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의학석사 학위논문

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

만성비부비동염 표현형과 내재형에 따른

싸이토카인과 수술 전후 증상과의 상관관계

2023년 2월

서울대학교 대학원

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한 선 아

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

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이 논문을 의학석사 학위논문으로 제출함
2022년 10월

서울대학교 대학원
의학과 이비인후과학 전공
한 선 아

한선아의 의학석사 학위논문을 인준함
2023년 1월

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초 록

만성비부비동염은 전통적으로 표현형, 즉 비용종을 동반한 경우와 동반하지 않은 경우로 분류되어왔다. 최근 제2형 면역반응을 표적으로 하는 생물학적 제제의 사용이 비용종을 동반한 만성비부비동염에도 승인되면서 만성비부비동염의 내재형을 파악하는 것에 대한 중요성이 강조되고 있다. 특정 임상 증상을 통해 표현형, 또는 내재형과의 연관성을 규명하는 경우 증상을 통해 치료 계획을 적립하는데 중요할 수 있다. 따라서 본 연구를 통해 수술 전후 증상과 만성비부비동염의 표현형, 내재형, 그리고 그와 관련이 있는 싸이토카인과의 연관성을 분석하고자 하였다. 양측 만성비부비동염에 대해 부비동내시경수술을 시행하는 환자들을 대상으로 수술 중 절제된 비용종 및 사골동 점막의 싸이토카인을 분석하였다. IFN-γ, IL-5, IL-17와 같은 싸이토카인을 통해 제 1형, 2형, 3형 면역반응이 있는 것으로 정의하고 내재형을 파악하였다. 수술 전, 그리고 수술 후 1년째 증상은 22개의 항목으로 이루어진 Sinonasal Outcome Test (SNOT-22) 설문지를 통해 확인하였다. 만성비부비동염의 표현형과 내재형에 따른 증상을 비교 분석 및

주성분분석을 시행하였고 싸이토카인과 특정 증상 또는 수술후 증상의 호전과 연관성이 있는지 분석하였다. 비용종을 동반한 비부비동염과 비용종을 동반하지 않은 비부비동염에서 모두 제2형과 3형 면역반응이 혼합된 내재형이 가장 흔했다. 주성분분석에서는 제2형과 제2형이 아닌 내재형을 가장 뚜렷히 구분하는 증상은 이과적 증상/안면통 증상에 해당되었으며 제2형 면역반응을 보이지 않은 군에서 수술 후 이과적 증상/안면통 증상이 크게 호전되었다. 호중구 염증반응과 관련이 있는 IL-17, MMP-9, MPO와 같은 싸이토카인은 이통이나 안면통과 같은 만성비부비동염 증상 또는 그 증상의 호전과 유의한 양의 상관관계를 보여 호중구성 염증반응이 이러한 증상 발현에 기여하는 것으로 생각된다. 만성비부비동염 환자에서 안면통과 이동이 동반될 경우 이러한 싸이토카인을 표적으로 하는 치료제의 가능성에 대해서도 탐색이 필요할 것으로 생각된다.

주요어 : 만성비부비동염, 표현형, 내재형, 싸이토카인, 임상증상

학 번 : 2021-29678

목 차

표 목차

그림 목차

Introduction

Chronic rhinosinusitis (CRS) is a chronic inflammatory disease involving the nasal mucosa and paranasal sinuses affecting about 7% to 12% of the population [1-3]. Classically, CRS has been classified according to the presence of polyps: CRS with nasal polyps (CRSwNP) and CRS without nasal polyps (CRSsNP). However, with deeper understanding of the pathophysiology of CRS and the advent of biological treatments, the importance of identifying inflammatory endotypes has been highlighted [4]. CRS patients experience diverse symptoms which can affect their quality of life. 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 [5-9]. In the European Position Paper on Rhinosinusitis and Nasal Polyps (EPOS) 2020 guideline, olfactory dysfunction is listed as one of the 5 criteria used to determine type 2 inflammation in CRS, which is an indication for using biologics. Improvement in olfactory function is also one of the criteria used to evaluate the response to biological treatments [10]. The association of clinical symptoms with endotypes may be important especially in the era of biological treatments, as these 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 [11-13]. 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 [14]. Thus, symptoms indicative of endotypes may be helpful in selecting candidates for these treatments. Through this study, we investigated the relationship of pre-and post-operative symptom changes with endotypes or phenotypes of CRS and the cytokines that are related to these clinical presentations.

