Correlations of pre- and post-operative symptoms with cytokines in different phenotypes and endotypes of chronic rhinosinusitis

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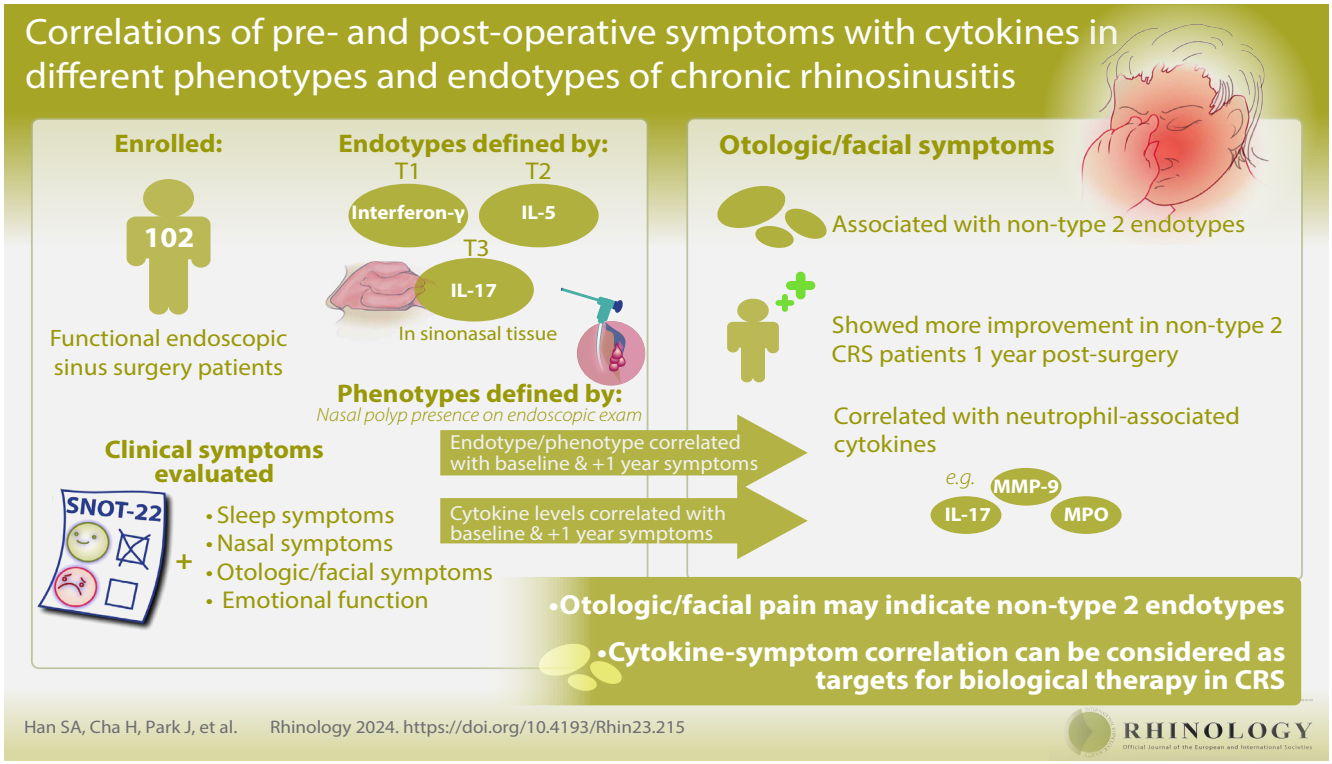

Abstract

Background: Recognising inflammatory endotypes in chronic rhinosinusitis (CRS) has become more important, especially with the advent of biological treatments. In this study, we investigated the correlations of pre- and post-operative symptoms with cytokine positivity in different endotypes and phenotypes of CRS. **Methodology**: In total, 102 patients undergoing routine functional endoscopic sinus surgery were enrolled. The endotype classification (type 1, 2, or 3 CRS) was defined based on positivity for interferon-γ, interleukin (IL)-5, or IL-17 respectively, in sinonasal tissue samples. Clinical symptom scores were evaluated pre- and post-operatively using the 22-item Sinonasal Outcome Test and its four symptom subdomains: sleep, nasal, otologic/facial symptoms, and emotional function. Symptoms were compared between endotypes and phenotypes, and exploratory factor analysis (EFA) based on principal component analysis (PCA) was performed. The correlations of cytokine levels with baseline symptoms and changes in symptoms after 1 year were analysed. **Results**: Symptoms in the otologic/facial pain category were associated with non-type 2 endotypes in PCA and confirmatory analysis. Non-type 2 CRS patients exhibited significantly more improvement in facial symptoms 1 year after surgery. Neutrophil-associated cytokines, such as IL-17, matrix metalloproteinase 9, and myeloperoxidase, were significantly correlated with baseline otologic/facial pain symptoms and changes in those symptoms after surgery. **Conclusions**: Otologic/facial pain symptoms may be indicative of non-type 2 endotypes. Neutrophil-associated cytokines, such as IL-17, MMP-9, and MPO, were significantly correlated with these symptoms. The establishment of links between specific symptoms and certain cytokines may help use and develop biological therapies for CRS.

Key words: chronic rhinosinusitis, clinical symptoms, cytokines, endotypes, phenotypes

Introduction

Chronic rhinosinusitis (CRS) has been classically categorised according to the presence of polyps as CRS with nasal polyps (CRSwNP) and CRS without nasal polyps (CRSsNP). However, with a deeper understanding of the pathophysiology of CRS and the advent of biological treatments, the importance of identifying the inflammatory endotype has been highlighted [1,2]. CRS patients experience diverse symptoms that can affect their quality of life, and specific symptoms have been correlated with phenotypes or endotypes of CRS. For example, it has been widely accepted that olfactory loss is a more characteristic symptom of CRSwNP than CRSsNP and has also been associated with type 2 inflammation [3-7]. In the European Position Paper on Rhinosinusitis and Nasal Polyps (EPOS) 2020 guideline, olfactory dysfunction is listed as one of the five criteria used to determine type 2 inflammation in CRS, which is an indication for using biological treatments. Improvement in olfactory function is also a criterion used to evaluate the response to biological treatments [8]. The association of clinical symptoms with endotypes may be especially important in the era of biological treatments, as these treatments target specific inflammatory pathways. Currently, the biological treatments that have been approved for the treatment of CRSwNP by the US Food and Drug Administration (omalizumab, mepolizumab and dupilumab) are targeted at type 2 inflammation [9-11]. Therapeutics that target type 1 or type 3 inflammatory pathways have been approved for use in other inflammatory diseases and have the potential to be applied to CRS treatment [12]. Thus, symptoms indicative of endotypes may be helpful in selecting candidates for these treatments. In this study, we investigated the correlations of pre- and post-operative symptoms in various endotypes or phenotypes of CRS with positivity for the cytokines related to these clinical presentations.

