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Original Article

Inflammatory Endotypes of Chronic Rhinosinusitis in the Korean Population: Distinct Expression of Type 3 Inflammation

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ABSTRACT

Purpose: Cluster analyses on inflammatory markers of chronic rhinosinusitis (CRS) in Asians from multicenter data are lacking. This multicenter study aimed to identify the endotypes of CRS in Koreans and to evaluate the relationship between the endotypes and clinical parameters.

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Disclosure

There are no financial or other issues that might lead to conflict of interest.

Methods: Nasal tissues were obtained from patients with CRS and controls who underwent surgery. The endotypes of CRS were investigated by measuring interleukin (IL)-5, interferon (IFN)-γ, IL-17A, IL-22, IL-1β, IL-6, IL-8, matrix metalloproteinase-9, eotaxin-3, eosinophil cationic protein, myeloperoxidase (MPO), human neutrophil elastase (HNE), periostin, transforming growth factor-β1, total immunoglobulin E (IgE), and staphylococcal enterotoxin (SE)-specific IgE. We performed hierarchical cluster analysis and evaluated the phenotype, comorbidities, and Lund-Mackay computed tomography (LM CT) score in each cluster.
Results: Five clusters and 3 endotypes were extracted from 244 CRS patients: cluster 1 had no upregulated mediators compared to the other clusters (mild mixed inflammatory CRS); clusters 2, 3, and 4 had higher concentrations of neutrophil-associated mediators including HNE, IL-8, IL-17A, and MPO (T3 CRS); and cluster 5 had higher levels of eosinophil-associated mediators (T2 CRS). SE-specific IgE was undetectable in T3 CRS and had low detectable levels (6.2%) even in T2 CRS. The CRS with nasal polyps (CRSwNP) phenotype and LM CT scores showed no significant differences between T2 and T3 CRS, while the incidence of comorbid asthma was higher in T2 CRS than T3 CRS. In T3 clusters, higher levels of neutrophilic markers were associated with disease severity and CRSwNP phenotype.
Conclusions: In Koreans, there is a distinct T3 CRS endotype showing a high proportion of CRSwNP and severe disease extent, along with T2 CRS.

Keywords: Asians; biomarker; cluster analysis; nasal polyps; sinusitis; multicenter study; surgery

INTRODUCTION

Chronic rhinosinusitis (CRS) is an inflammatory disease of the sinonasal mucosa with duration of at least 12 weeks; its prevalence is 12% in the United States,[1] 10.9% in Europe,[2] and 7%–8.4% in Korea.[3,4] CRS is a vastly heterogeneous disorder with a wide spectrum of inflammatory patterns. Moreover, the immunological features of CRS vary across different geographic locations and racial backgrounds, further complicating the heterogeneity of CRS.[5-7] Studies in Caucasian patients have demonstrated that CRS with nasal polyps (CRSwNP) is characterized by eosinophilic and type 2 (T2) inflammation, with the expression of interleukin (IL)-4, IL-5, IL-13, and immunoglobulin E (IgE), whereas type 1 (T1) inflammation with interferon (IFN)-γ expression and type 3 (T3) inflammation with IL-17A expression predominate in CRS without nasal polyps (CRSsNP).[8-13] In contrast, approximately half of the Asian CRSwNP patients demonstrate non-eosinophilic inflammation involving T1 and T3 inflammation as well as neutrophilic inflammation.[14-16]

Given the immunological heterogeneity of CRS, several studies have performed endotyping of CRS based on inflammatory profiles using cluster analysis to identify diverse clinical and immunological features of CRS and determine therapeutic arms.[5,6,15,17-21] Tomassen *et al.*[21] carried out a cluster analysis of 14 inflammatory markers in 173 CRS patients in Europe and identified 10 clusters demonstrating distinct endotypes. In Chinese populations,[15,18] Lou *et al.*[18] showed that CRSwNP patients could be classified into 5 phenotypes based on the predominant presence of plasma cells, lymphocytes, neutrophils, and mixed inflammatory cells. Additionally, Liao *et al.*[15] subclassified Chinese CRS patients into 7 clusters and documented their associations with treatment outcomes; their classification included 28 clinical indicators and 39 cellular/molecular markers as cluster variables. Although that study integrated various multidimensional characteristics of CRS, the 7 clusters did not completely reflect the pure

inflammatory endotype as they included clinical parameters such as the presence of nasal polyps. In addition, cluster analyses based on multicenter data in Asia are lacking.

Considering different immunologic profiles of CRS between Western and Asian countries, further studies involving cluster analysis with inflammatory markers for Asian populations from multicenter data are warranted to achieve a better understanding of the endotypes of CRS. Hence, the present multicenter study aimed to identify endotypes of CRS in the Korean population and sought to assess the relationships between the endotype and nasal polyp phenotype, comorbid asthma, and disease extent.

MATERIALS AND METHODS

Subjects and sample collection

Healthy controls and patients with CRS were recruited from the otolaryngology-head and neck clinics at 19 institutions in Korea in this prospective multicenter study. The diagnosis of asthma was based on medical history and lung function tests, as confirmed by allergy specialists. Preoperative computed tomography (CT) scans were analyzed according to the grading system of Lund-Mackay CT (LM CT) scores.[22] This study was approved by the ethics committees of all individual institutions and that of Seoul National University Hospital, Boramae Medical Center (20-2019-100), and written informed consent was obtained from all subjects before sampling. Ethmoid tissues were obtained from control subjects and CRSsNP patients, and nasal polyp tissues from CRSwNP patients. Further details are provided in the **Supplementary Data S1**.