Methods

Patients and tissue samples

The diagnosis of CRS was based on clinical history, endoscopy and computed tomography (CT) findings according to the definition of CRS in the EPOS 2020 [10]. 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 polyps (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 Center (IRB No. 30-2019-136). Exclusion criteria employed were: 1) those younger than 18 years; 2) use of antibiotics, systemic or topical corticosteroids or other immune-modulating drugs in the 4 weeks before surgery; 3) those diagnosed with unilateral rhinosinusitis, antrochoanal polyp, allergic fungal rhinosinusitis, cystic fibrosis, or immotile ciliary disease. Endotype classification for type 2 CRS was defined as having interleukin (IL)-5 positivity, while type 1 and type 3 were defined as having interferon (IFN)-γ positivity and IL-17 positivity, respectively. Positivity was defined as having levels more than mean plus two standard deviation of the respective cytokines in normal controls. Patients were divided into phenotypes according to the presence of nasal polyps in endoscopic examination. Atopic status was determined by measuring the IgE levels of 6 common aeroallergens using the ImmunoCAP® assay (Phadia, Uppsala, Sweden). When IgE levels greater than 0.35 IU/mL were detected to any of the allergens, the subjects were determined atopic. Asthma status was defined as that

diagnosed by an allergist through clinical history and lung function and/or provocation tests. Clinical symptom score was measured by the 22-item Sinonasal Outcome Test (SNOT-22) [15]. The SNOT-22 score was divided further into four symptom subdomains: sleep symptoms (questionnaire items 11 through 18), and nasal symptoms (items 1 through 6, 21 and 22), otologic and facial pain symptoms (items 7 to 10), and emotional function (items 19 and 20) [16]. SNOT-22 questionnaire was repeated in subjects who were followed up until one year postoperatively. Systemic medication including corticosteroids or antibiotics were not prescribed during the one month preceding one-year follow up. Lund-Mackay (LM) score was calculated from the preoperative CT [17].

Measurement of cytokines in tissue homogenates

Sample preparation and cytokine analysis was performed as previously described [18-20]. Tissue homogenates were analyzed for levels of B-cell activating factor (BAFF), bone morphogenic protein (BMP)-2, BMP-7, matrix metalloproteinase (MMP)-9, CC Motif Chemokine (CC) 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 multiplex immunoassay (R&D systems LXSAHM Human Premixed Multi-Analyte Kit). IL-22, human neutrophil elastase (HNE) (R&D systems DuoSet ELISA DY782, DY9167-05) and transforming growth factor (TGF)-β1 were also analyzed (R&D systems LTGM100 Magnetic Luminex performance Assay TGF-beta 1 Kit). All cytokines were normalized to the total protein level.

Statistical analysis

Statistical analyses were performed using GraphPad Prism version 8.4.3 (GraphPad Softward, La Jolla, CA, USA), and R software version 4.2.0 (R Foundation for statistical Computing, Vienna, Austria). 2-tailed Mann-Whitney U test was used for comparison of continuous variables between two groups, and chi-square test was used for the comparison of categorical variables. Spearman correlation coefficients (r) were calculated through correlation analysis.

Results

Endotypes of CRS

A total of 102 subjects including 69 CRSwNP patients and 33 CRSsNP patients who had been enrolled consecutively were analyzed. Endotype classification was determined according to IL-5, IL-17 and IFN- γ positivity. Type 2, 3 mixed endotypes were the most frequent in both CRSwNP and CRSsNP patients (32% and 52% respectively). In the CRSwNP group there were no patients with only type 1 endotype while 23% showed only the type 2 endotype and 10% showed only type 3 endotype. Similarly in the CRSsNP group, there no patients with type 1 endotype only while 9% of them had type 2 inflammation only and 21% had type 3 inflammation only. Nineteen percent of patients with CRSwNP and 15% of CRSsNP patients were not classified into any of the endotypes as none of the three cytokines met positivity definition. Seven percent of CRSwNP patients had type 1 and type 3 mixed inflammation, and 9% had all of type 1, 2, and 3 inflammation combined. None of the CRSsNP patients showed type 1, 3 mixed or type 1, 2, 3 mixed endotypes, but 3% of them were type 1, 2 mixed endotype (Figure 1).