Materials and methods

Patients and tissue samples

The diagnosis of CRS was based on clinical history, endoscopy, and computed tomography (CT) findings according to the EPOS 2020 definition of CRS [8]. Patients undergoing routine functional endoscopic sinus surgery (FESS) for bilateral CRS who provided informed consent to participate were enrolled in the study. Ethmoid mucosa was obtained from CRS patients and nasal polyp (NP) tissue was obtained from CRSwNP patients. The study was approved by the Institutional Review Board of Seoul Metropolitan Government-Seoul National University Boramae Medical Centre (IRB No. 30-2019-136). The exclusion criteria employed were: 1) those younger than 18 years; 2) patients using antibiotics, systemic or topical corticosteroids, or other immune-modulating drugs in the 4 weeks before surgery; and 3) patients diagnosed with unilateral rhinosinusitis, antrochoanal polyps, allergic fungal rhinosinusitis, cystic fibrosis, or immotile ciliary disease. The type 2 CRS endotype was defined as interleukin (IL)-5 positivity, while type 1 and type 3 CRS were defined as interferon (IFN)-γ positivity and IL-17 positivity, respectively. Positivity was defined as having levels more than the mean plus two standard deviations of the respective cytokine level in normal controls (the mean value of controls was derived from a previous study [13]). Patients were divided into phenotypes according to the presence of nasal polyps in an endoscopic examination. Atopic status was determined by measuring the immunoglobulin E (IgE) levels of six common aeroallergens using the ImmunoCAP® assay (Phadia, Uppsala, Sweden). When IgE levels greater than 0.35 IU/mL were detected for any of the allergens, the subjects were defined as having atopic disease. Asthma status was defined based on a diagnosis by an allergist through a clinical history and lung function and/or provocation tests. Clinical symptom scores were measured using the 22-item Sinonasal Outcome Test (SNOT-22) [14]. The SNOT-22 scores were divided further into four symptom subdomains: sleep symptoms (questionnaire items 11 through 18), nasal symptoms (items 1 through 6, 21 and 22), otologic/facial pain symptoms (items 7 to 10), and emotional function (items 19 and 20) [15]. The SNOT-22 questionnaire was repeated in subjects who were followed up until 1 year post-operatively. Item 21 of the questionnaire was analysed separately to assess olfactory symptoms. Systemic medications, including corticosteroids or antibiotics, were not prescribed during the 1 month preceding the 1-year follow-up visit. The Lund-Mackay (LM) score was calculated from the preoperative CT [16].

Measurement of cytokines in tissue homogenates

Sample preparation and cytokine analysis were performed as previously described [17-19]. Tissue homogenates were analysed for levels of B-cell activating factor (BAFF), bone morphogenic protein (BMP)-2, BMP-7, matrix metalloproteinase (MMP)-9, CC motif chemokine ligand (CCL)-26 (eotaxin-3), CCL-17 (thymus and activation-regulated chemokine, TARC), IL-1β, IL-5, IL-6, IL-8, IL-13, IL-17, IFN-γ, periostin, and myeloperoxidase (MPO) through a multiplex immunoassay (LXSAHM Human Premixed Multi-Analyte Kit, R&D Systems, Minneapolis, MN, USA). IL-22, human neutrophil elastase (HNE) (DuoSet ELISA DY782, DY9167-05, R&D Systems) and transforming growth factor (TGF)-β1 were also analysed (LTGM100 Magnetic Luminex Performance Assay TGF-beta 1 Kit, R&D Systems). All cytokine levels were normalised to the total protein level.

Statistical analysis

The statistical analysis was performed using GraphPad Prism version 8.4.3 (GraphPad Software, La Jolla, CA, USA), and R software version 4.2.0 (R Foundation for Statistical Computing, Vienna, Austria). Exploratory factor analysis (EFA) of mixed data based on principal component analysis (PCA) was performed using

FactoMineR package [20] on R. The two-tailed Mann-Whitney U test was used to compare continuous variables between two groups, and the chi-square test was used to compare categorical variables. Spearman correlation coefficients (r) were calculated in the correlation analysis.

Results

Endotypes of CRS

In total, 102 subjects, including 69 CRSwNP patients and 33 CRSsNP patients who had been enrolled consecutively, were analysed. The type 2/3 mixed endotype was the most frequent in both CRSwNP and CRSsNP patients (32% and 52%, respectively). In the CRSwNP group there were no patients with only the type 1 endotype while 23% showed only the type 2 endotype and 10% showed only the type 3 endotype. Similarly, in the CRSsNP group, there were no patients with only the type 1 endotype, while 9% of patients had only type 2 inflammation and 21% had only type 3 inflammation. Nineteen percent of the patients with CRSwNP and 15% of CRSsNP patients were not classified into any of the endotypes because they showed positivity for none of the three cytokines. Seven percent of the CRSwNP patients had type 1/3 mixed inflammation, and 9% had all three types of inflammation (types 1, 2, and 3). None of the CRSsNP patients showed type 1/3 mixed or type 1/2/3 mixed endotypes, but 3% of them had the type 1/2 mixed endotype (Figure 1 and S1).

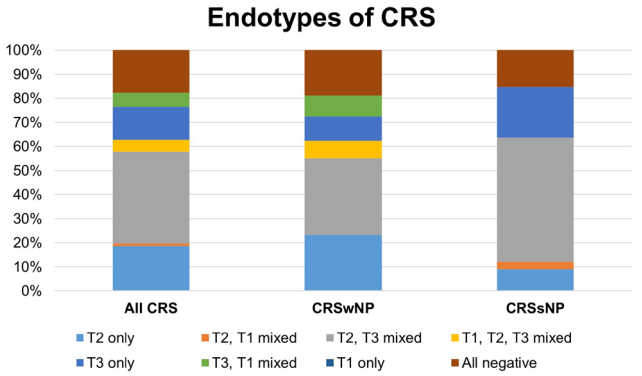

Figure 1. Inflammatory endotypes in CRS patients. T1, type 1inflammation; T2, type 2 inflammation; T3, type 3 inflammation.

Symptoms according to endotypes of CRS

To investigate the relationship between clinical symptoms and the endotypes of CRS, we performed EFA on the endotypes and scores for the individual items on the SNOT-22 questionnaire based on PCA. The first and second dimensions accounted for 23.39% and 8.90% of the variance, respectively (Figure 2A, B). Questionnaire item number that contributed the most to the first dimension were 19 ("sadness") 20 ("embarrassed"), 21 ("sense of taste/smell") which contributed 8.51%, 10.84%, and 9.82% respectively. Items 19 and 20 were both positively correlated with both the 1st and 2nd dimensions while item 21 was positively correlated to dimension 1 (r = 0.56) but negatively correlated with dimension 2 (r = -0.58). Otologic/facial pain symptoms including items 7 ("ear fullness") and 9 ("ear pain"), each contributing 6.75% and 6.08% respectively, followed. Item 21, representing olfactory symptoms, contributed the most to the second dimension (27.84%). Multiple comparison of scores of items 19, 20 and 21, which contributed most to dimensions 1 and 2, were compared according to endotypes as a confirmatory analysis. As the endotypes were not clearly differentiated by dimensions 1 and 2, confirmatory analysis also showed that items contributing most to these dimensions did not show significant difference between endotypes (Figure S4A). Although dimension 3 accounted for lower percentage of variance than the first and second dimensions, it made a clearer distinction between endotypes. Dimensions 3 and 4 accounted for 8.03% and 7.67% of variance, respectively (Figure 2C, D). Individuals with type 2 endotype were positively correlated with dimension 3 while non-type 2 endotype patients were negatively correlated (Figure 2C). Item 10 ("facial pain/ pressure") and item 8 ("dizziness"), which are in the category of otologic/facial pain symptoms, contributed the most to dimension 3 (7.12% and 4.39%), and were both negatively correlated with dimension 3 (r = -0.41 and r = -0.32 respectively). Confirmatory analysis comparing scores of items 8 and 10 between type 2 and non-type 2 endotypes showed that score for item 8 did not show a significant difference (p = 0.188) while the median score of item 10 was higher in non-type 2 endotype patients (p = 0.025) (Figure S4B).