Measurement of inflammatory markers in nasal tissue

We selected 16 inflammatory markers based on previous reports.[21,23] The protein concentrations of tissue extracts were determined using the Pierce 660 nm Protein Assay Kit (Thermo Fisher Scientific ., Waltham, MA, USA). Enzyme-linked immunosorbent assays for IL-22 and human neutrophil elastase (HNE) were performed with commercially available kits (all from R&D Systems, Minneapolis, MN, USA). Multiplex cytokine analysis kits (IL-1β, IL-5, IL-6, IL-8, IL-17A, IFN-γ, eotaxin-3, myeloperoxidase [MPO], matrix metalloproteinase [MMP]-9, TGF-β1, and periostin) were purchased from R&D Systems, and data were collected using a Luminex 100 reader (Luminex, Austin, TX, USA). Data analysis was conducted using MasterPlex QT version 2.0 (MiraiBio, Alameda, CA, USA). The levels of total IgE, specific IgE to staphylococcal enterotoxins (SE-IgE [SEA, SEB, SEC, and TSST-1]), and eosinophil cationic protein (ECP) in nasal tissue homogenates were measured using the ImmunoCAP assay (Thermo Fisher Scientific). A SE-IgE level > 0.35 IU/mL for at least one SE-specific IgE (SEA, SEB, SEC, and TSST-1) was defined as SE-specific IgE positive. All assay procedures mentioned were run in duplicate according to the manufacturer's protocol. All protein levels in the tissue homogenates were normalized to the concentration of total protein. Further details are provided in the **Supplementary Data S1**.

Statistical analysis

Sixteen cluster variables that reflect the inflammatory profiles of CRS were selected (**Table 1**). We performed hierarchical cluster analysis based on the correlation ratio and exploratory factor analysis (EFA) of the mixed data.[24] Orthogonal rotation with the varimax method was performed, and only variables with factor loadings > 0.4 were retained. Ward's minimum variance was used for hierarchical clustering.[25] For cluster analysis of individual cases, the

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Table 1. The inflammatory markers used as cluster variables

Marker	Interpretation of increased levels
IFN-γ	Type 1 inflammation
IL-5	Type 2 inflammation
IL-17A	Type 3 inflammation
IL-22	Cytokine for epithelial homeostasis
IL-1β	Proinflammatory action
IL-6	Proinflammatory action
IL-8	Neutrophilic chemotaxis
MPO	Neutrophilic activity marker
HNE	Neutrophilic activity marker
MMP-9	Marker for extracellular matrix homeostasis
ECP	Eosinophilic activity marker
Eotaxin-3	Eosinophilic chemotaxis
Total IgE	Adaptive immunity marker for type 2 inflammation
SE-IgE	Marker for superantigen effect on local mucosa
TGF-β1	Fibrosis/regulatory T-cell activation
Periostin	Marker for tissue remodeling in type 2 inflammation

Cutoff value of 0.35 IU/mL is given for SE-IgE that was analyzed as categorical.
IFN, interferon; IL, interleukin; MPO, myeloperoxidase; HNE, human neutrophil elastase; MMP, matrix metalloproteinase; ECP, eosinophil cationic protein; IgE, immunoglobulin E; SE, staphylococcal enterotoxin; TGF, transforming growth factor.

Euclidean distance was calculated, which allowed for both ordinal and binomial data. Next, the optimal number of clusters was determined by the NbClust R software package, which uses a variety of indices to find the optimal number of clusters ranging from 3 to 10 in a partitioning of a data set during the clustering process.[26] Further details are provided in the **Supplementary Data S1**. All statistical analyses were conducted using R for Windows version 4.0.3 (R Foundation for Statistical Computing, Vienna, Austria). A *P* value less than 0.05 was considered significant.

RESULTS

Characteristics of the study cohort

Of the 287 CRS patients and 27 control participants recruited, 244 and 27, respectively, who had complete clinical data and an adequate amount of tissue collected were enrolled in all of the intended analyses. Among the 244 CRS patients, 208 had CRSwNP (85.2%), 36 had CRSsNP (14.8%), and 20% had comorbid asthma (41/205). The demographic characteristics and inflammatory profiles of participants are demonstrated in **Supplementary Table S1**. Most of the inflammatory markers used in the cluster analysis had significantly higher levels in the CRSwNP or CRSsNP groups than in the control group. However, there were no significant differences in the levels of some inflammatory markers including IFN-γ, MMP-9, HNE, and periostin. In addition, the TGF-β1 level was higher in the control group than in the CRSwNP or CRSsNP groups.

Clustering outcomes

Five clusters were established as the optimal number through hierarchical clustering (**Fig. 1**). EFA retained 6 dimensions accounting for 68.5% of all variances in the data (**Supplementary Table S2**), and the first and second dimensions accounted for 37.2% of the total variance (**Fig. 2A and B**). Individual patient data are presented in dimensions categorized by cluster, and the clusters were separated from each other (**Fig. 2B and D**). A modified heatmap with inflammatory markers was generated to characterize the clusters (**Fig. 3**).

