12.12	2.01	6.28	2.75	
2	6.82	6.04	7.62	4.59	
3	26.52	14.77	21.52	25.69	
4	27.27	35.57	33.63	47.71	
5	27.27	41.61	30.94	19.27	
No of unscheduled visits	0.38 ± 0.18	0.79 ± 0.13	0.17 ± 0.12	0.24 ± 0.17	0.061
No of exacerbations requiring systemic steroid	0.78 ± 0.33	1.70 ± 0.23	0.38 ± 0.22	0.63 ± 0.30	< 0.05
No of admissions	0.10 ± 0.38	0.21 ± 0.77	0.024 ± 0.15	0.022 ± 0.15	< 0.05

†Numeric data are expressed as mean ± SD.
*Including allergic rhinitis, allergic conjunctivitis, atopic dermatitis, pollinosis, food allergies, drug allergies, anaphylaxis, and urticaria
‡Specific IgE responsiveness to common inhaled allergens
ACQ, asthma control questionnaire; AQLQ, asthma quality of life questionnaire; ASK-20, adherence starts with knowledge 20; FEV_1, forced expiratory volume in the first second; FeNO, fractional exhaled nitric oxide; GERD, gastroesophageal reflux disease; GINA, Global Initiative for Asthma; SAS, sleep apnea syndrome

Cluster 4: Elderly, late-onset, and asthma-COPD overlap (ACO)-dominant T2-high asthma

This cluster was the smallest (n = 109, 17.8%) and was a male-dominant late-onset cluster. Cluster 4 patients had a markedly high smoking status (35.93 ± 25.40 pack-year) and the highest prevalence of comorbidities with COPD, hypertension, and diabetes among the four clusters. IgE levels were the highest and pulmonary function was assessed by FEV_1 % predicted, and FEV_1/FVC was the worst. The proportion of patients with GINA treatment step 4, especially users of bronchodilators such as long-acting β2 agonist and long-acting muscarinic antagonists, was the highest in Cluster 4.

Biomarkers

The serum biomarkers of the four clusters are shown in **Table 4**. Cluster 1, which included the youngest patients with allergic asthma, showed significantly higher serum interferon (IFN)-γ, IL-7, IL-12p70, and IL-33 levels than all the other clusters. IL-4, IL-13, and matrix metalloproteinase (MMP)-12 levels were also elevated in Cluster 1 but did not reach statistical significance. No cytokine was significantly elevated in Cluster 2 owing to the high use of OCS and biologics in this cluster; however, MMP-1, MMP-2, and platelet-derived growth factor (PDGF)-BB tended to be high. Similarly, although none of the biomarkers were characteristic of Cluster 3, IFN-γ, IL-5, IL-12p70, and periostin levels seemed to be elevated. ACO-dominant Cluster 4 had the highest serum levels of IL-1RA, IL-18, MMP-3, MMP-8, ST2, and YKL40 and tended to show high periostin levels.

Table 4. Biomarker levels of moderate to severe asthma among the T2-high asthma clusters.

	Cluster 1 (n = 42)	Cluster 2 (n = 83)	Cluster 3 (n = 89)	Cluster 4 (n = 47)	Significance (*P* value)
Summary	The youngest, Early-onset, Atopic, T2-high asthma	Long duration, Eosinophilic, Low lung function, T2-high asthma	Elderly Female-dominant, Late-onset, T2-high asthma	Elderly, Late-onset, ACO-dominant, T2-high asthma	
Eotaxin [pg/mL]	172.45 ± 21.91†	185.34 ± 15.59	175.34 ± 15.05	168.08 ± 20.72	0.86
IFN-γ [pg/mL]	319.36 ± 100.94	52.28 ± 71.81	114.09 ± 69.34	28.94 ± 95.42	< 0.05
IL-1RA [ng/mL]	1.34 ± 0.075	1.31 ± 0.054	1.17 ± 0.052	1.44 ± 0.071	< 0.01
IL-2 [pg/mL]	234.86 ± 47.11	128.94 ± 33.51	131.08 ± 32.36	55.70 ± 44.53	0.22
IL-4 [pg/mL]	66.26 ± 10.55	49.76 ± 7.51	48.73 ± 7.25	26.94 ± 9.98	0.082
IL-5 [pg/mL]	3.57 ± 0.58	2.96 ± 0.41	4.05 ± 0.40	2.22 ± 0.55	0.063

Table 4. (Continued)