Comparison of symptoms between type 2 and non-type 2 CRS

The symptoms of patients were further analysed according to type 2 CRS and non-type 2 CRS as defined by IL-5 positivity. Sixty-four (62.7%) subjects had type 2 CRS, while 38 (37.3%) subjects had non-type 2 CRS. There was no significant difference in the baseline demographics and clinical characteristics, such as age, sex, LM CT scores, initial SNOT-22 scores, or serum total IgE levels, between groups. The number of patients with atopic status was higher in the type 2 CRS group than in the non-type 2 CRS group (p = 0.020), as was also observed for the number of patients with asthma (20 [31.3%] in the type 2 CRS group vs. 4 [10.5%] in the non-type 2 CRS group; p = 0.018). The blood eosinophil percentage was significantly higher in the type 2 CRS group (p = 0.002) than in the non-type 2 CRS group (Table 1). Symptom scores were compared between type 2 CRS and non-type 2 CRS patients. The baseline total SNOT-22 scores and subdomain scores were not significantly different between the two groups. Olfactory symptoms, as determined by SNOT-22 item number 21, were also not significantly different between groups (Figure 3A). However, non-type 2 CRS patients exhibited

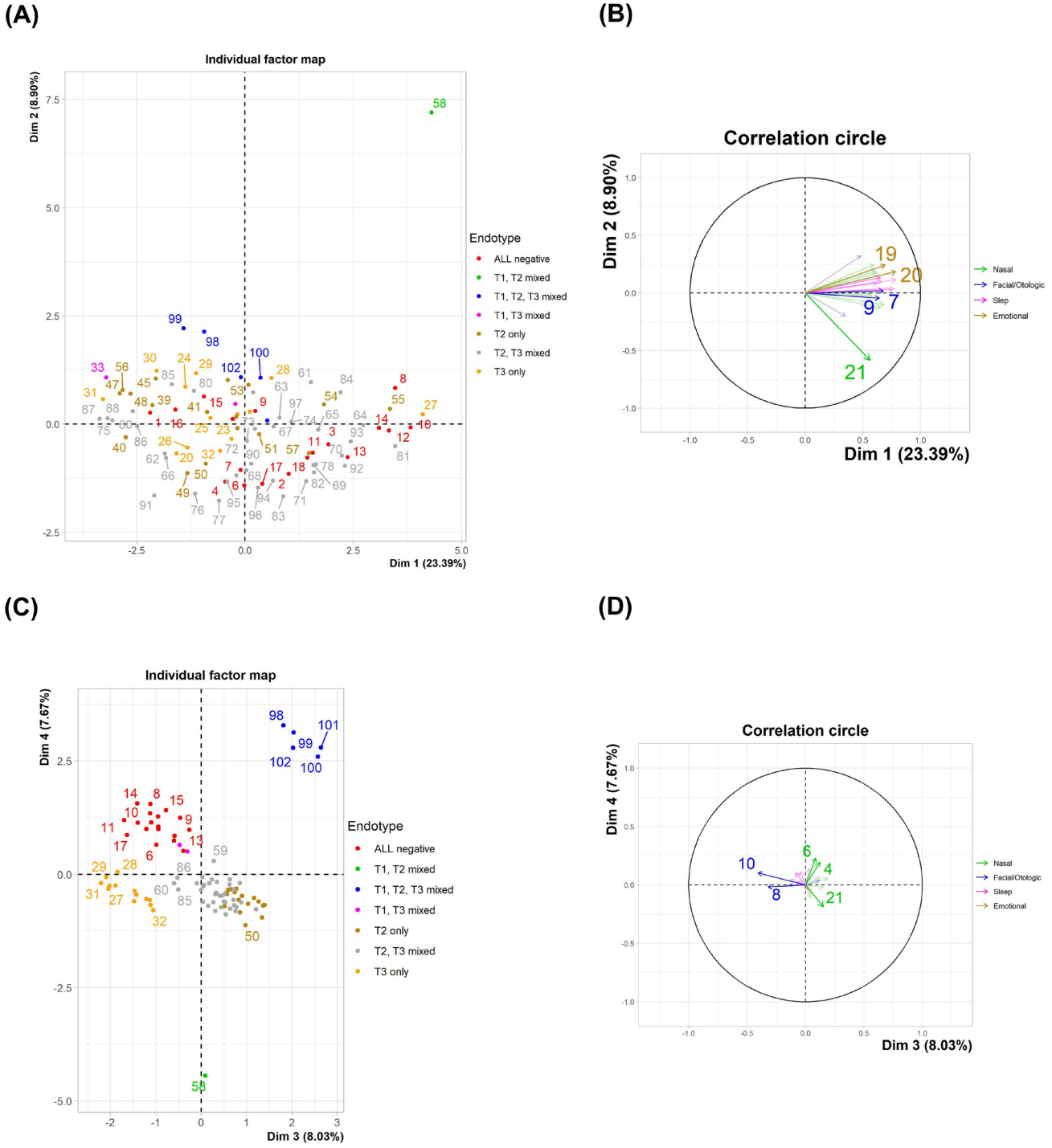

Figure 2. Exploratory factor analysis based on principal component analysis according to endotypes and score of individual item on SNOT-22. Each dot on the individual factor map (A, C) indicates individual patients and are colored based on their endotypes. Arrows on the correlation circle (B, D) indicate items on SNOT-22 and their correlation with each dimension. (A) Individual patients against dimensions 1 and 2. (B) SNOT-22 items against dimensions 1 and 2. (C) Individual patients against dimensions 3 and 4. (D) SNOT-22 items against dimensions 3 and 4. Item 21 is an item of the nasal symptom subdomain that relates to olfactory symptoms.

significantly more symptom improvement at 1 year after surgery in the facial symptom domain than patients with type 2 CRS (1.77 vs. 3.26; p = 0.033) (Figure 3B).

To clarify whether certain cytokines were related to baseline symptoms and changes in symptoms, a correlation analysis was performed. In non-type 2 CRS, TGF-β1 was significantly positively associated with the emotional domain score of the baseline SNOT-22 (r = 0.41, p = 0.02), while IL-17 levels showed a signifi-

Table 1. Demographics and clinical characteristics of type 2 CRS and non-type 2 CRS patients.

	type 2 CRS (n=64)	non-type 2 CRS (n=38)	p-value [a]
Sex (female)	15 (23.4%)	12 (31.6%)	0.487
Age (yr)	49.4±14.2	47.3±17.2	0.638
Atopic (number)	31 (48.4%)	9 (23.7%)	0.020*
Asthma (number)	20 (31.3%)	4 (10.5%)	0.018*
Lund-Mackay score	14.6±5.0	15.2±4.4	0.349
Initial SNOT-22	38.7±20.3	41.9±22.9	0.686
Current smoking(number)	14 (21.9%)	10 (26.3%)	0.635
Blood eosinophil (%)	6.28±3.97	4.52±4.55	0.002**
Total IgE	399.7±710.3	265.8±518.0	0.142

[a] Comparison between type 2 CRS and non-type 2 CRS using Mann-Whitney U tests or chi-square tests. *p<0.05. **p<0.01. CRS, chronic rhinosinusitis; SNOT-22, 22-item Sinonasal Outcome Test.