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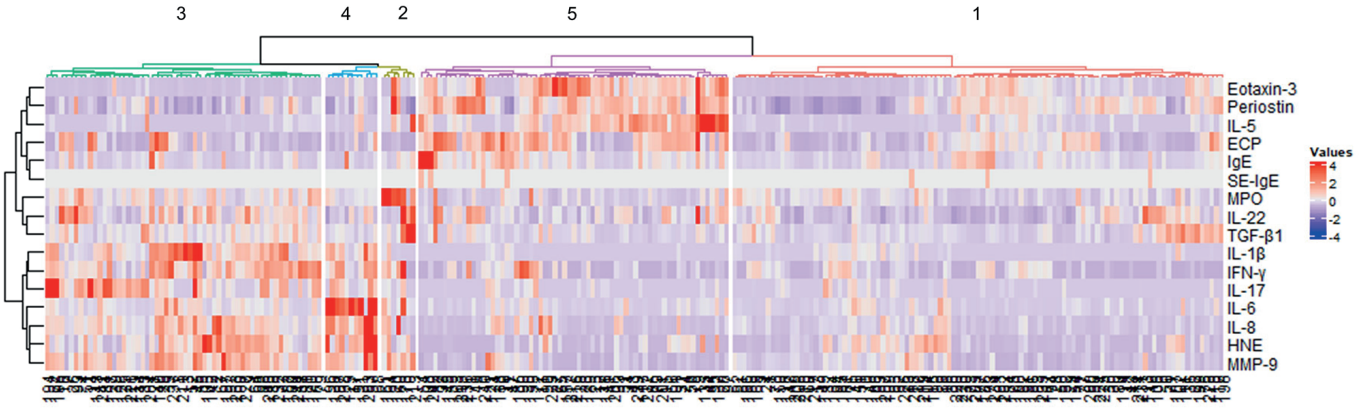

Fig. 1. Heatmap of the hierarchical clustering. The dendrogram on the left side shows the cluster variables, and the dendrogram on the bottom shows the 244 CRS patients in this study. Five clusters of CRS patients were obtained, as illustrated on the top of the dendrogram.
CRS, chronic rhinosinusitis; IL, interleukin; ECP, eosinophil cationic protein; IgE, immunoglobulin E; SE, staphylococcal enterotoxin; MPO, myeloperoxidase; TGF, transforming growth factor; IFN, interferon; HNE, human neutrophil elastase; MMP, matrix metalloproteinase.

Cluster 1: mild mixed inflammation (mild mixed group; n = 103, 42.2%)

Cluster 1 was the largest of the 5 clusters (42.2%). The levels of inflammatory markers were not significantly higher in this cluster than in the other clusters (**Fig. 3**). However, the levels of T2 (ECP, IL-5, eotaxin-3, and total IgE) and T3 (IL-17A and IL-8) inflammatory markers as well as of proinflammatory markers (IL-6, IL-1β, and MMP-9) were significantly higher in cluster 1 than in the control group.

Clusters 2, 3, and 4: T3 inflammation (T3 group; n = 76, 31.1%)

Clusters 2, 3, and 4 exhibited neutrophilic inflammation with T3 inflammatory profiles mixed with T1 inflammation and accounted for about one-third of all patients (31.1%). Cluster 2 accounted for 2.9% of all patients and showed mild T3 inflammation with the highest levels of MPO and TGF-β1. The patients in this cluster showed the lowest proportion of CRSwNP and atopy among the clusters. Cluster 3 accounted for 23.8% of all patients and the largest proportion among the T3 clusters (clusters 2, 3, and 4). Cluster 3 showed T1 inflammation as well as moderate T3 and proinflammatory profiles (IL-6, IL-1β, and MMP-9). Cluster 4 exhibited more severe T3 inflammation, characterized by higher levels of IL-8 and IL-6 than cluster 3. The proportion of CRSwNP in cluster 4 was 100%, and no patients in cluster 4 had asthma. In addition, patients in cluster 4 showed the greatest disease severity, as determined by the LM CT score, among the clusters (**Supplementary Table S3**). To sum up, stratification of T3 inflammation was observed, and these clusters were also correlated to the CRSwNP phenotype and disease extent.

Cluster 5: T2 inflammation (T2 group; n = 65, 26.6%)

Cluster 5, which accounted for 26.6% of all patients, showed typical T2 inflammatory profiles with elevated ECP, IL-5, eotaxin-3, and total IgE levels. The level of periostin, a marker of tissue remodeling in T2 inflammation, was also significantly higher in cluster 5 than in the other clusters and controls. The positivity of SE-specific IgE was 6.2%, which was the highest among the clusters. The proportion of CRSwNP was 91%, and this cluster had the highest proportion of asthmatic patients (34%).