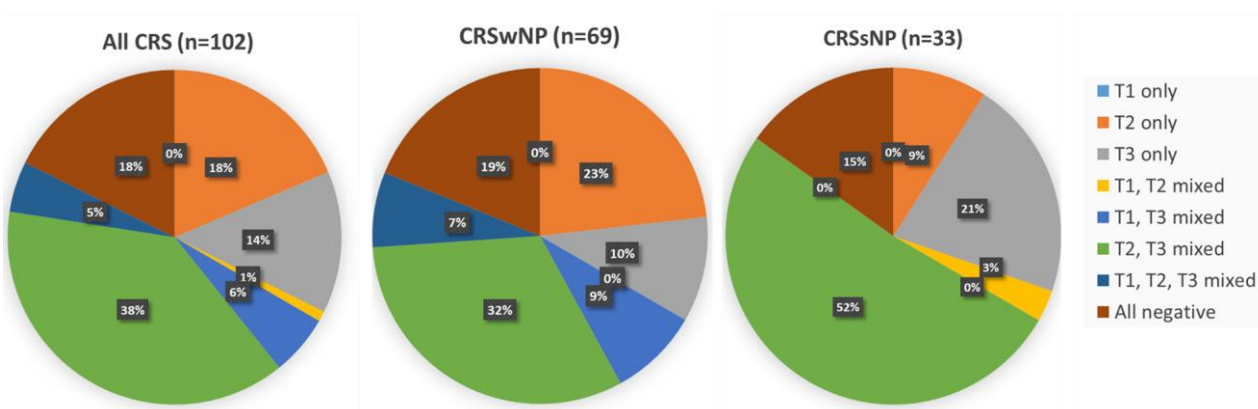

Figure 1. Inflammatory endotypes in CRS patients
T1, type 1inflammation; T2, type 2 inflammation; T3, type 3 inflammation

Symptoms according to endotypes of CRS

To determine the relationship between clinical symptoms and the endotypes of CRS, we performed principal component analysis (PCA) of the endotypes and scores to the individual items on the SNOT-22 questionnaire. The first and second dimensions accounted for 23.39% and 8.92% of the variance respectively (Figure 2A, B). Questionnaire item number 20 (“A feeling of shame”), 21 (“Difficulty to feel ‘smells’ or ‘tastes’), and otologic/facial pain symptoms including items 7 (“A feeling of full or stuffed ear”) and 9 (“Ear ache”) contributed the most to the first dimension. Item 21, representing olfactory symptoms, contributed the most to the second dimension. Dimensions 3, 4 accounted for 8.07% and 7.70% of variance, respectively (Figure 2C, D), which was less in comparison to the first and second dimensions. However, dimensions 3 and 4 made more clear distinction between endotypes. Item 10 (“Facial pain or pressure”) and 8 (“Dizziness or vertigo”), which are of otologic/facial pain symptoms category contributed the most to dimension 3 which differentiated between type 2 and non-type 2 endotypes, and were more associated with the non-type 2 endotypes.