	Cluster 1 (n = 42)	Cluster 2 (n = 83)	Cluster 3 (n = 89)	Cluster 4 (n = 47)	Significance (*P* value)
IL-6 [pg/mL]	3.00 ± 0.57	3.29 ± 0.41	3.19 ± 0.39	2.66 ± 0.54	0.22
IL-7 [pg/mL]	20.79 ± 1.43	17.88 ± 1.02	16.49 ± 0.98	15.56 ± 1.35	< 0.05
IL-8 [pg/mL]	14.84 ± 2.14	12.68 ± 1.52	13.48 ± 1.47	11.73 ± 2.03	0.79
IP-10 [pg/mL]	16.02 ± 1.43	16.83 ± 1.01	18.80 ± 0.98	21.82 ± 1.35	0.14
IL-12p70 [pg/mL]	367.43 ± 70.51	180.55 ± 50.16	259.86 ± 48.44	82.91 ± 66.66	< 0.05
IL-13 [pg/mL]	313.30 ± 39.90	250.24 ± 28.39	224.57 ± 27.41	177.67 ± 37.72	0.072
IL-17A [pg/mL]	6.21 ± 0.80	4.88 ± 0.57	5.31 ± 0.55	4.10 ± 0.75	0.29
IL-18 [pg/mL]	183.46 ± 15.93	158.02 ± 11.33	186.69 ± 10.95	214.45 ± 15.06	< 0.01
IL-25 [pg/mL]	147.61 ± 17.50	111.44 ± 12.45	114.05 ± 12.02	92.86 ± 16.55	0.67
IL-33 [pg/mL]	71.94 ± 20.45	18.83 ± 14.54	26.38 ± 14.05	8.07 ± 19.33	< 0.001
Leptin [ng/mL]	31.07 ± 4.05	32.72 ± 2.88	31.35 ± 2.78	20.59 ± 3.83	0.082
MCP-1 [pg/mL]	386.01 ± 34.82	414.90 ± 24.77	379.53 ± 23.92	411.95 ± 32.91	0.17
MIP-1α [pg/mL]	116.28 ± 14.84	91.61 ± 10.55	93.08 ± 10.19	76.81 ± 14.03	0.59
MIP-1β [pg/mL]	261.09 ± 33.39	239.73 ± 23.75	263.72 ± 22.94	225.68 ± 31.56	0.75
MMP-1 [ng/mL]	4.70 ± 0.64	5.72 ± 0.46	4.20 ± 0.44	4.84 ± 0.61	0.098
MMP-2 [ng/mL]	249.05 ± 11.46	285.96 ± 8.15	279.82 ± 7.88	262.98 ± 10.84	0.060
MMP-3 [ng/mL]	29.23 ± 2.96	28.04 ± 2.11	23.76 ± 2.03	31.56 ± 2.80	< 0.05
MMP-8 [ng/mL]	1.49 ± 0.15	1.42 ± 0.10	0.97 ± 0.10	1.62 ± 0.14	< 0.001
MMP-12 [pg/mL]	67.41 ± 11.65	56.21 ± 8.29	47.43 ± 8.00	34.28 ± 11.01	0.088
Periostin [ng/mL]	321.20 ± 29.83	363.18 ± 21.22	398.89 ± 20.49	422.80 ± 28.20	0.061
PDGF-BB [ng/mL]	8.80 ± 0.55	9.04 ± 0.39	7.70 ± 0.38	8.39 ± 0.52	0.099
RANTES [ng/mL]	33.52 ± 2.43	36.95 ± 1.73	34.24 ± 1.67	33.78 ± 2.30	0.64
ST2 [ng/mL]	8.40 ± 0.75	7.59 ± 0.54	7.88 ± 0.52	9.41 ± 0.71	< 0.05
TARC [ng/mL]	0.85 ± 0.16	0.78 ± 0.11	0.78 ± 0.11	1.16 ± 0.15	0.89
TIMP-1 [ng/mL]	132.52 ± 4.95	138.85 ± 3.52	136.53 ± 3.40	150.82 ± 4.68	0.12
TGF-β [ng/mL]	19.46 ± 1.03	20.11 ± 0.73	17.83 ± 0.71	19.34 ± 0.97	0.23
TNF-α [pg/mL]	10.12 ± 1.79	6.10 ± 1.27	6.64 ± 1.23	3.65 ± 1.69	0.16
YKL40 [ng/mL]	47.67 ± 14.53	56.39 ± 10.34	90.75 ± 9.98	119.69 ± 13.74	< 0.0001
Urine LTE4 [pg/mg creatinine]	218.20 ± 88.73	306.69 ± 63.87	124.21 ± 62.03	216.74 ± 84.87	0.20

†Numeric data expressed as geometric mean ± geometric SD.
IFN, interferon; IL, interleukin; LTE4, leukotriene E4; MCP-1, monocyte Chemotactic protein 1; MIP-1, macrophage inflammatory protein 1; MMP, matrix metalloproteinase; PDGF, platelet-derived growth factor; RANTES, Regulated on activation, normal T cell expressed and secreted; ST2, suppression of tumorigenicity 2; TARC, Thymus and Activation-Regulated Chemokine; TIMP, tissue inhibitor of metalloproteinase