Table 2. Demographics and clinical characteristics of CRSwNP and CRSsNP patients.

	CRSwNP (n=69)	CRSsNP (n=33)	p-value [a]
Sex (female)	12 (17.4%)	15 (45.5%)	**0.004****
Age (yr)	49.4±14.7	47.3±16.7	0.451
Atopic (number)	26 (37.7%)	14 (42.4%)	0.670
Asthma (number)	19 (27.5%)	10 (30.3%)	0.817
Lund-Mackay score	16.0±4.5	12.4±4.5	**0.001****
Initial SNOT-22	38.1±19.0	42.5±24.6	0.433
Current smoking(number)	19 (27.5%)	5 (15.1%)	0.216
Blood eosinophil (%)	5.64±4.26	5.55±4.22	0.726
Total IgE	395.9±736.4	257.6±399.8	0.584

[a] Comparison between CRSwNP and CRSsNP using Mann-Whitney U tests or chi-square tests. *p<0.05. **p<0.01. CRS, chronic rhinosinusitis; CRSwNP, CRS with nasal polyps; CRSsNP, CRS without nasal polyps; SNOT-22, 22-item Sinonasal Outcome Test.

cant positive correlation with the baseline otologic/facial pain domain scores in type 2 CRS patients (r = 0.42, p = 0.002) (Figure S2A and B). As for changes in symptoms after surgery, MPO had a significant positive correlation with improvement in otologic/facial pain symptoms in patients with type 2 CRS (r = 0.423, p = 0.040). MMP-9 showed a significant positive correlation with improvement in otologic/facial pain symptoms in non-type 2 CRS patients (r = 0.445, p = 0.049) (Figure S2C and D).

Comparison of symptoms between CRSwNP and CRSsNP

Subjects were categorised according to phenotypes of CRS. Sixty-nine (67.6%) patients had NP, while 33 (32.4%) patients did not. The proportion of female patients was significantly higher in the CRSsNP group than in the CRSwNP group (p = 0.004), and baseline LM CT scores were higher in CRSwNP patients than in CRSsNP patients (p = 0.001). Other demographic and baseline characteristics did not differ significantly between groups (Table 2).

PCA of the individual items on the SNOT-22 questionnaire according to CRS phenotypes was performed to determine which questionnaire items were related to CRS phenotypes. Dimensions 1 and 2 explained 35.64% and 15.34% of the variance, respectively. Baseline olfactory symptoms (item 21) contributed the most to dimension 2, which differentiated between the two phenotypes (Figure 4). The results of this EFA were confirmed in the comparison of baseline symptom scores. While other symptom domains showed no difference between groups, olfactory symptoms (i.e., the median score of item 21) were significantly higher in CRSwNP patients than in CRSsNP patients (p = 0.019) (Figure 5A, B).

The improvement of olfactory symptoms at 1 year after surgery was also significantly greater in CRSwNP patients than in CRSsNP patients (2.28 vs. 0.8; p = 0.016). To determine whether certain cytokines were correlated with baseline symptom domains, a correlation analysis was conducted according to phenotypes. The TGF-β1 (r = -0.35, p = 0.009) and IFN-γ (r = -0.27, p = 0.047)

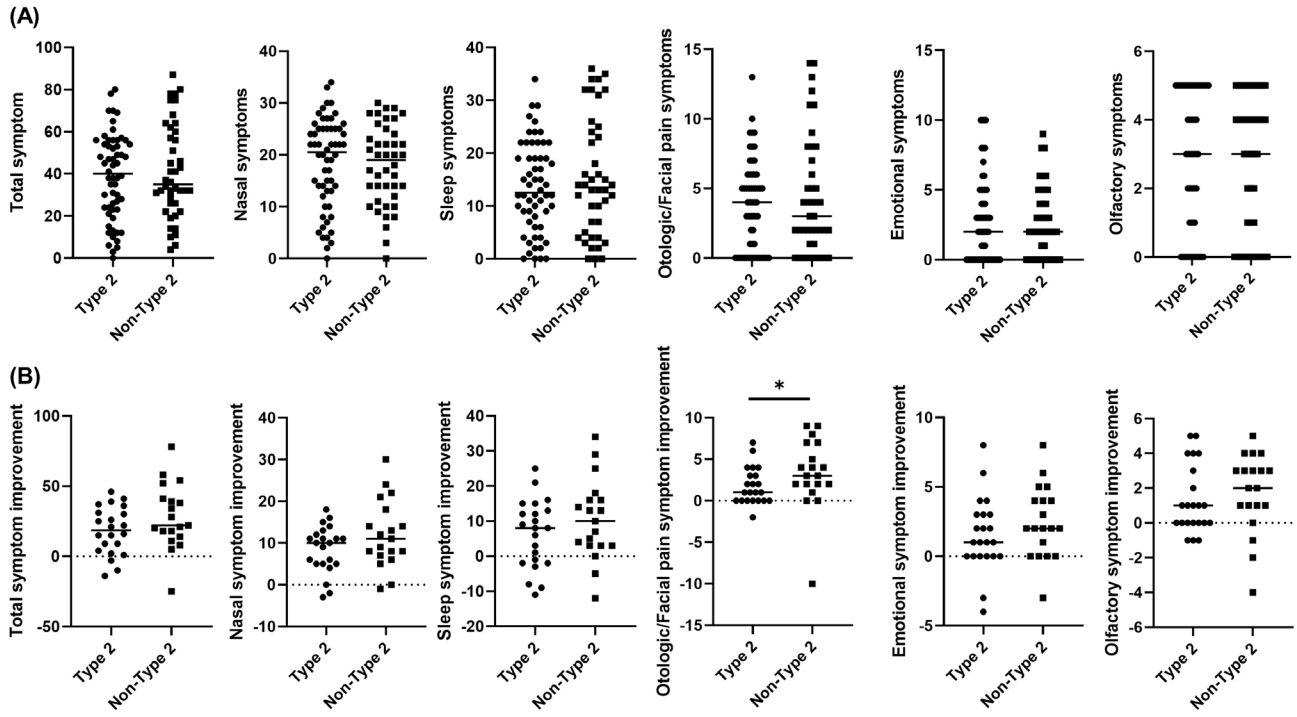

Figure 3. Comparison of symptoms in type 2 and non-type 2 CRS patients. (A) Baseline symptoms (B) Symptom improvement at 1 year after surgery (*$p < 0.05$).