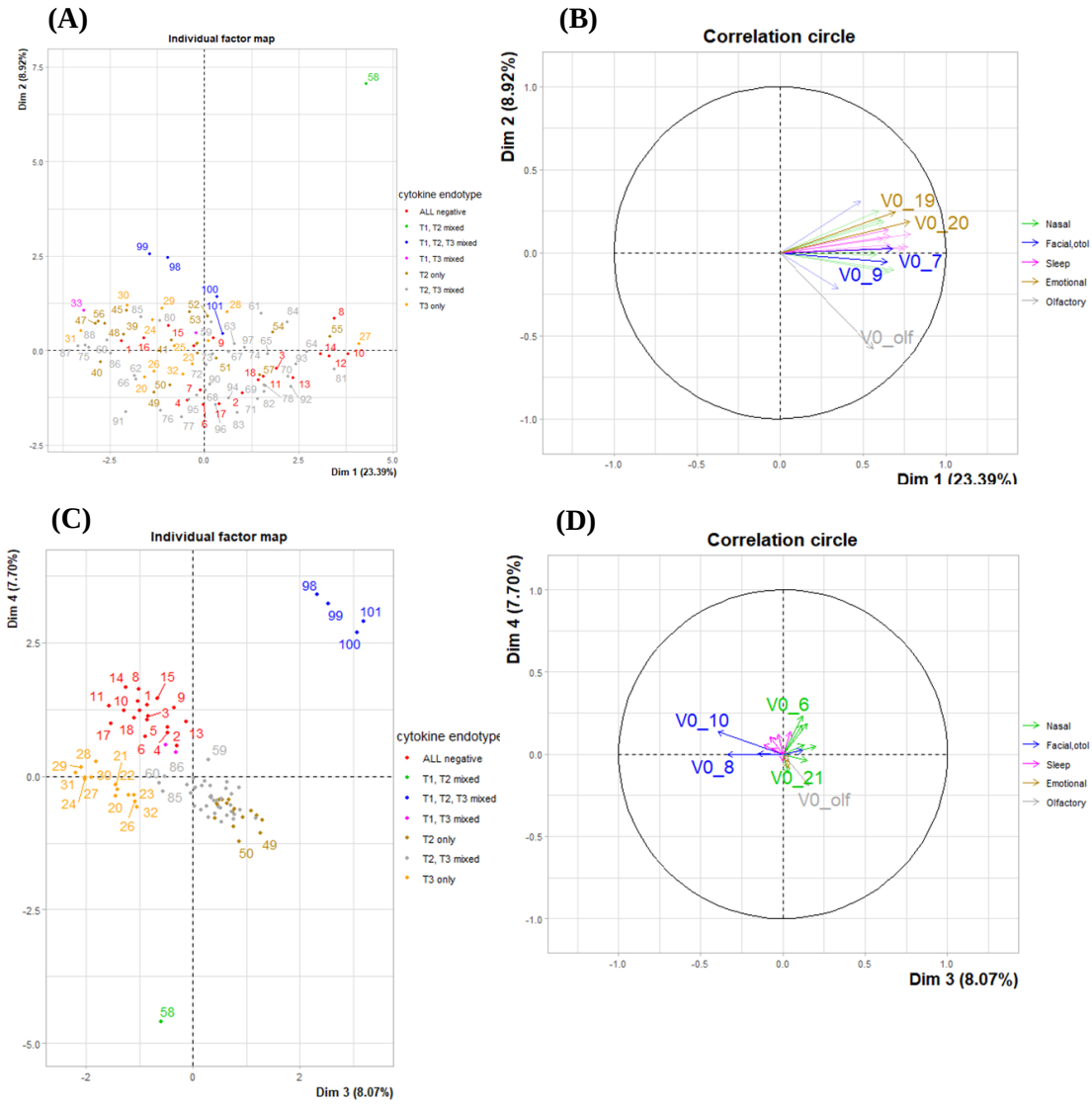

Figure 2. Principal component analysis according to endotypes and score of individual item on SNOT-22. **(A, B)** Dimensions 1, 2; **(C, D)** Dimensions 3, 4
Each plot on (A) and (C) denotes an individual subject

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

The symptoms of patients were further analyzed according to type 2 CRS and

non-type 2 CRS as defined by IL-5 positivity. Sixty-four (62.7%) subjects were type 2 CRS patients, while 38 (37.3%) subjects were non-type 2 CRS patients. There was no difference in the baseline demographics and clinical characteristics such as age, sex, LM CT scores, initial SNOT-22 scores, and serum total IgE between groups. Number of patients with atopic status were higher in the type 2 CRS group compared to non-type 2 CRS group (p = 0.020) as with the number of patients with asthma: 20 (31.3%) in type 2 CRS group vs. 4 (10.5%) in non-type 2 CRS group (p = 0.018). Blood eosinophil percentage was significantly higher in the type 2 CRS group (p = 0.002) than non-type 2 CRS group (Table 1).

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
Sex (female)	15 (23.4%)	12 (31.6%)	0.4866
Age (yr)	49.4±14.2	47.3±17.2	0.6381
Atopic (number)	**31 (48.4%)**	**9 (23.7%)**	**0.0204**
Asthma (number)	**20 (31.3%)**	**4 (10.5%)**	**0.0176**
Lund-Mackay score	14.6±5.0	15.2±4.4	0.3491
Initial SNOT-22	38.7±20.3	41.9±22.9	0.6856
Current smoking(number)	14 (21.9%)	10 (26.3%)	0.6354
Blood eosinophil (%)	**6.28±3.97**	**4.52±4.55**	**0.0019**
Total IgE	399.7±710.3	265.8±518.0	0.1422

Symptoms scores were compared between type 2 CRS and non-type 2 CRS patients. Baseline total SNOT-22 scores and subdomain scores were not different between two groups. Olfactory symptom as determined by SNOT-22 item number 21 was also not different between groups (Figure 3A). However, when changes in symptoms at 1 year after surgery were compared between groups, non-type 2 CRS patients exhibited significantly more symptom improvement in the facial symptom

domain than type 2 CRS (1.77 vs. 3.26; p = 0.033) (Figure 3B).