Endotypes of CRS derived from cluster analysis

To characterize the 3 endotype groups elicited from cluster analysis, we compared the cytokine levels between these groups and controls, which were standardized using skewing

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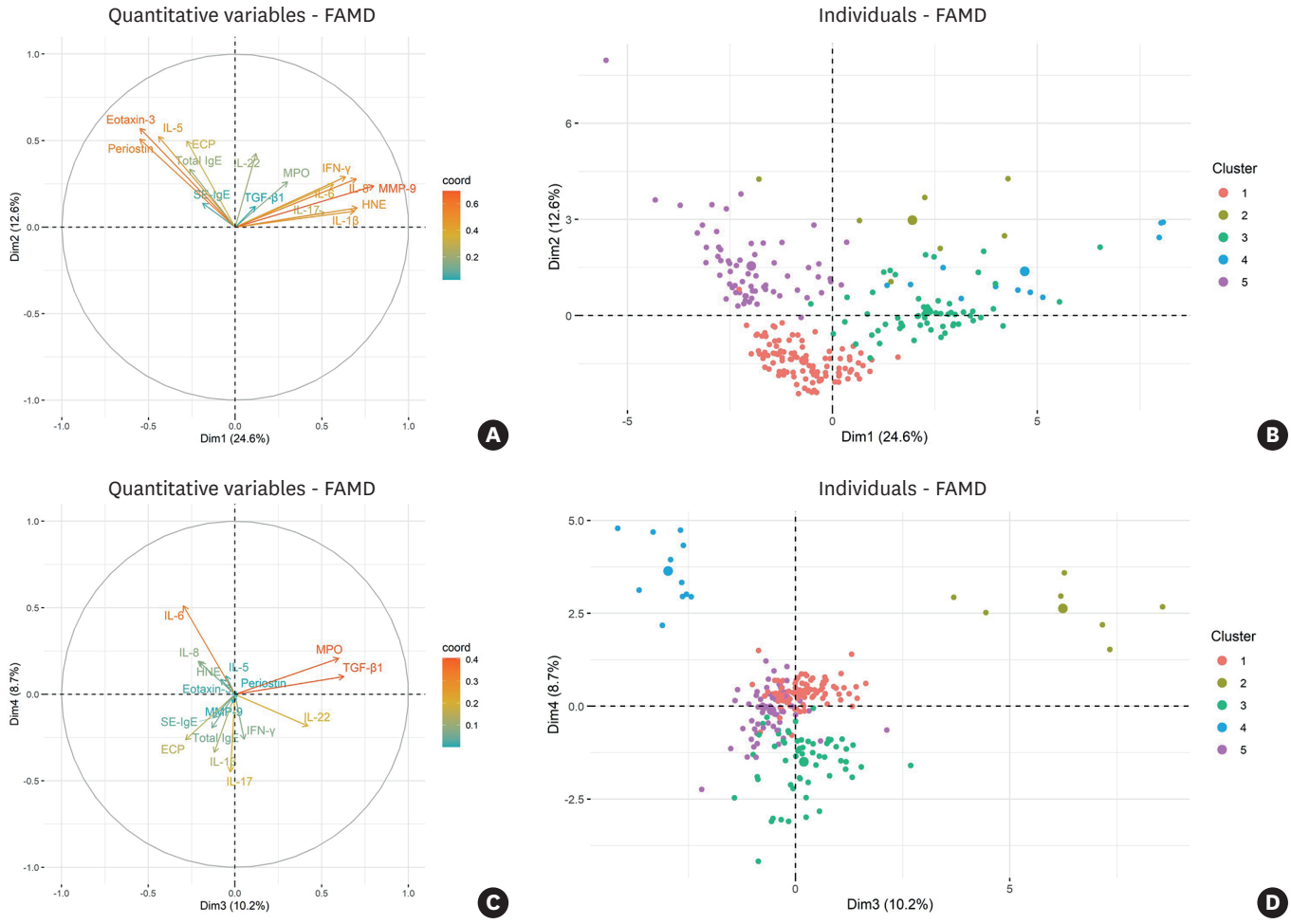

Fig. 2. Exploratory factor analysis based on principal component analysis and multiple correspondence analysis. (A) The first and second dimensions accounted for 24.6% and 12.6% of total variance, respectively. Arrows are displayed for inflammatory markers used as cluster variables against the first 2 dimensions. (B) Individual patients were categorized by the cluster against the first 2 dimensions. Dots are displayed for individual patients, and they are divided into 5 colors based on the cluster. (C) The third and fourth dimensions accounted for 10.2% and 8.7% of the total variance, respectively. (D) Individual patients were categorized by the cluster against the third and fourth dimensions.
FAMD, factor analysis of mixed data; IL, interleukin; ECP, eosinophil cationic protein; IgE, immunoglobulin E; SE, staphylococcal enterotoxin; MPO, myeloperoxidase; TGF, transforming growth factor; IFN, interferon; HNE, human neutrophil elastase; MMP, matrix metalloproteinase.

degree (**Fig. 4**). In total CRS patients, MPO, IL-8, IL-6, IL-1β, IL-17A, ECP, IL-5, eotaxin-3, and total IgE levels were higher than in controls, while IFN-γ, MMP-9, and TGF-β1 levels were lower in total CRS patients than in controls. The mild mixed inflammation group had higher levels of IL-8, IL-6, IL-1β, IL-17A, ECP, IL-5, eotaxin-3, and total IgE than controls, whereas the mild mixed group had lower levels of IFN-γ, MMP-9, IL-22, and TGF-β1 than controls. In the T3 group, MPO, HNE, IL-8, IL-6, IL-1β, IL-17A, IFN-γ, MMP-9, ECP, IL-5, and total IgE levels were higher than in controls, while the levels of TGF-β1 and periostin were lower in the T3 group than in controls. Notably, the skewing degree of neutrophilic and proinflammatory markers (MPO, HNE, IL-8, IL-6, IL-1β, IL-17A, IFN-γ, and MMP-9) were markedly higher, and the levels of IFN-γ and MMP-9 were higher in the T3 group than in controls, unlike the mild mixed inflammation group. As expected, the T2 group showed higher levels of IL-8, IL-6, IL-17A, ECP, IL-5, eotaxin-3, periostin, and total IgE, whereas IFN-γ, MMP-9, and TGF-β1 levels were lower in the T2 group than in controls. Altogether, the T2 group showed a similar inflammatory pattern to that of the mild mixed inflammation group; however, the