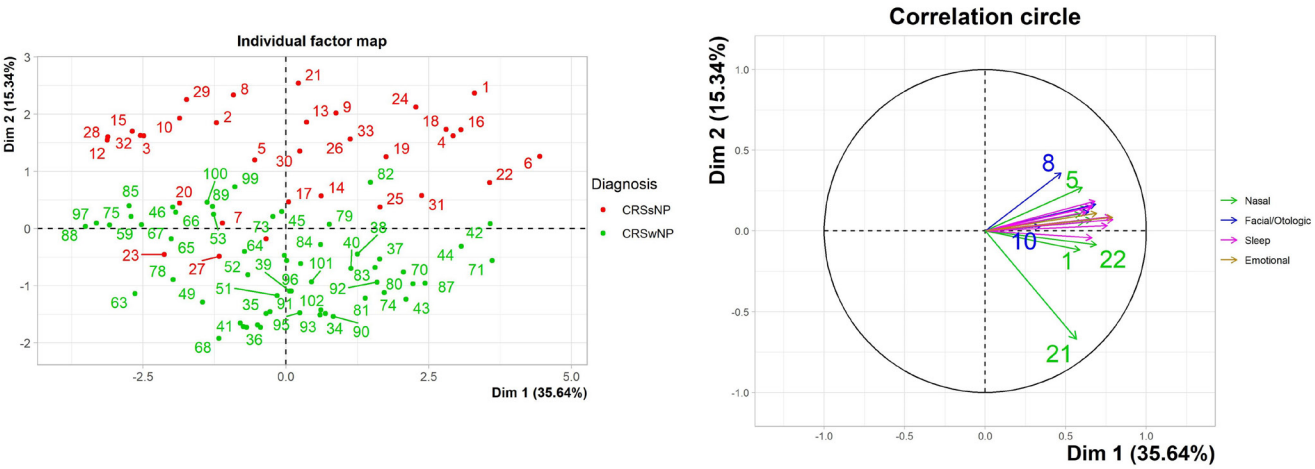

Figure 4. Exploratory factor analysis based on principle component analysis according to phenotypes and scores of individual item on SNOT-22. Each dot on the individual factor map indicates individual patients against dimensions 1 and 2 and are colored based on their phenotypes. Arrows on the correlation circle indicate items on SNOT-22 and their correlation with dimensions 1 and 2. Item 21 is an item of the nasal symptom subdomain that relates to olfactory symptoms.

cytokines were significantly negatively correlated with the sleep domain of SNOT-22 in CRSwNP patients. In CRSsNP patients, multiple cytokines (e.g., IL-17, IL-8, MMP-9, and BAFF) showed positive correlations with the otologic/facial pain symptom domain of the SNOT-22 (Figure S3A and B). Similar to type 2 CRS, MPO was positively correlated with improvement in otologic/facial pain symptoms in CRSwNP patients ($r = 0.428$, $p = 0.029$). In CRSsNP patients, MMP-9 showed positive correlations with improvement in the total SNOT-22 score ($r = 0.546$, $p = 0.019$), sleep ($r = 0.575$, $p = 0.012$), otologic/facial pain symptoms ($r = 0.559$, $p = 0.016$), and emotional symptoms ($r = 0.543$, $p = 0.020$) (Figure S3C and D).

Discussion

In this study, we investigated the association of tissue cytokine positivity with baseline symptoms and symptom improvement at 1 year after surgery according to CRS endotypes. Generally, loss of smell is associated with CRSwNP, which mainly com-

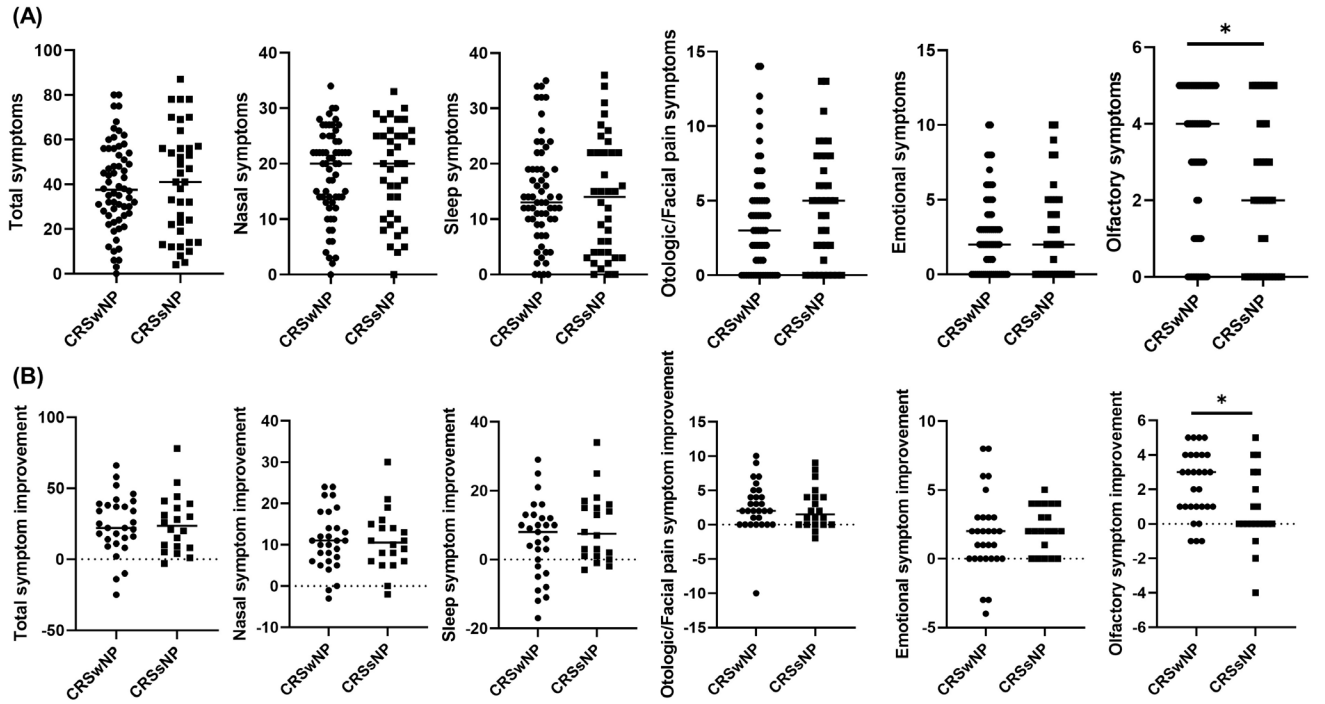

Figure 5. Comparison of symptoms in CRSwNP and CRSsNP patients. (A) Baseline symptoms (B) Symptom improvement (*p < 0.05).

prises the type 2 endotype in Western populations [3-7]. In the EPOS 2020 guideline, olfactory loss is one of the criteria used to determine whether to use biological treatment targeted at type 2 inflammation and assess its response [8]. In comparison, non-type 2 CRS is not as strongly associated with specific symptoms, and no monoclonal antibody targeted at the type 1 or type 3 inflammatory pathway has been approved for use in CRS. The patients with type 2 CRS in our study showed clinical characteristics similar to what has already been established.

In the EFA on the endotypes and symptoms using PCA, otologic/facial pain symptoms contributed the most to the dimension that differentiated between type 2 and non-type 2 CRS. In the confirmatory analysis item 10 ("facial pain/pressure") was significantly higher in non-type 2 endotype patients. IL-17 was positively correlated with the otologic/facial pain domain of the baseline SNOT-22 score; MPO was positively correlated with improvements of the score in this domain in type 2 CRS patients. MMP-9 was positively correlated with improvements in the otologic/facial pain symptom domain in non-type 2 CRS patients. Although some studies have investigated associations between the endotype and clinical outcomes, they were limited to evaluating only the absence or presence of symptoms, or assessing SNOT-22 post-operatively only, and none made correlations with cytokine levels [21, 22]. To the best of our knowledge, this is the first study to associate endotypes with clinical symptoms pre- and post-operatively and further correlate them with cytokine profiles. The results of this study may provide clues for determining other endotypes through symptoms and insights into new therapeutic targets.