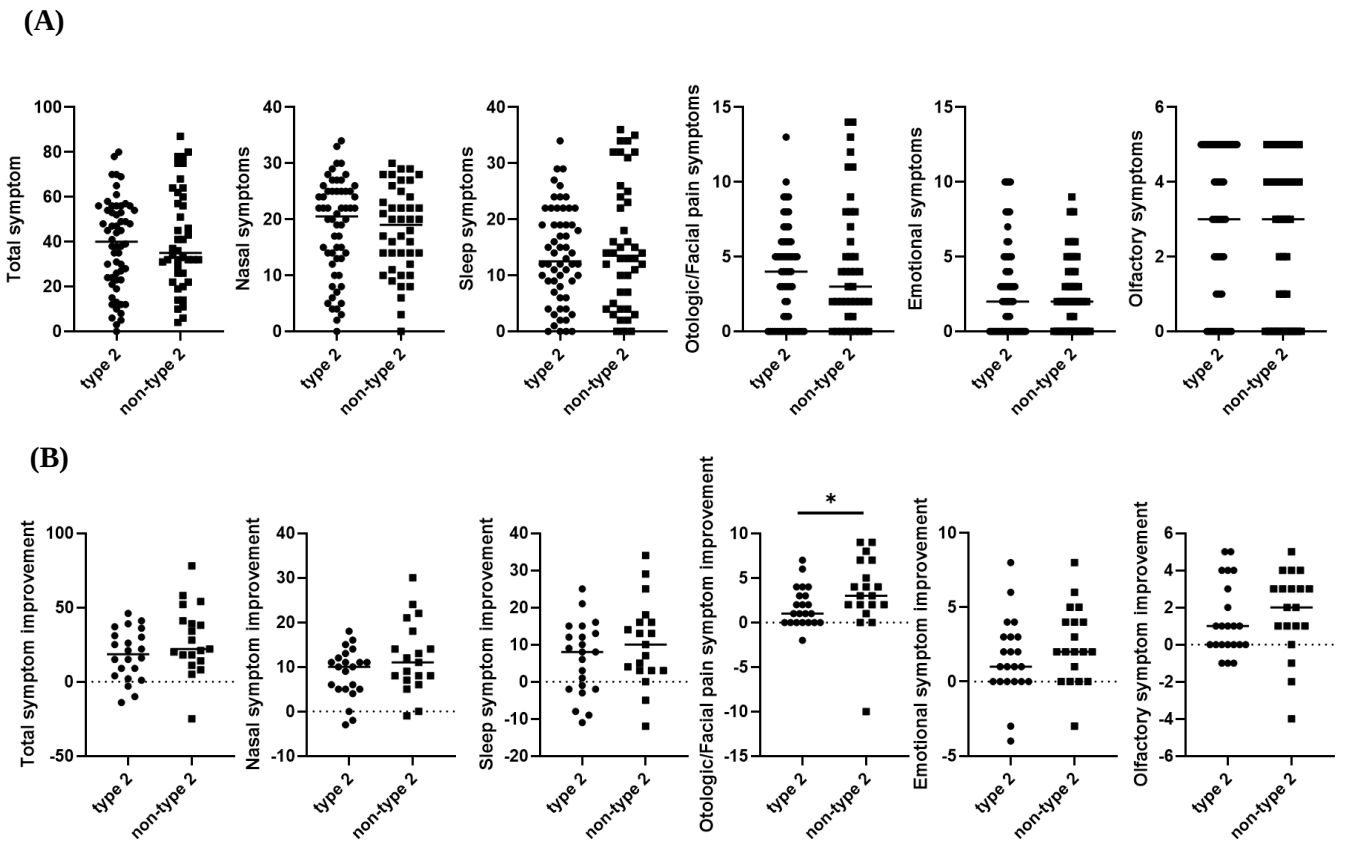

Figure 3. Comparison of symptoms in type 2 and non-type 2 CRS patients.
(A) Baseline symptoms **(B)** Symptom improvement at 1 year after surgery (*p < 0.05)

To elucidate whether certain cytokines were related with baseline symptoms and change in symptoms, correlation analysis was performed. In non-type 2 CRS, TGF-β1 was significantly positively associated with emotional domain score of baseline SNOT-22 (r = 0.41, p = 0.02) while IL-17 levels showed significant positive correlation with the otologic/facial pain domain scores of baseline SNOT-22 in type 2 CRS patients (r = 0.42, p = 0.002) (Figure 4A and B). As for change in symptoms after surgery, MPO had significant positive correlation with

improvement in otologic/facial pain symptom domain in 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 4C and D).