Discussion

The present study revealed the characteristics of T2-high asthma, defined as blood eosinophils ≥ 300 /μL and/or FeNO ≥ 25 ppb which accounted for 61.5%, using data from a nationwide asthma cohort study in Japan.[5] The patients with T2-high asthma were older, included less female, had later asthma onset, had longer asthma duration, had more sinusitis and SAS, and had a lower prevalence of heart disease and mental disorder. They had higher levels of blood eosinophils, total IgE, specific IgE for molds, and lower pulmonary function. Serum TARC and urinary LTE4 levels were higher, while serum ST2 levels were lower in this group. In addition, there were four phenotypes included in T2-high asthma: Cluster 1 (youngest, early-onset, and atopic), Cluster 2 (long duration, eosinophilic, and low lung function), Cluster 3 (elderly, female-dominant, and late-onset), and Cluster 4 (elderly, late-onset, and asthma-COPD overlap-dominant).

The cutoff values for blood eosinophils and FeNO for defining T2-high asthma have not yet been established, and the ratio of T2-high and T2-low asthma varies depending on the cutoffs.[6-8] Based on the trends in cutoff values for biomarkers in clinical studies[9-11] and to clearly reveal the characteristics of T2-high asthma, we adopted the cutoffs at FeNO ≥ 25 ppb and blood eosinophils ≥ 300 /μL in this study. Approximately 80% of patients with mild asthma demonstrate blood or airway eosinophilia with a concomitant increase in FeNO;[12] however, up to 95% of severe asthma patients have evidence of eosinophilia.[9] The present findings were slightly lower than those of previous reports, which may have been influenced by the high eosinophil cutoffs. Patients with T2-high asthma were older and interestingly, despite the later disease onset, asthma duration was longer in T2-high than in T2-low asthma. These results indicate more severe disease conditions in T2-high asthma, which can also be estimated from the decreased lung function in patients with T2-high asthma. This may have resulted from the greater degree of airway inflammation in T2-high asthma, as inferred from elevated blood eosinophils in T2-high asthma.

Regarding comorbidities, although T2 inflammation is considered to contribute to allergy and that T2-high asthma in our study showed significantly higher serum IgE levels than T2-low asthma, allergic comorbidities were equally common in T2-low asthma. This result suggests that allergy and T2-high asthma differ, indicating that interpreting total IgE levels as a biomarker of T2 inflammation is inadequate. Serum IgE levels are reported to be affected by tobacco smoke, neoplasms, use of some drugs, immune diseases, and/or infectious diseases aside from atopic diseases, which may have affected the results of this study.[13-15] However, only the mold-specific IgE was significantly more prevalent in T2-high asthma than in T2-low asthma, suggesting that mold has other mechanisms of action on pathogenesis in asthma, especially severe asthma.[16] Other than allergic comorbidities, T2-high asthma more often coexisted with SAS, less with heart diseases and mental disorders. Reports of the pathogenic role of asthma in SAS and the contribution of SAS to asthma exacerbation suggest a bidirectional relationship;[17,18] however, further mechanisms have to be clarified by which T2 inflammation is related to SAS. Eosinophils are reported to play a protective role in cardiac function, and this may have affected the decrease in heart disease among patients with T2-high asthma.[19]

In the present study, after adjusting for confounding factors, serum TARC and urinary LTE4 levels were found to be more related to T2-high asthma than IL-4, IL-5, and IL-13, which have central roles in T2 inflammation[2] (**Table 2**). The low serum levels of IL-4 and IL-5 may indicate that the levels of these T2 cytokines are more important at the inflammation sites than in the blood. TARC, produced by macrophages, dendritic cells, endothelial cells, keratinocytes, and fibroblasts, is a chemokine that attracts Th2 cells and eosinophils and is detected in the sera and sputum of patients with asthma.[20] In an ovalbumin-induced murine model of asthma, TARC suppression using anti-TARC antibody has been reported to reduce airway hyper-responsiveness and eosinophil inflammation.[21] In addition, urinary LTE4, a sensitive biomarker for detecting total body cysteinyl leukotriene production, is a potent inflammatory lipid mediator, and an increase in urinary LTE4 levels has been reported to correlate with the degree of airflow limitation in adults with acute asthma.[22] Biologically, LTE4 elicits migration, cytokine production from ILC2, and induces pulmonary inflammation by activating inflammatory cell types.[23] Thus, it is reasonable that TARC and urinary LTE4 are potent biomarkers for T2-high asthma. Conversely, the levels of serum ST2 were lower in T2-high asthma than in T2-low asthma. ST2 in serum functions as a decoy receptor for IL-33 and exerts a beneficial inhibitory effect on IL-33/ST2L signaling.[24] In T2-high asthma in which pathogenesis IL-33 may be involved, ST2 may have been consumed for neutralization of IL-33; however, the precise role and dynamics of serum ST2 need to be further elucidated.