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Cluster	N (%)	MPO (ng/mg)	HNE (ng/mg)	IL-8 (pg/mg)	IL-6 (pg/mg)	IL-1β (pg/mg)	IL-17A (pg/mg)	IFN-γ (pg/mg)	MMP-9 (ng/mg)	IL-22 (pg/mg)	TGF-β1 (pg/mg)	ECP (pg/mg)	IL-5 (pg/mg)	Eotaxin-3 (pg/mg)	periostin (ng/mg)	Total IgE (kU/g)	SE-IgE (%)	prop. CRSwNP (%)	prop. Asthma (%)	prop. Atopy (%)	prop. AERD (%)	LMCT
1	103 (42.2)	37	183	**128**	**8**	**0.9**	**0.4**	*2.5*	*5.1*	*38*	*693*	**154**	**1.6**	**53**	43.0	**19**	2.9	83	20	42	3	14
2	7 (2.9)	**299**	323	**568**	**35.4**	**1.9**	**1.2**	10.6	**18.1**	104	3425	24	**9.1**	**118**	59.2	12	0	71	0	29	0	15
3	58 (23.8)	**61**	**508**	**456**	**18.6**	**11.8**	**5.3**	**10.3**	**16.4**	66	*871*	**137**	**1.1**	19	*37.0*	**13**	0	83	11	30	2	16
4	11 (4.5)	**45**	**1059**	**1089**	**136.1**	**9.6**	**3**	9.9	**24.2**	39	*554*	**139**	**2.2**	34	32.5	19	0	100	0	36	0	18
5	65 (26.6)	34	121	**174**	**9.3**	0.3	**0.5**	*3.5*	*4.4*	54	*562*	**307**	**14.1**	**206**	**71.1**	**32**	6.2	91	34	43	10	16

Concentration significantly higher than 4 or more other clusters
Concentration significantly higher than 3 or more other clusters
Concentration significantly higher than 2 or more other clusters

Fig. 3. Modified heat map of inflammatory markers and clinical features according to clusters. Rows indicate clusters of patients with CRS, and columns define inflammatory markers used for cluster analysis (cellular markers, chemotaxis-associated markers, and others). Mean values for each marker are provided for each cluster. Phenotypes, the presence of asthma as a comorbidity, and disease extent according to clusters are demonstrated, although these parameters were not used in the cluster analysis. To characterize clusters, a multiple-group comparison for between-cluster differences is visualized with color coding, as shown in the legend. In addition, differences in inflammatory markers between each cluster and controls were demonstrated with bold character and italic characters; bold and italic characters mean higher and lower levels than controls, respectively.
CRS, chronic rhinosinusitis; MPO, myeloperoxidase; HNE, human neutrophil elastase; IL, interleukin; IFN, interferon; MMP, matrix metalloproteinase; TGF, transforming growth factor; ECP, eosinophil cationic protein; IgE, immunoglobulin E; SE, staphylococcal enterotoxin; prop. CRSwNP, proportion of chronic rhinosinusitis with nasal polyps; AERD, aspirin exacerbated respiratory disease; LM CT, Lund-Mackay computed tomography.

high skewing degree of eosinophilic markers and significantly higher periostin levels than the control group were noted. Additionally, IL-1β levels were not significantly different from those of the controls.

Next, we analyzed the demographics and clinical characteristics of patients with each endotype (**Table 2**). The proportion of patients with a history of asthma was higher in the T2 group than in the T3 group ($P = 0.002$). In addition, the T2 group had higher incidence of serum and tissue eosinophilia than the other groups. Considering the extent of disease, the LM CT scores were higher in the T3 and T2 groups than in the mild mixed inflammation group ($P = 0.018$ and $P = 0.035$, respectively). To investigate the inflammatory mediators associated with disease extent, we evaluated the correlation between the LM CT score and each cytokine level according to endotype (**Supplementary Table S4**). In all patients, IL-8, IL-17A, and IL-5 levels were weakly but positively correlated to the LM CT score, and IL-22 level was inversely correlated to the LM CT score. In the mild mixed inflammation group, IL-22 and TGF-β1 levels were inversely correlated to the LM CT score. IL-8 level was positively correlated to the LM CT score in the T3 group. However, no inflammatory mediators showed significant correlations to the LM CT score in the T2 group.

DISCUSSION

In this study, the inflammatory endotypes of CRS in the Korean population were derived from multicenter data by cluster analysis based on inflammatory markers, unbiased by phenotype data. Patients were clustered by the inflammatory markers, and 5 clusters were derived: one cluster with mild mixed inflammation (cluster 1), 3 clusters with T3 inflammation (clusters 2, 3, and 4), and one cluster with moderate T2 inflammation (cluster 5).

In the present study, it was found that there was a difference in inflammatory CRS endotypes between the previous cluster analysis in Europe[21] and the present Korean multicenter study.