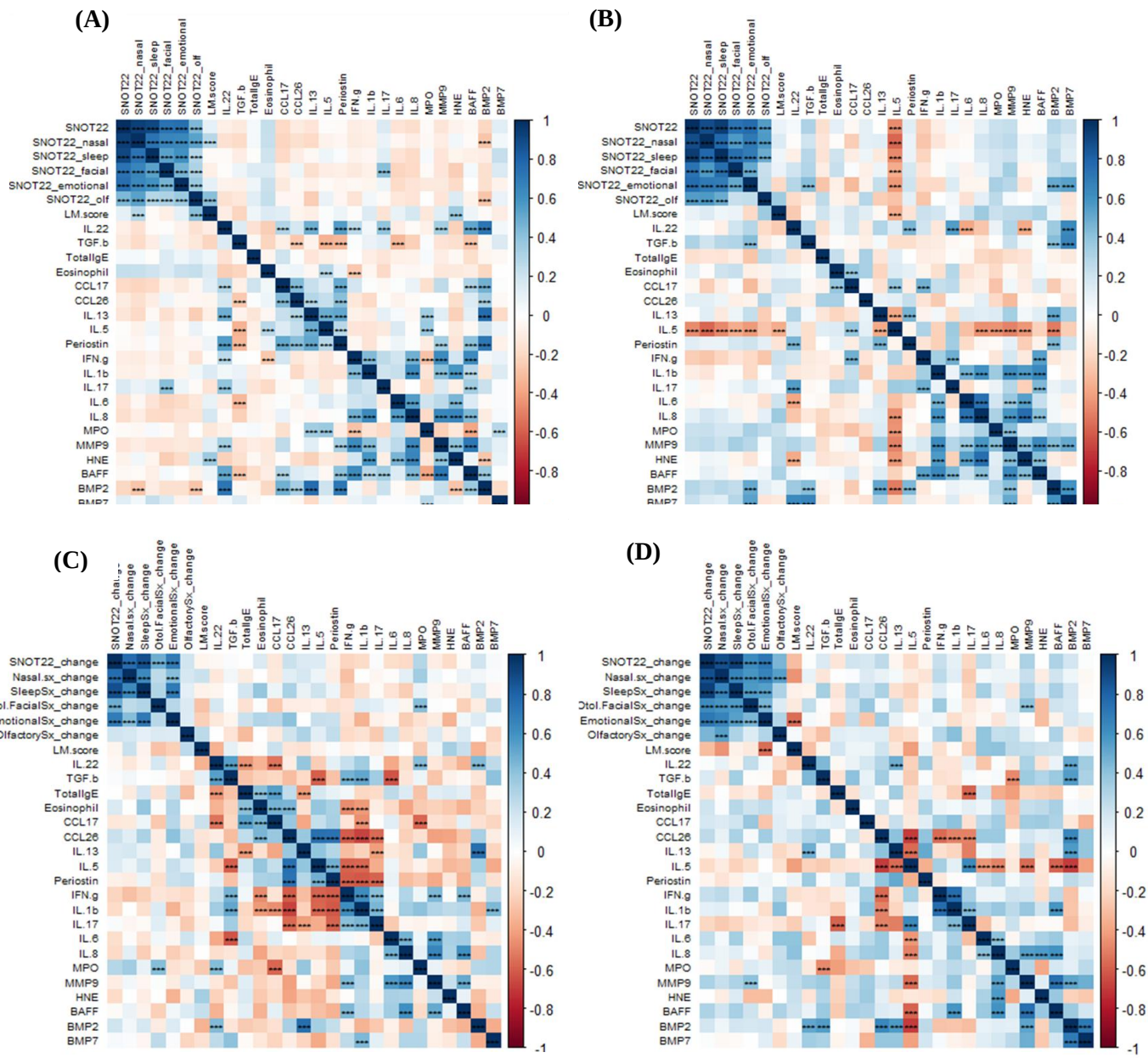

Figure 4. Correlation analysis of symptoms with cytokines in type 2 and non-type 2 CRS shown as correlation matrix. **(A)** Type 2 CRS: baseline symptoms and cytokines **(B)** Non-type 2 CRS: baseline symptoms and cytokines **(C)** Type 2 CRS: change in symptoms and cytokines **(D)** Non-type 2 CRS: change in symptoms and cytokines