The present study further revealed four clinical phenotypes among T2-high asthma. Asthma phenotyping has been studied extensively in the world, and the main phenotypes include: early-onset allergic, late-onset nonallergic eosinophilic, and late-onset nonallergic non-eosinophilic.[5,25-27] The present study adds detailed phenotypes of T2-high asthma to the existing asthma phenotypes, and supports the importance of disease-onset and atopic status in determination of the phenotypes. A recent report from 10 countries in North America, Europe, and Asia classified severe asthma patients by using biomarkers into five clusters.[28] They found that their clusters 2 and 5, which showed the highest blood eosinophil levels, were linked with frequent exacerbations and low lung function, respectively, which is similar to the results of our Cluster 2. Thus, the present findings may be applied universally to some extent.

The present findings of serum and urinary cytokines among four clusters were of special interest. Cluster 1 had the highest levels of IL-7 and IL-33, potent activators of ILC2 to produce abundant Th2 cytokines, and tended to have high levels of IL-4, IL-13, indicative of the activation of ILC2 in the airways involved in the pathogenesis of Cluster 1.[29] In addition, Th1 inflammatory cytokines, such as IFN-γ and IL-12p70, may complicate the pathogenesis of Cluster 1. Although no significant elevation of biomarkers could be detected for Cluster 2, this may be due to the most frequent use of OCS and biologics; MMP-1, MMP-2, and PDGF-BB tended to be elevated in Cluster 2. These cytokines may lead to airway remodeling and result in worse asthma control.[30,31] Despite the lack of significant biomarkers in Cluster 3, the levels of IFN-γ and IL-12p70, in addition to IL-5 and periostin, tended to be elevated, which may suggest mixed pathogenesis of type 1 inflammation. Interestingly, IL-1RA, IL-18, MMP-3, MMP-8, YKL-40, and ST2, which are reported to be involved in the pathogenesis of COPD,[32-34] were significantly elevated in cluster 4, the ACO-dominant cluster. The results of the present cluster analysis with distinctive characteristics and the distribution of biomarkers in T2-high asthma may be useful in precision medicine in the future.

The present study has some limitations. First, the present study was performed in Japan, and the number of participants was relatively small. Since there are regional differences in asthma pathogenesis,[35] validation studies should be performed in different regions of the world. In addition, since the definition of T2-high and T2-low asthma is still controversial, consensus on the definition is needed in the future.

In summary, T2-high asthma in Japan defined by blood eosinophils ≥ 300 /μL and/or FeNO ≥ 25 ppb was characterized by older age, less female sex, longer asthma duration, and lower pulmonary function, with higher serum TARC and urinary LTE4 levels and lower serum ST2 levels than T2-low asthma. Cluster analysis of T2-high asthma further identified four distinct phenotypes, in which eosinophil-dominant Cluster 2 was the most severe phenotype. The results of the present study may be useful for the identification of T2-high phenotypes by using serum and urinary biomarkers, which may lead to phenotype-dependent precision medicine in the future.

Acknowledgments

The authors thank the NHOM-Asthma study group (NHOM-Asthma study group: Maho Suzukawa, Ken Ohta, Yuma Fukutomi, Hiroya Hashimoto, Takeo Endo, Masahiro Abe, Yosuke Kamide, Makoto Yoshida, Yoshihiro Kikuchi, Toshiyuki Kita, Kenji Chibana, Yasushi Tanimoto, Kentaro Hyodo, Shohei Takata, Toshiya Inui, Masahide Yasui, Yoshinori Harada, Toshio Sato, Yumi Sakakibara, Yoshiaki Minakata, Yoshikazu Inoue, Shinji Tamaki, Tsutomu Shinohara, Kazutaka Takami, Motofumi Tsubakihara, Masahide Oki, Kentaro Wakamatsu, Masahide Horiba, Gen Ideura, Koko Hidaka, Akiko M. Saito, Nobuyuki Kobayashi, and Masami Taniguchi) and all the patients and employees of the participating hospitals. Dr. Kazufumi Takada, Dr. Shizuka Watanabe, Ms. Sayaka Igarashi, Mr. Isao Asari, and Mr. Masaaki Minegishi for their technical assistance. The authors also greatly appreciate Mses. Mariko Yoshizawa and Taeko Kawabe for their excellent secretarial work.

Conflict of Interest

Maho Suzukawa received Grants from AstraZeneca, GlaxoSmithKline, Kyorin, Kyowa Kirin, Daiichi Sankyo, and Shionogi, honoraria for lectures from AstraZeneca, Novartis Pharma, GlaxoSmithKline, and Sanofi.

Funding Source

None.