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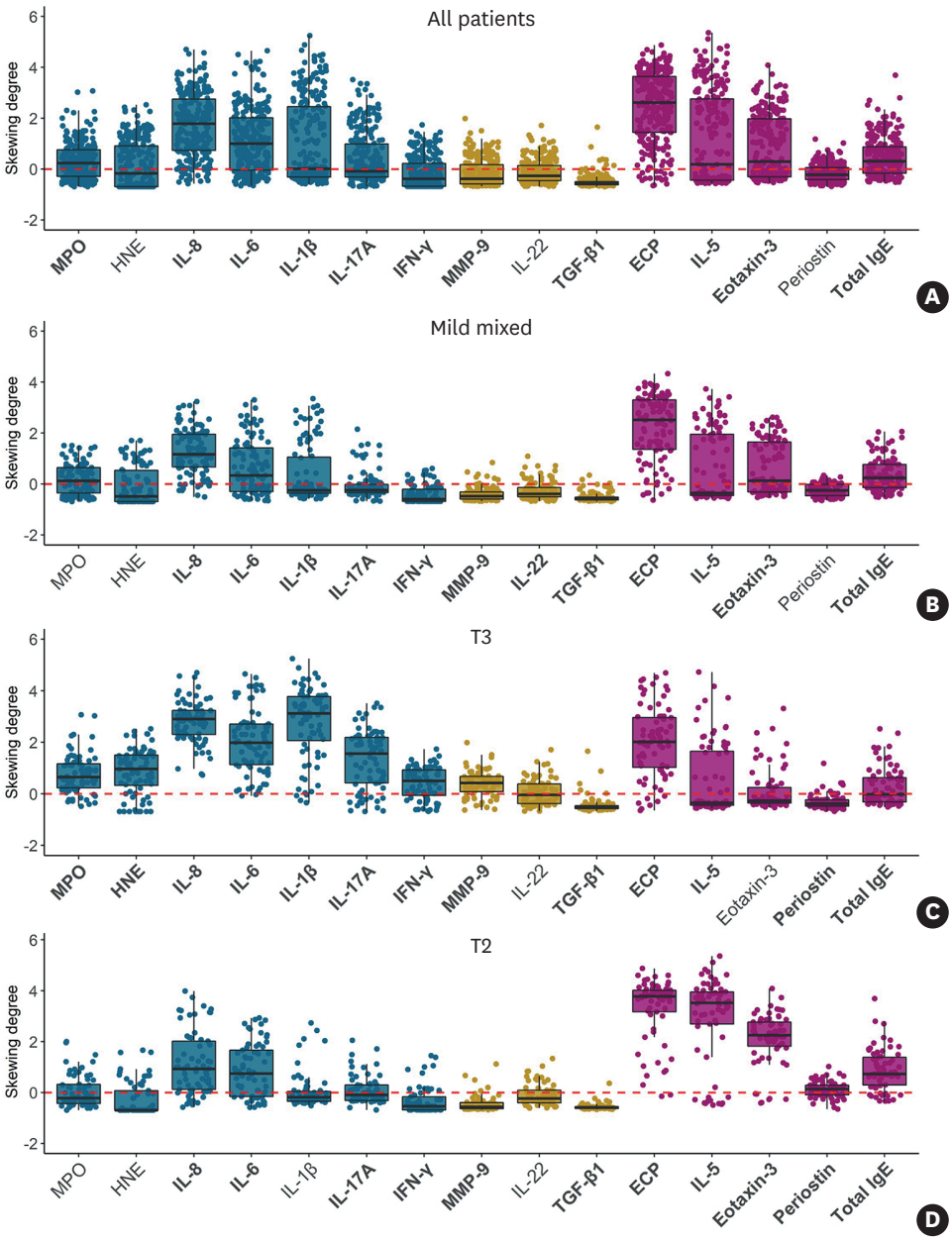

Fig. 4. Standardized cytokine levels in CRS patients compared to controls using skewing degree. Dots display the values for individual patients, and a boxplot was made to demonstrate the median value, interquartile range, and 95% confidence interval of skewing degree for each cytokine. Bold text on the *x*-axis denotes a cytokine that is significantly different from the controls in the original values. (A) All patients. (B) Mild mixed group. (C) T3 group. (D) T2 group.
CRS, chronic rhinosinusitis; T, type; MPO, myeloperoxidase; HNE, human neutrophil elastase; IL, interleukin; IFN, interferon; MMP, matrix metalloproteinase; TGF, transforming growth factor; ECP, eosinophil cationic protein; IgE, immunoglobulin E.

The previous cluster analysis demonstrated that CRS could be categorized into non-T2, moderate, and severe T2 inflammation, with a clear increase in CRSwNP phenotype and comorbid asthma.[21] Unlike that study, we showed that CRS could be classified into mild mixed, T3, and moderate T2 inflammation in the Korean population. In Western populations, T2 inflammation plays a major role in determining the inflammatory profile, and markedly eosinophilic inflammation is accompanied by substantial levels of neutrophilic inflammation

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Table 2. Demographics and inflammatory profiles of endotype groups