Comparison of symptoms between CRSwNP and CRSsNP

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

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

	CRSwNP (n=69)	CRSsNP (n=33)	*p*-value
Sex (female)	**12 (17.4%)**	**15 (45.5%)**	**0.004**
Age (yr)	49.4±14.7	47.3±16.7	0.4512
Atopic (number)	26 (37.7%)	14 (42.4%)	0.6698
Asthma (number)	19 (27.5%)	10 (30.3%)	0.8167
Lund-Mackay score	**16.0±4.5**	**12.4±4.5**	**0.0012**
Initial SNOT-22	38.1±19.0	42.5±24.6	0.4327
Current smoking(number)	19 (27.5%)	5 (15.1%)	0.2158
Blood eosinophil (%)	5.64±4.26	5.55±4.22	0.7259
Total IgE	395.9±736.4	257.6±399.8	0.5836

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 symptom, item 21, contributed the most to dimension 2 which differentiated between the two phenotypes (Figure 5).

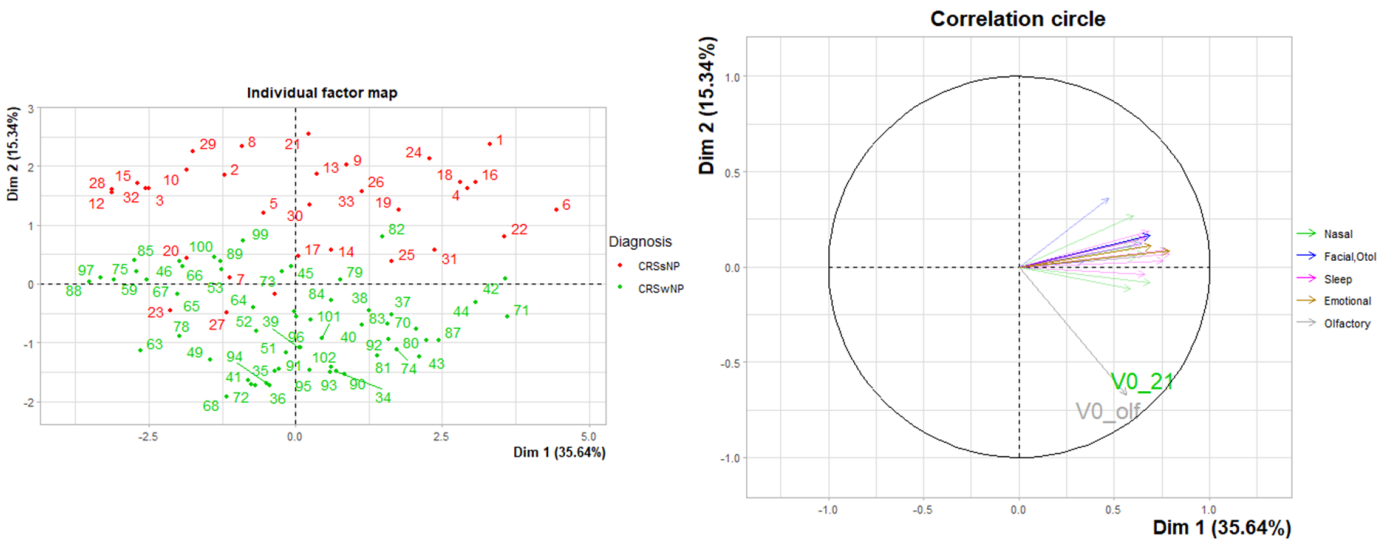

Figure 5. Principle component analysis according to phenotypes and scores of individual item on SNOT-22

Comparison of baseline symptom scores showed similar results: while other symptom domains showed no difference between groups, the olfactory symptom, the mean score of item 21 were significantly higher in CRSwNP patients compared to CRSsNP patients (p = 0.019) (Figure 6A, B).