Author contributions

- Sahoko Imoto: Investigation, Data Curation, Writing – Original Draft.
- Maho Suzukawa: Conceptualization, Methodology, Supervision, Project administration.
- Yuma Fukutomi: Investigation, Resources.
- Nobuyuki Kobayashi: Investigation, Resources.
- Masami Taniguchi: Investigation, Resources.
- Takahide Nagase: Conceptualization, Methodology.
- Ken Ohta: Conceptualization, Methodology.

References

1. Boonpiyathad T, Sözener ZC, Satitsuksanoa P, Akdis CA. Immunologic mechanisms in asthma. Semin Immunol. 2019;46:101333.
2. Lambrecht BN, Hammad H, Fahy JV. The Cytokines of Asthma. Immunity. 2019;50(4):975-91.
3. Samitas K, Zervas E, Gaga M. T2-low asthma: current approach to diagnosis and therapy. Curr Opin Pulm Med. 2017;23(1):48-55.
4. Nagase H. Severe asthma in Japan. Allergol Int. 2019;68(2):167-71.
5. Suzukawa M, Ohta K, Fukutomi Y, Hashimoto H, Endo T, Abe M, et al. Classifications of moderate to severe asthma phenotypes in Japan and analysis of serum biomarkers: A Nationwide Cohort Study in Japan (NHOM Asthma Study). Allergol Int. 2023;72(1):63-74.
6. Couillard S, Jackson DJ, Wechsler ME, Pavord ID. Workup of Severe Asthma. Chest. 2021;160(6):2019-29.
7. Sze E, Bhalla A, Nair P. Mechanisms and therapeutic strategies for non-T2 asthma. Allergy. 2020;75(2):311-25.
8. Rasmussen SM, Halvard Hansen ES, Stensrud T, Radon K, Wolfarth B, Kurowski M, et al. Asthma endotypes in elite athletes: A cross-sectional study of European athletes participating in the Olympic Games. Allergy. 2022;77(7):2250-3.
9. Jackson DJ, Busby J, Pfeffer PE, Menzies-Gow A, Brown T, Gore R, et al. Characterisation of patients with severe asthma in the UK Severe Asthma Registry in the biologic era. Thorax. 2021;76(3):220-7.
10. Reddel HK, Bacharier LB, Bateman ED, Brightling CE, Brusselle GG, Buhl R, et al. Global Initiative for Asthma Strategy 2021: executive summary and rationale for key changes. Eur Respir J. 2022;59(1).
11. Chen M, Shepard K, 2nd, Yang M, Raut P, Pazwash H, Holweg CTJ, et al. Overlap of allergic, eosinophilic and type 2 inflammatory subtypes in moderate-to-severe asthma. Clin Exp Allergy. 2021;51(4):546-55.
12. Berry M, Morgan A, Shaw DE, Parker D, Green R, Brightling C, et al. Pathological features and inhaled corticosteroid response of eosinophilic and non-eosinophilic asthma. Thorax. 2007;62(12):1043-9.
13. Bahna SL, Heiner DC, Myhre BA. Immunoglobulin E pattern in cigarette smokers. Allergy. 1983;38(1):57-64.
14. Waldmann TA, Bull JM, Bruce RM, Broder S, Jost MC, Balestra ST, et al. Serum immunoglobulin E levels in patients with neoplastic disease. J Immunol. 1974;113(1):379-86.