Characteristics	Mild mixed (n = 103, 42.2%)	Type 3 (n = 76, 31.1%)	Type 2 (n = 65, 26.6%)	*P* value
Age (yr)	51 (42–60)	53 (36–62)	49 (41–57)	0.647
Sex, ratio (male:female)	82:21	45:31	43:22	**0.010**
Presence of nasal polyp (%)	85/103 (82.5)	64/76 (84.2)	59/65 (90.8)	0.325
Asthma (%)	16/82 (19.5)	5/64 (7.8)	20/59 (33.9)	**0.001**
AERD (%)	3/95 (3.2)	1/70 (1.4)	6/63 (9.5)	0.088
Atopy (%)	42/101 (41.6)	22/72 (30.6)	27/63 (42.9)	0.245
Serum eosinophil (%)	3.2 (1.5–5.8)	1.7 (1.1–3.3)	5.5 (3.0–8.5)	< **0.001**
Tissue eosinophil count (cells/HPF)	4 (1–18)	1 (0–5)	17 (4–33)	< **0.001**
Revision surgery (%)	15/103 (14.6)	16/76 (21.1)	9/65 (13.8)	0.458
Lund-Mackay score	14 (10–18)	17 (13–20)	15 (12–21)	0.010
SNOT-22	32 (22–50)	37 (25–55)	39 (26–53)	0.250
IFN-γ (pg/mg)	1.1 (0–4.5)	9.7 (5.4–14.8)	1.7 (0–4.8)	< **0.001**
IL-5 (pg/mg)	0 (0–2.4)	0 (0–1.9)	11.7 (5.1–18.0)	< **0.001**
IL-17A (pg/mg)	0 (0–0)	3.2 (0.6–6.6)	0 (0–0)	< **0.001**
IL-22 (pg/mg)	30 (20–49)	55.3 (31.0–84.7)	43 (29–63)	< **0.001**
IL-1β (pg/mg)	0 (0–1.0)	7.8 (2.6–15.0)	0 (0–0)	< **0.001**
IL-6 (pg/mg)	3.2 (1.5–9.4)	16.6 (7.0–34.1)	4.8 (1.9–12.0)	< **0.001**
IL-8 (pg/mg)	79 (48–174)	448 (241–625)	63 (28–185)	< **0.001**
MPO (ng/mg)	31 (15–51)	52 (34–89)	21 (13–37)	< **0.001**
MMP-9 (ng/mg)	4.1 (2.0–6.7)	16.4 (11.6–21.1)	2.6 (1.2–5.5)	< **0.001**
HNE (ng/mg)	65 (0–299)	458 (233–787)	0 (0–188)	< **0.001**
ECP (pg/mg)	98 (28–275)	55 (21–166)	350 (149–430)	< **0.001**
Eotaxin-3 (pg/mg)	19 (11–88)	12 (10–22)	163 (105–271)	< **0.001**
Total IgE (kU/g)	12 (8–24)	8 (6–14)	22 (11–31)	< **0.001**
SE-IgE (%, positivity)	3/103 (2.9)	0/76 (0)	4/65 (6.2)	0.082
TGF-β1 (pg/mg)	493 (290–734)	705 (508–972)	425 (312–627)	< **0.001**
Periostin (ng/mg)	44.2 (25.2–60.3)	32.8 (23.4–45.3)	70.3 (55.2–81.7)	< **0.001**

Value is presented as median with interquartile range. Bold text indicates statistical significance ($P < 0.05$).
AERD, aspirin exacerbated respiratory disease; HPF, high-power field; SNOT-22, sino-nasal outcome test-22; IFN, interferon; IL, interleukin; MPO, myeloperoxidase; HNE, human neutrophil elastase; MMP, matrix metalloproteinase; ECP, eosinophil cationic protein; IgE, total immunoglobulin E; SE, staphylococcal enterotoxin; TGF, transforming growth factor.

in CRSwNP patients. However, CRSwNP in China and Korea revealed that only a small percentage of CRSwNP patients (4.4%–11.2%) had both high eosinophilic and neutrophilic inflammation.[23,27,28] A divergence between T3 and T2 inflammation was consistently observed in CRS studies in Asian populations, including this cluster analysis,[15,28,29] suggesting that both T3 and T2 inflammation may play major roles in determining the inflammatory profiles of CRS in Asian populations.

In the T3 group (clusters 2, 3, and 4), the skewing degrees of neutrophilic and proinflammatory mediators were prominent. Furthermore, elevated levels of IFN-γ, a T1 marker, were also present in the T3 group, which was not found in the mild mixed and T2 groups. The T3 group consisted of 3 clusters, and stratification of T3 inflammatory patterns was observed among these clusters (mild T3, cluster 2; moderate T3, cluster 3; and severe T3, cluster 4). Moreover, the stratification of T3 inflammation also correlated to phenotype and disease extent. Unlike our findings, in the study of Liao *et al.*,[15] clusters derived from molecular and cellular factors did not show significant differences in a number of clinical characteristics. However, our study showed that the clusters generated by only inflammatory markers correlated to phenotypes.

In the mild mixed and T2 groups, although MPO expression is commonly consistent with HNE expression in neutrophils, our previous study revealed that MPO was upregulated in the controlled CRSwNP patients, but HNE-positive cells and HNE levels were upregulated

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in the refractory CRSwNP patients.[23] Cluster 2, which exhibited high MPO and relatively low HNE levels, had a low proportion of CRSwNP and low LM CT scores, and cluster 4, which had relatively low MPO and high HNE levels, showed a high proportion of CRSwNP and high LM CT scores, which is consistent with the results of our previous study.[23] In addition, IL-8 levels, which were associated with poor disease outcomes in the study of Liao *et al.*,[15] were also high in cluster 4 and were significantly correlated with disease extent in the T3 groups.