First, we confirmed that the clinical characteristics of type 2 CRS patients in our study were consistent with previously established results [1,8,23]. Atopic status, the prevalence of asthma, and the blood eosinophil percentage were all higher in type 2 CRS patients than in the other groups (Table 1). However, the baseline olfactory symptoms were not worse in type 2 CRS patients. Furthermore, in the EFA based on PCA involving endotypes, the dimension that differentiated between type 2 and non-type 2 CRS explained only 7.67% of the variance, and item 21 ("olfactory symptoms") contributed less than items 8 ("dizziness") and 10 ("facial pain/pressure") to this dimension (Figure 2C, D). Furthermore, olfactory loss in type 2 CRS patients has been associated with various cytokines in previous studies [3, 5-7]. The correlation analysis in our study, however, revealed no cytokine that was significantly associated with baseline olfactory symptoms in type 2 CRS.

Both the CRSwNP phenotype and the type 2 endotype have been associated with olfactory dysfunction. In a study by Tomassen et al., clustering of CRS patients based on tissue cytokines resulted in 10 clusters, and the high IL-5 cluster was associated with the NP phenotype and high asthma prevalence [24]. In a similar study in a Chinese population, the patients were clustered into 7 clusters, one of which comprised CRSwNP patients with a high prevalence of allergic rhinitis and asthma and was associated with highest hyposmia scores [25]. In the EFA using PCA according to phenotypes, item 21 ("sense of taste/smell") contributed the most to dimension 2, which explained 15.34% of the

variance (Figure 4). This result was corroborated in the confirmatory analysis showing olfactory symptom score was significantly higher in the CRSwNP patients (Figure 5A), demonstrating that olfactory symptoms are correlated with CRSwNP. These results may suggest that the conductive type of olfactory loss due to NPs contributed to olfactory symptoms in CRS patients more than type 2 inflammation in this population.

Otologic/facial pain symptoms were associated with non-type 2 CRS in this study and also showed associated with neutrophilic markers (e.g., IL-17, MPO, and MMP-9) at baseline and improvement after surgery. Symptoms are an important part of the definition of CRS [8]. Although symptoms may not always be reflective of disease severity assessed by objective measures [26], CRS symptoms can affect patients' quality of life, and some symptoms can affect patients more than others [27]. The results of EFA based on PCA in our study revealed that the symptoms that contributed the most to dimension 3, which distinguished between type 2 and non-type 2 endotypes, were in the otologic/facial pain symptom domain (Figure 2C, D) and symptom score for item 10 was significantly worse in non-type 2 CRS patients in the confirmatory analysis (Figure S4B). Non-type 2 CRS patients experienced significant improvements in otologic/facial pain symptoms at a 1-year follow-up. Stevens and colleagues observed that type 2 inflammation was associated with smell/taste loss, while type 3 inflammation was associated with pus and purulent nasal drainage [22]. In that study, purulent discharge was associated with only the single type 3 endotype, while intraoperative pus was also associated with the mixed type 3 endotype. Pus and purulent discharge are often associated with bacterial infection and type 3 immunity. Type 3 immunity is associated with protection against extracellular bacteria and increased neutrophil recruitment [28]. Varying rates of facial pain or pressure as symptoms of sinusitis have been reported [29,30]. Negative pressure caused by obstruction of the osteomeatal complex, which is one of the main factors in the pathophysiology of non-type 2 CRS, has been suggested as a possible cause of facial pain [31]. Various nociceptive neurons are distributed within the sinonasal mucosa [32], and neutrophilic cytokines may play a role in stimulating these neurons and eliciting pain [33]. As the non-type 2 CRS patients experienced significantly more improvement in otologic/facial symptoms than the type 2 CRS patients, it can be postulated that these symptoms can be significantly alleviated by surgery in non-type 2 CRS.

Biological treatment targeted at IL-17 or other neutrophilic inflammatory mediators may also help alleviate intractable otologic/facial pain symptoms in CRS patients, although the exact pathophysiological mechanisms have yet to be fully elucidated. Monoclonal antibodies targeting IL-17 have already been approved for treatment in psoriasis [34,35]. As biologicals targeting type 2 inflammation have been effective in improving olfaction [36], those targeting IL-17, which has been identified as a cytokine associated with osteitis in CRS [37], may also have potential for the alleviation of facial pain symptoms.

One limitation of this study is that the number of patients enrolled in this study did not allow an analysis according to all endotypes, including mixed endotypes. Another possible limitation is that olfactory symptoms were determined through a single item on the SNOT-22 questionnaire without psychophysical olfactory tests. Inconsistent results have been reported regarding whether subjective reporting of olfactory function correlates with psychophysical test results [38, 39]; however, patients' subjective symptoms may be more reflective of effects on their quality of life. The possibility also exists that other aetiologies, such as migraine, might have caused otologic and facial pain symptoms in some patients. Furthermore, it was not possible for all patients to receive the same treatment, which might have affected the symptoms at the 1-year follow-up, although all patients did receive standard treatment. Additionally, as the samples were obtained from patients undergoing surgery at a tertiary referral hospital in South Korea, the results may not be generalizable as endotypes may differ according to different regions in the world. About 80% of nasal polyps in Europe and the US are known to show type 2 endotypes, while only up to 60% of CRSwNP in Korea show type 2 signature [40]. However, the percentage of patients showing eosinophilic polyps is increasing [41] and since neutrophilic inflammation is also known to play a role in severe type 2 inflammation [42], these findings may provide clues to CRS in other populations. To overcome the limitations of this study, multi-center, multi-national studies with a prospective design are warranted to corroborate our findings and to investigate in more detail the possible differences according to populations and mixed endotypes.

Conclusion

We suggest that otologic and facial pain symptoms may be indicative of non-type 2 endotypes and help in distinguishing them from type 2 CRS. Neutrophilic inflammation may be a culprit in eliciting these symptoms. Further research on how neutrophilic cytokines result in these symptoms is warranted. The cytokines related to neutrophilic inflammation should be considered as targets for therapy, which may help relieve intractable otologic and facial pain symptoms in CRS patients.

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None.

Authorship contribution

Study conception or design: DWK, SNH; Acquisition of data: SAH, HKC, JAP, Do Won K, HTR, SKY, SNH, DWK; Analysis and interpretation of data: SAH, AJ, SNH, DWK, JYK, SHO.

All authors were involved in drafting the work or reviewing it critically for important intellectual content and approved of the

final version to be published, and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or the integrity of any part of the work are appropriately investigated and resolved.

Conflict of interest

The authors declare no conflict of interest relevant to this article.

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Ethics approval and consent to participate

The study was approved by the Institutional Review Board of Seoul Metropolitan Government-Seoul National University Boramae Medical Centre (IRB No. 30-2019-136).