15. Kelkar PS, Li JT. Cephalosporin allergy. N Engl J Med. 2001;345(11): 804-9.
16. Denning DW, O'Driscoll BR, Hogaboam CM, Bowyer P, Niven RM. The link between fungi and severe asthma: a summary of the evidence. Eur Respir J. 2006;27(3):615-26.
17. Emilsson Ö I, Bengtsson A, Franklin KA, Torén K, Benediktsdóttir B, Farkhooy A, et al. Nocturnal gastro-oesophageal reflux, asthma and symptoms of OSA: a longitudinal, general population study. Eur Respir J. 2013;41(6):1347-54.
18. Wang Y, Liu K, Hu K, Yang J, Li Z, Nie M, et al. Impact of obstructive sleep apnea on severe asthma exacerbations. Sleep Med. 2016;26:1-5.
19. Yang C, Li J, Deng Z, Luo S, Liu J, Fang W, et al. Eosinophils protect pressure overload- and β-adrenoreceptor agonist-induced cardiac hypertrophy. Cardiovasc Res. 2022;cvac060.
20. Hoshino M, Nakagawa T, Sano Y, Hirai K. Effect of inhaled corticosteroid on an immunoreactive thymus and activation-regulated chemokine expression in the bronchial biopsies from asthmatics. Allergy. 2005;60(3):317-22.
21. Kawasaki S, Takizawa H, Yoneyama H, Nakayama T, Fujisawa R, Izumizaki M, et al. Intervention of thymus and activation-regulated chemokine attenuates the development of allergic airway inflammation and hyperresponsiveness in mice. J Immunol. 2001;166(3):2055-62.
22. Green SA, Malice MP, Tanaka W, Tozzi CA, Reiss TF. Increase in urinary leukotriene LTE4 levels in acute asthma: correlation with airflow limitation. Thorax. 2004;59(2):100-4.
23. Salimi M, Stöger L, Liu W, Go S, Pavord I, Klenerman P, et al. Cysteinyl leukotriene E(4) activates human group 2 innate lymphoid cells and enhances the effect of prostaglandin D(2) and epithelial cytokines. J Allergy Clin Immunol. 2017;140(4):1090-100.e11.
24. Palmer G, Lipsky BP, Smithgall MD, Meininger D, Siu S, Talabot-Ayer D, et al. The IL-1 receptor accessory protein (AcP) is required for IL-33 signaling and soluble AcP enhances the ability of soluble ST2 to inhibit IL-33. Cytokine. 2008;42(3):358-64.
25. Haldar P, Pavord ID, Shaw DE, Berry MA, Thomas M, Brightling CE, et al. Cluster analysis and clinical asthma phenotypes. Am J Respir Crit Care Med. 2008;178(3):218-24.
26. Moore WC, Meyers DA, Wenzel SE, Teague WG, Li H, Li X, et al. Identification of asthma phenotypes using cluster analysis in the Severe Asthma Research Program. Am J Respir Crit Care Med. 2010;181(4): 315-23.
27. Lefaudeux D, De Meulder B, Loza MJ, Peffer N, Rowe A, Baribaud F, et al. U-BIOPRED clinical adult asthma clusters linked to a subset of sputum omics. J Allergy Clin Immunol. 2017;139(6):1797-807.
28. Denton E, Price DB, Tran TN, Canonica GW, Menzies-Gow A, FitzGerald JM, et al. Cluster Analysis of Inflammatory Biomarker Expression in the International Severe Asthma Registry. J Allergy Clin Immunol Pract. 2021;9(7):2680-8.e7.
29. Martinez-Gonzalez I, Steer CA, Takei F. Lung ILC2s link innate and adaptive responses in allergic inflammation. Trends Immunol. 2015; 36(3):189-95.
30. Dolhnikoff M, da Silva LF, de Araujo BB, Gomes HA, Fernezlian S, Mulder A, et al. The outer wall of small airways is a major site of remodeling in fatal asthma. J Allergy Clin Immunol. 2009;123(5):1090-7, 7.e1.
31. Yamashita N, Sekine K, Miyasaka T, Kawashima R, Nakajima Y, Nakano J, et al. Platelet-derived growth factor is involved in the augmentation of airway responsiveness through remodeling of airways in diesel exhaust particulate-treated mice. J Allergy Clin Immunol. 2001;107(1):135-42.
32. Ghosh N, Choudhury P, Kaushik SR, Arya R, Nanda R, Bhattacharyya P, et al. Metabolomic fingerprinting and systemic inflammatory profiling of asthma COPD overlap (ACO). Respir Res. 2020;21(1):126.
33. Elkington PT, Friedland JS. Matrix metalloproteinases in destructive pulmonary pathology. Thorax. 2006;61(3):259-66.
34. Huang Q, Li CD, Yang YR, Qin XF, Wang JJ, Zhang X, et al. Role of the IL-33/ST2 axis in cigarette smoke-induced airways remodelling in chronic obstructive pulmonary disease. Thorax. 2021; thoraxjnl-2020-214712.
35. Nyenhuis SM, Krishnan JA, Berry A, Calhoun WJ, Chinchilli VM, Engle L, et al. Race is associated with differences in airway inflammation in patients with asthma. J Allergy Clin Immunol. 2017;140(1):257-65.e11.

Supplemental material

Supplementary Table 1. Comparison of baseline characteristics between T2-high asthma on OCS or biologics versus T2-high asthma without OCS or biologics, and T2-low asthma.

Variables	T2-high on OCS or biologics (n = 85)	vs T2-high without OCS or biologics (n = 675)	*P* value	vs T2-low (n = 476)	*P* value
Age at enrolment, years	63.69 ± 1.43[†]	62.49 ± 0.56	0.47	58.84 ± 0.76	< 0.05
Sex, % female	57.65	55.70	0.82	68.90	< 0.05
BMI, kg/m^2	23.70 ± 0.45	24.12 ± 0.20	0.47	24.06 ± 0.20	0.48
Age of asthma onset, years	42.37 ± 2.27	44.82 ± 0.87	0.33	41.19 ± 1.10	0.66
Asthma duration, years	18.34 ± 1.46	15.48 ± 0.59	0.095	13.90 ± 0.69	< 0.01
Family history of asthma, %	33.33	30.82	0.69	33.08	> 0.99
Smoking status, pack-year	4.75 ± 1.21	11.10 ± 0.78	< 0.01	10.07 ± 1.06	< 0.05

Supplementary Table 1. (Continued)