The T2 group (cluster 5) was characterized by a predominant elevation in T2 inflammatory mediators. Periostin, which is known to be induced mainly by T2 cytokines,[30] was also prominently increased in the T2 group. The positivity of SE-specific IgE was 6.2%, which was the highest among the clusters. In a previous multi-continent study that revealed the difference in the positivity of SE-specific IgE according to regional factors, the positivity of SE-IgE was high in European and Oceanian countries and Japan (26%–56%), but low in China (3%–4%).[6] Similar to the Chinese population,[6] we found that the positivity of SE-IgE was 2.9% in the overall CRS patients. It is known that the presence of SE-IgE is associated with intense eosinophilic inflammation associated with elevated IgE concentrations,[31] and a previous cluster analysis[21] showed that the most severe T2 cluster had a high positivity of SE-IgE (100%). Given this, we could hypothesize that the low positivity of SE-IgE in the T2 group indicates that there is a small population of individuals with severe T2 CRSwNP in Korea and China. The observation of relatively low levels of IL-5, a key cytokine in T2 inflammation, compared with CRS in Western countries[6,21] may also support this idea. In the present study, comorbid asthma was most common in the T2 group (34%), but this proportion was comparable to the "IL-5 moderate-expression group" in the study of Tomassen *et al.*[21] (27%–37%). Interestingly, 20% of patients in the mild mixed group had comorbid asthma, which was the second-highest rate among the clusters. Although the levels of eosinophilic and T2 inflammatory markers in the mild mixed group were not as markedly high as in the T2 group, levels of these markers were higher in the mild mixed group than in controls, suggesting that T2 inflammation might contribute to the relatively high proportion of comorbid asthma in the mild mixed group. Because of the absence of the severe form of T2 inflammation, we speculated that a mild form of T2 inflammation (which may be mixed with T3) was presented as cluster 1, and a moderate form of T2 inflammation was presented as cluster 5. Altogether, the predominance and severity of T2 inflammation in CRS may be less common in Asian countries than in Western countries. Future studies are warranted.

It is known that the severity of T2 inflammation plays a main role in clinical outcomes and disease severity of CRS, especially in Western countries[5,6,9,21] However, another major finding of our study is that the disease extent of the T3 group was comparable to that of the T2 group, and the proportion of CRSwNP in cluster 4 (severe T3 inflammation) was 100%, indicating that T3 inflammation may be a main driver determining disease severity and T2 inflammation. In other words, endotyping by only T2 inflammation could not reflect clinical features, such as the phenotype and disease extent, in the Korean population. Supporting our findings, our previous study showed that non-eosinophilic nasal polyps with tissue neutrophilia and eosinophilic nasal polyps without tissue neutrophilia had similar refractoriness (50% vs. 51%, respectively).[23] Moreover, the presence of non-eosinophilic CRS with poor clinical outcomes in another cluster analysis in the Chinese population[15] may support the important role of neutrophil-associated inflammation in severe CRS in Asians.

Furthermore, we sought to investigate whether any factors in each endotype group were significantly correlated to the extent of disease. Interestingly, IL-22 and TGF-β1 showed

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significant inverse correlations with the LM CT score in the mild mixed group, whereas IL-8 was positively correlated to the LM CT score in the T3 group, suggesting that biomarkers may differ according to endotype. Additionally, IL-5 was positively correlated to the LM CT score in all CRS patients, although the relationship was not significant in the T2 group. This could be explained by the absence of severe T2 inflammation in this study, which did not lead to stratification in the T2 group (cluster 5) as clearly as in the T3 group. The presence of different biomarkers according to endotype was also demonstrated in a previous cluster analysis,[15] and these findings emphasize the heterogeneity of CRS and the necessity of endotype-based management.

This study has some limitations. First, the long-term clinical outcomes were not assessed. Secondly, the number of patients in cluster 2 (mild T3 group) and cluster 4 (severe T3 group) was small, and further characterization of these patients is needed. Lastly, although distinct inflammatory mediators showed significant correlations with disease extent in each endotype group, most correlation coefficients were relatively low ($r < 0.3$). This may be because complex interactions between inflammatory mediators determine the severity and extent of disease, rather than the effects of a single mediator.

In conclusion, distinct inflammatory endotypes were derived in our cluster analysis, which correlated with phenotype, comorbid asthma, and disease severity: mild mixed, T3, and moderate T2 inflammation. Furthermore, the divergence between T3 and T2 inflammation observed in this cluster analysis indicates that both T3 and T2 inflammation may play important roles in the pathophysiology of CRS in Asian populations. The inflammatory endotypes and their relevant clinical features in the Korean population may provide a better understanding of the pathophysiology of CRS and aid in optimizing therapeutic approaches to CRS in Asians.

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SUPPLEMENTARY MATERIALS

Supplementary Data S1
Methods

Click here to view

Supplementary Table S1
Demographics and inflammatory profiles of participants

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Supplementary Table S2

Coordinates of given variables in the first 6 dimensions from the results of factor analysis for mixed data

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Supplementary Table S3

Demographics and inflammatory profiles of the clusters

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Supplementary Table S4

Correlation between Lund-Mackay score and each cytokine according to endotypes

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