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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SUPPLEMENTARY MATERIAL

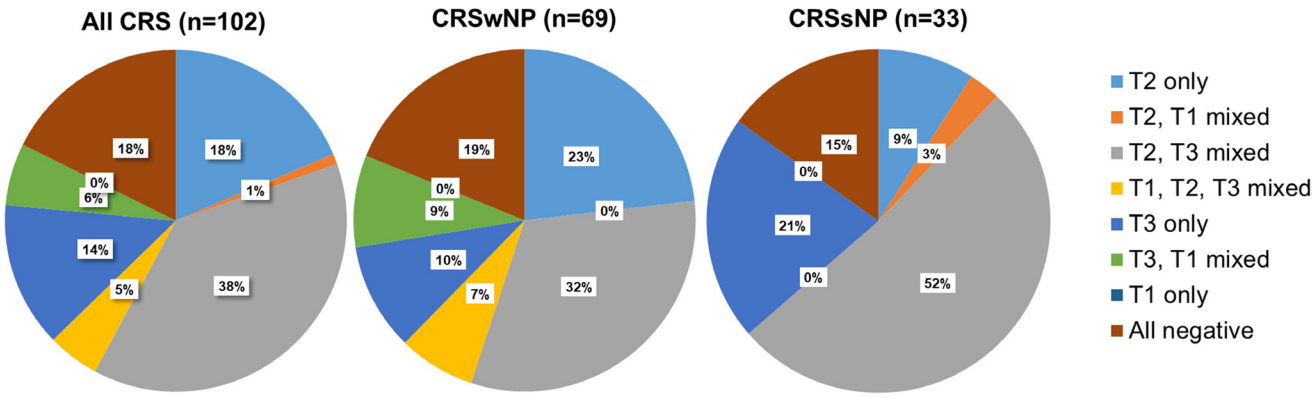

Figure S1. Inflammatory endotypes in CRS patients. T1, type 1inflammation; T2, type 2 inflammation; T3, type 3 inflammation.

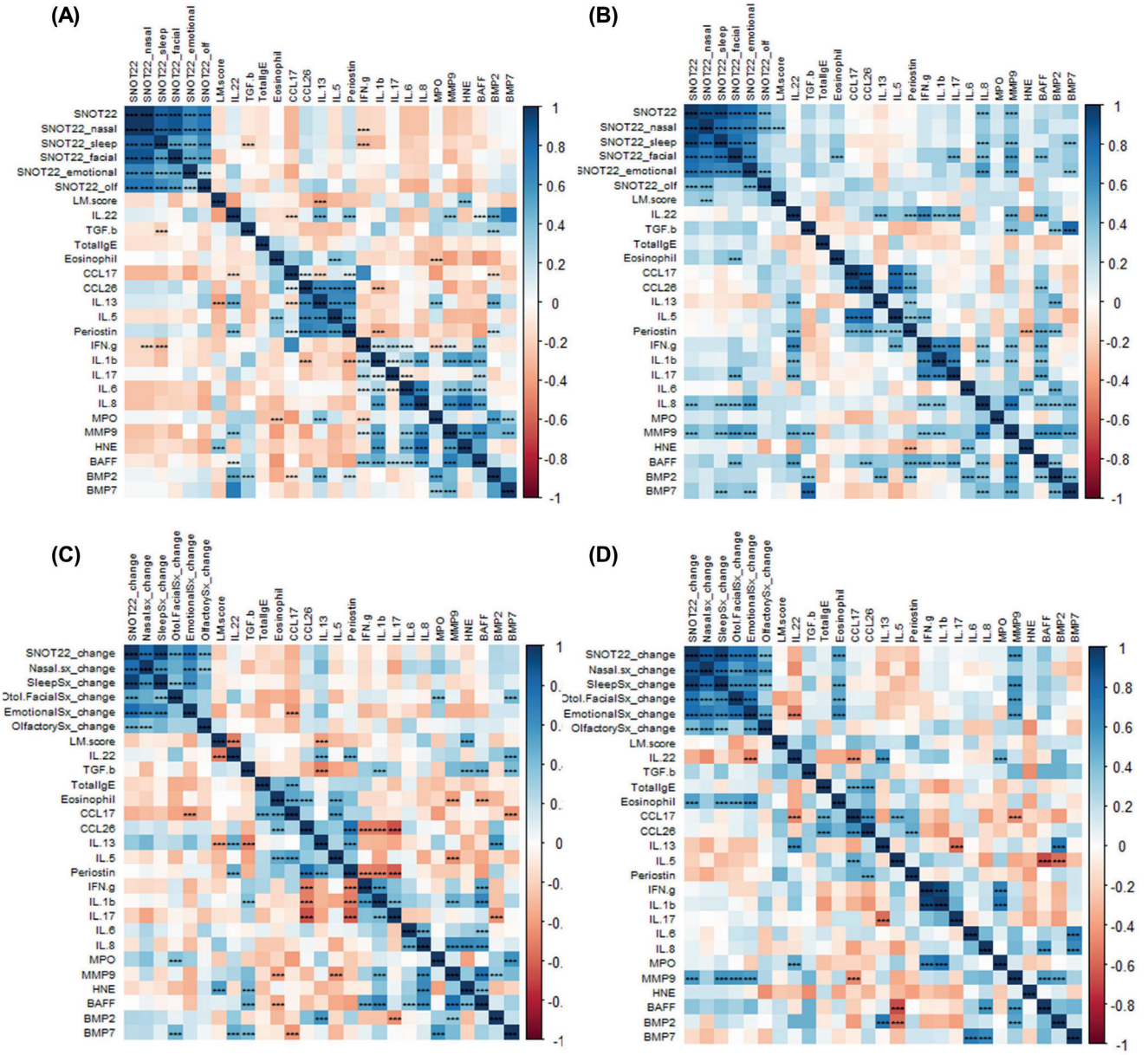

Figure S2. Correlation analysis of SNOT-22 symptom domains with cytokines in type 2 and non-type 2 CRS shown as correlation matrix. (A) Type 2 CRS: baseline symptoms and cytokines (B) Non-type 2 CRS: baseline symptoms and cytokines (C) Type 2 CRS: change in symptoms and cytokines (D) Non-type 2 CRS: change in symptoms and cytokines. Blue and red indicate positive and negative correlation, respectively. Correlation coefficient is shown on the right side of each figure. (*p < 0.05).