Variables	T2-high on OCS or biologics (n = 85)	vs T2-high without OCS or biologics (n = 675)	*P* value	vs T2-low (n = 476)	*P* value
Comorbidities, %					
Sinusitis	50.60	43.79	0.24	36.68	< 0.05
Allergic disease*	70.59	62.81	0.19	63.87	0.27
COPD	6.33	6.34	> 0.99	6.82	> 0.99
GERD	20.24	15.67	0.273	20.13	> 0.99
SAS	9.52	8.60	0.84	5.41	0.14
Heart disease	4.76	5.07	> 0.99	8.01	0.3734
Osteoporosis	8.33	6.30	0.48	9.31	> 0.99
Aspirin-induced asthma	15.19	6.07	< 0.01	4.52	< 0.01
Mental disorder	6.41	9.53	0.53	17.08	< 0.05
Laboratory findings					
WBC count, /μL	6836.00 ± 238.70	6274.00 ± 65.65	< 0.01	6105.00 ± 85.69	< 0.01
Blood eosinophils, /μL	332.30 ± 47.36	369.90 ± 13.19	0.36	116.00 ± 3.65	< 0.0001
Blood neutrophils, /μL	4172.00 ± 213.60	3781.00 ± 102.40	0.075	4030.00 ± 129.90	0.57
Total IgE, IU/mL	654.90 ± 166.30	530.80 ± 48.03	0.39	305.30 ± 34.62	< 0.01
Atopy‡, %	58.82	60.59	0.81	58.61	> 0.99
Specific IgE (dust mites), %	41.77	49.28	0.23	44.91	0.63
Specific IgE (molds), %	42.50	26.21	< 0.01	19.87	< 0.0001
Specific IgE (cats/ dogs), %	17.95	17.62	> 0.99	14.61	0.49
Specific IgE (pollen), %	62.50	59.62	0.72	55.51	0.27
Specific IgE (insects), %	27.14	32.55	0.41	30.38	0.67
FEV_1, mL	1981.00 ± 77.50	2137.00 ± 30.09	0.080	2281.00 ± 34.95	< 0.001
FEV_1 % pred, %	90.21 ± 2.26	92.08 ± 0.88	0.47	100.80 ± 1.03	< 0.0001
FEV_1/FVC, %	69.27 ± 1.21	70.63 ± 0.50	0.36	75.95 ± 0.55	< 0.0001
FeNO, ppb	58.59 ± 4.56	50.18 ± 1.55	0.071	14.80 ± 0.25	< 0.0001
The questionnaires score					
ACQ-6	1.07 ± 0.10	0.72 ± 0.030	< 0.001	0.69 ± 0.028	< 0.0001
AQLQ total score	5.44 ± 0.15	5.96 ± 0.039	< 0.001	5.89 ± 0.036	< 0.01
ASK-20	70.09 ± 0.90	72.10 ± 0.29	< 0.05	71.63 ± 0.036	0.38
No of unscheduled visits	0.72 ± 0.17	0.32 ± 0.068	< 0.01	0.44 ± 0.086	0.11
No of exacerbations requiring systemic steroid	2.06 ± 0.46	0.58 ± 0.098	< 0.0001	0.92 ± 0.16	< 0.01
No of admissions	0.11 ± 0.043	0.080 ± 0.032	0.5993	0.080± 0.029	0.54

†Numeric data expressed as geometric mean ± geometric SD.
*Including allergic rhinitis, allergic conjunctivitis, atopic dermatitis, pollinosis, food allergies, drug allergies, anaphylaxis, and urticaria
‡Specific IgE responsiveness to common inhaled allergen
ACQ, asthma control questionnaire; AQLQ, asthma quality of life questionnaire; ASK-20, adherence starts with knowledge 20; FEV_1, forced expiratory volume in the first second; FeNO, fractional exhaled nitric oxide; GERD, gastroesophageal reflux disease; SAS, sleep apnea syndrome

Supplementary Table 2. Comparison of biomarkers between T2-high asthma on OCS or biologics versus T2-high asthma without OCS or biologics, and T2-low asthma.