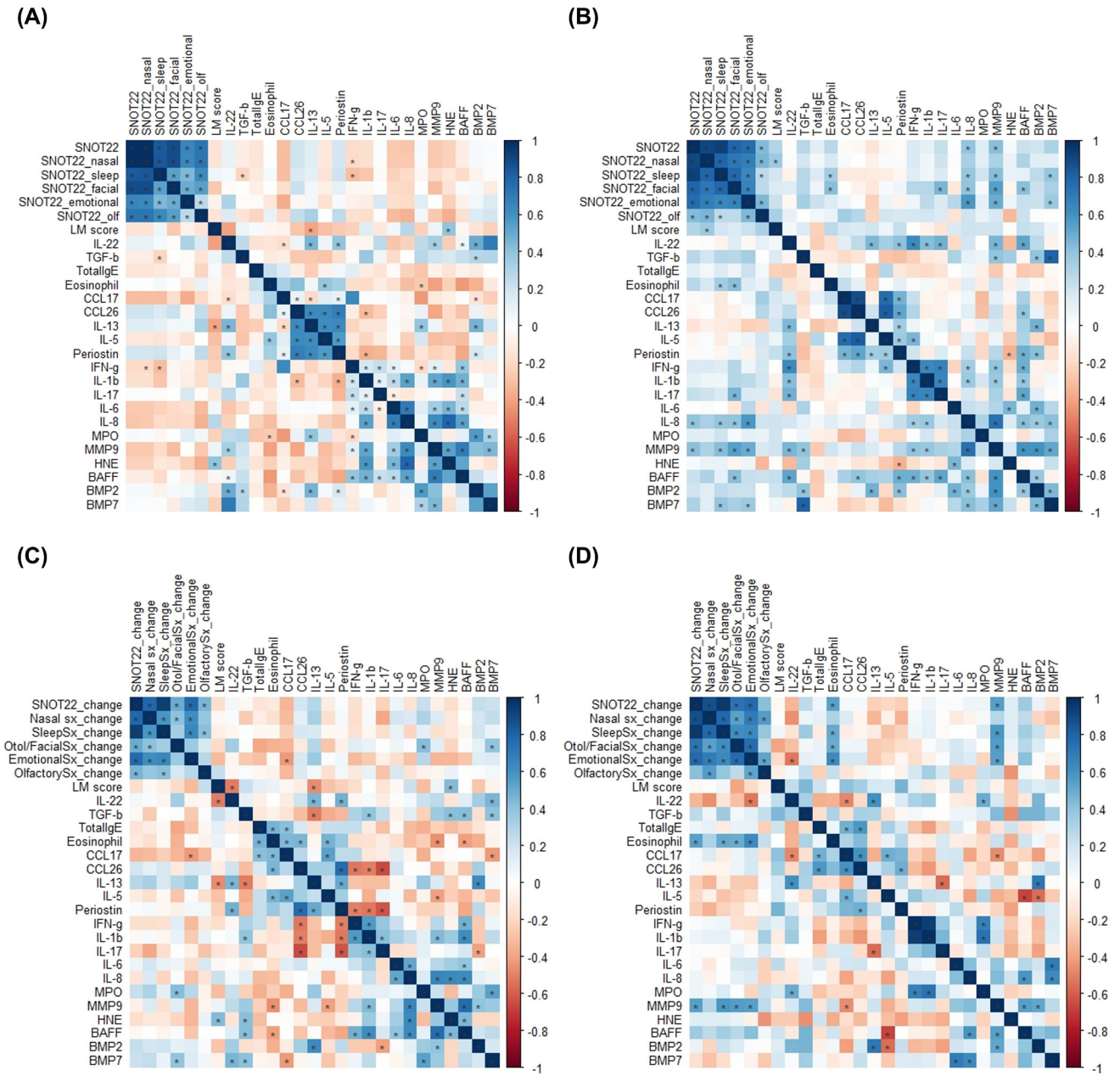

Figure S3. Correlation analysis of SNOT-22 symptom domains with cytokines in type 2 and non-type 2 CRS shown as correlation matrix. (A) CRSwNP: baseline symptoms and cytokines (B) CRSsNP: baseline symptoms and cytokines (C) CRSwNP: change in symptoms and cytokines (D) CRSsNP: change in symptoms and cytokines. Blue and red indicate positive and negative correlation, respectively. Correlation coefficient is shown on the right side of each figure. (*$p < 0.05$).

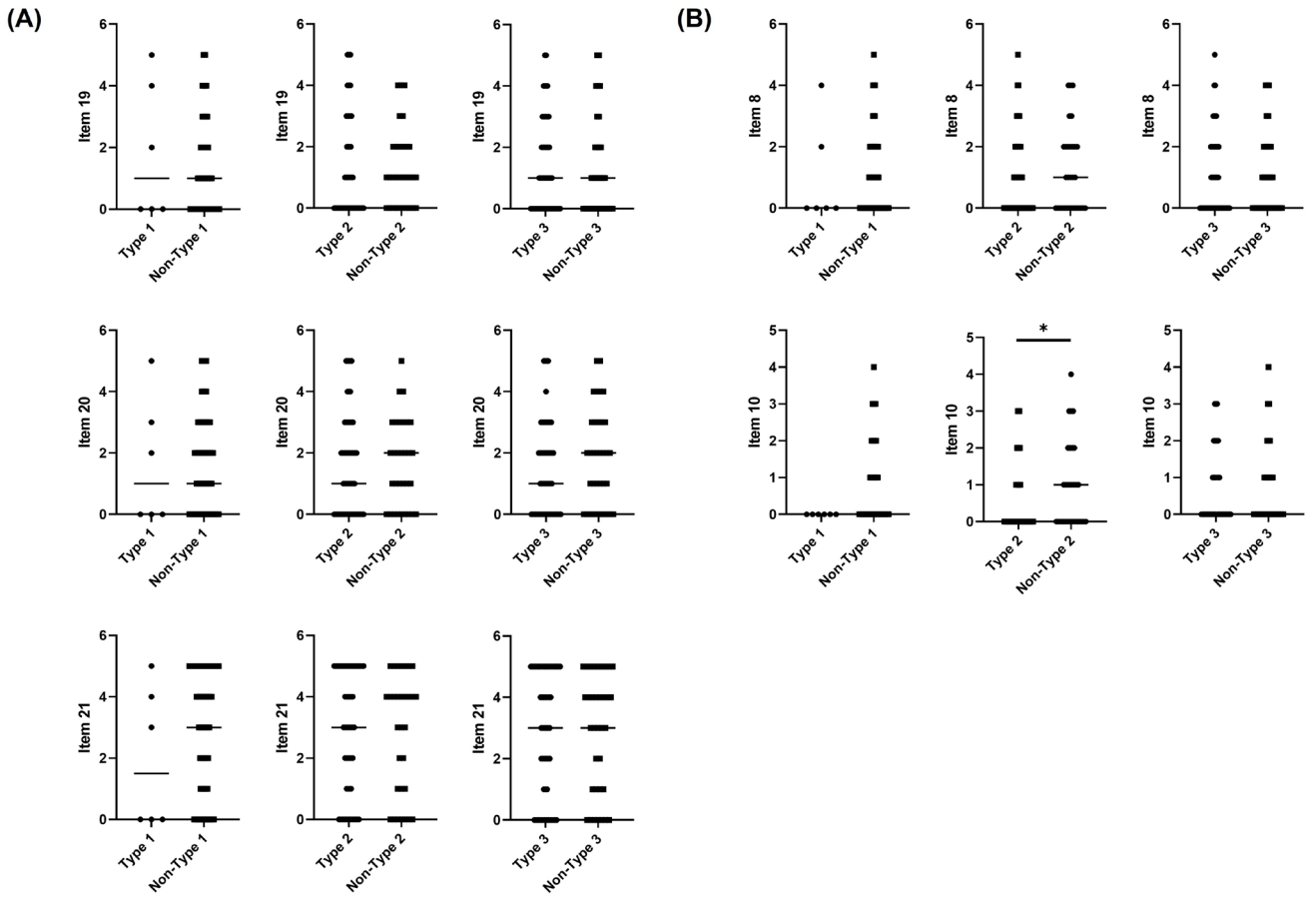

Figure S4. Confirmatory analysis comparing items of SNOT-22 between endotypes according to results from exploratory factor analysis based on principal component analysis (PCA). (A) Items 19, 20, 21 contributed to dimensions 1 and 2 in PCA (B) Items 8 and 10 contributed to dimensions 3 and 4 in PCA. (*$p < 0.05$).