Variables	T2-high on OCS or biologics (n = 73)	vs T2-high without OCS or biologics (n = 235)	*P* value	vs T2-low (n = 191)	*P* value
Eotaxin [pg/mL]	209.50 ± 24.89†	167.60 ± 6.86	< 0.05	182.20 ± 8.73	0.19
IFN-γ [pg/mL]	61.74 ± 15.62	117.00 ± 45.10	0.50	67.27 ± 13.20	0.81
IL-1RA [ng/mL]	1.46 ± 0.059	1.24 ± 0.030	< 0.001	1.28 ± 0.038	< 0.05
IL-2 [pg/mL]	145.30 ± 36.25	124.70 ± 18.82	0.60	123.10 ± 18.05	0.55
IL-4 [pg/mL]	62.71 ± 10.11	42.3 ± 3.89	< 0.05	50.29 ± 5.00	0.23
IL-5 [pg/mL]	5.22 ± 0.73	2.57 ± 0.12	< 0.0001	2.45 ± 0.11	< 0.0001
IL-6 [pg/mL]	3.06 ± 0.27	3.12 ± 0.25	0.89	2.97 ± 0.19	0.80
IL-7 [pg/mL]	17.47 ± 0.84	17.2 ± 0.63	0.82	17.35 ± 0.61	0.91
IL-8 [pg/mL]	14.74 ± 1.51	13.43 ± 1.04	0.52	13.64 ± 0.83	0.50
IP-10 [pg/mL]	17.81 ± 0.68	18.62 ± 0.66	0.52	17.67 ± 0.71	0.91
IL-12p70 [pg/mL]	193.30 ± 39.31	218.20 ± 30.21	0.67	189.80 ± 28.45	0.95
IL-13 [pg/mL]	242.70 ± 32.88	232.30 ± 15.66	0.76	240.80 ± 19.91	0.96
IL-17A [pg/mL]	5.36 ± 0.70	4.89 ± 0.29	0.47	6.40 ± 0.88	0.49
IL-18 [pg/mL]	169.50 ± 7.31	187.60 ± 7.13	0.18	190.30 ± 7.94	0.13
IL-25 [pg/mL]	106.60 ± 7.94	116.10 ± 7.61	0.51	116.00 ± 7.87	0.49
IL-33 [pg/mL]	19.32 ± 3.15	29.00 ± 9.16	0.56	22.86 ± 4.49	0.64
Leptin [ng/mL]	31.29 ± 3.15	28.46 ± 1.71	0.42	30.29 ± 1.63	0.76
MCP-1 [pg/mL]	444.80 ± 39.67	383.70 ± 10.22	< 0.05	377.30 ± 9.82	< 0.05
MIP-1α [pg/mL]	104.10 ± 10.31	90.96 ± 6.36	0.31	94.26 ± 5.04	0.34
MIP-1β [pg/mL]	227.00 ± 17.47	257.70 ± 14.52	0.27	250.00 ± 17.66	0.45
MMP-1 [ng/mL]	4.49 ± 0.36	4.90 ± 0.28	0.45	4.98 ± 0.24	0.27
MMP-2 [ng/mL]	277.92 ± 7.74	269.26 ± 5.14	0.40	263.05 ± 5.02	0.12
MMP-3 [ng/mL]	38.77 ± 2.87	23.59 ± 0.97	< 0.0001	26.18 ± 0.15	< 0.0001
MMP-8 [ng/mL]	1.25 ± 0.11	1.41 ± 0.068	0.23	1.32 ± 0.078	0.60
MMP-12 [pg/mL]	50.62 ± 7.81	52.26 ± 5.24	0.87	45.38 ± 4.68	0.56
Periostin [ng/mL]	364.13 ± 15.28	375.18 ± 13.30	0.66	359.63 ± 13.16	0.85
PDGF-BB [ng/mL]	8.29 ± 0.43	8.29 ± 0.23	0.99	7.91 ± 0.22	0.40
RANTES [ng/mL]	31.74 ± 1.58	35.11 ± 1.03	0.099	33.66 ± 1.14	0.36
ST2 [ng/mL]	8.60 ± 0.55	8.21 ± 0.32	0.55	8.85 ± 0.31	0.68
TARC [ng/mL]	0.99 ± 0.020	0.81 ± 0.039	0.17	0.67 ± 0.036	< 0.05
TIMP-1 [ng/mL]	137.19 ± 3.45	138.43 ± 2.19	0.78	132.15 ± 1.74	0.15
TGF-β [ng/mL]	18.23 ± 0.78	19.20 ± 0.43	0.27	19.06 ± 0.41	0.31
TNF-α [pg/mL]	6.32 ± 1.10	6.50 ± 0.76	0.91	6.17 ± 0.73	0.91
YKL-40 [ng/mL]	83.00 ± 15.71	75.90 ± 5.01	0.57	80.62 ± 10.67	0.90
Urinary LTE4 [pg/mg creatinine]	145.70 ± 15.28	221.80 ± 40.2	0.30	110.50 ± 7.60	< 0.05

†Numeric data expressed as geometric mean ± geometric SD.
IFN, interferon; IL, interleukin; LTE4, leukotriene E4; MCP-1, monocyte Chemotactic protein 1; MIP-1, macrophage inflammatory protein 1; MMP, matrix metalloproteinase; PDGF, platelet-derived growth factor; RANTES, Regulated on activation, normal T cell expressed and secreted; ST2, suppression of tumorigenicity 2; TARC, Thymus and Activation-Regulated Chemokine; TIMP, tissue inhibitor of metalloproteinase