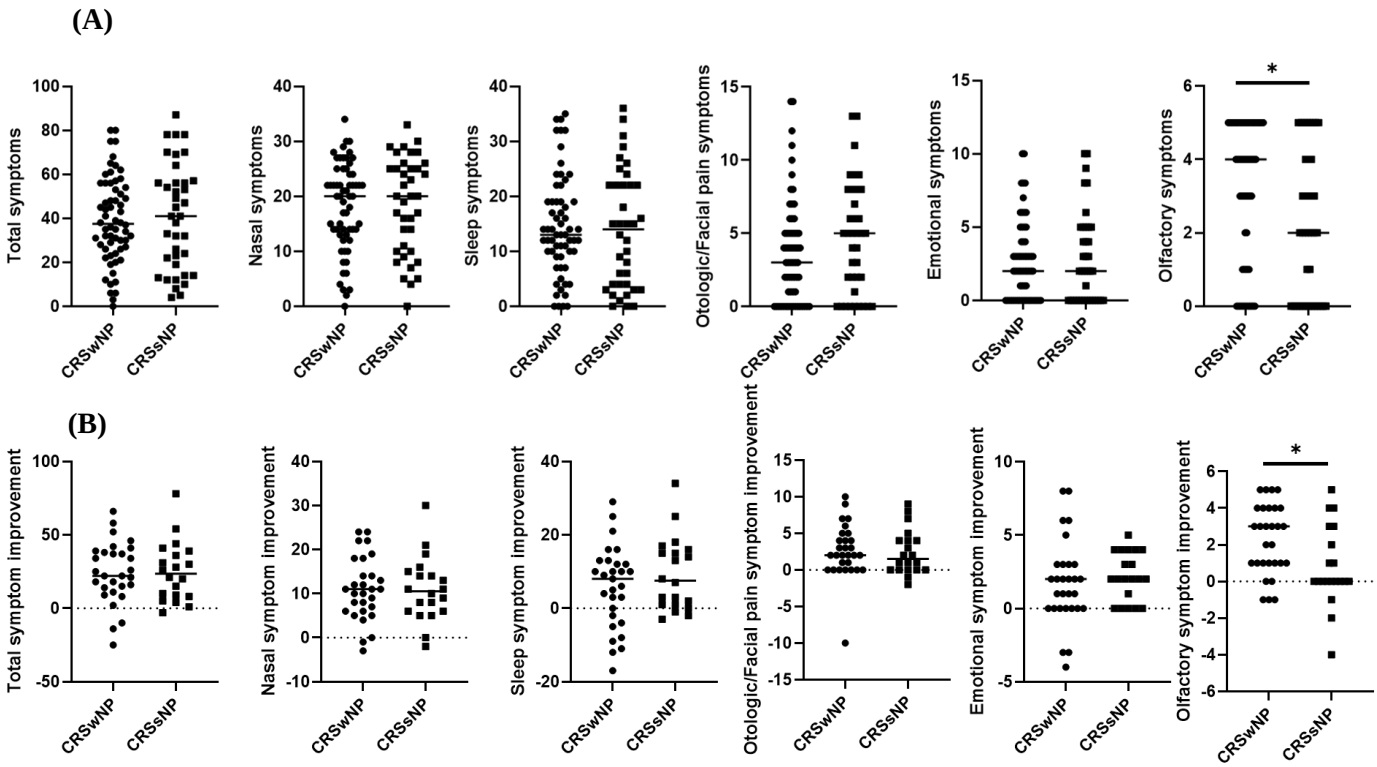

Figure 6. Comparison of symptoms in CRSwNP and CRSsNP patients.
(A) Baseline symptoms **(B)** Symptom improvement (*p < 0.05)

Improvement of olfactory symptom at1 year after surgery was also significantly greater in CRSwNP than in CRSsNP patients (2.28 vs. 0.8; p = 0.016). To determine whether certain cytokines show correlation with baseline symptoms domains, correlation analysis was conducted according to phenotypes. Cytokines TGF-β1 (r = -0.35, p = 0.009), and IFN-γ (r = -0.27, p = 0.047) were significantly negatively correlated with the sleep domain of SNOT-22 in CRSwNP patients. In CRSsNP patients multiple cytokines such as IL-17, IL-8, MMP-9, BAFF showed positive correlation with otologic/facial pain symptom domain of the SNOT-22 (Figure 7A and B). Similarly to type 2 CRS, MPO was positively correlated with improvement in otologic/facial pain symptom domain in CRSwNP (r = 0.428, p =

0.029). MMP-9 in CRSsNP showed positive correlation with improvement in total SNOT-22 score (r = 0.546, p = 0.019), sleep (r = 0.575, p = 0.012), otologic/facial pain symptom (r = 0.559, p = 0.016), and emotional symptom domains (r = 0.543, p = 0.020) (Figure 7C and D).

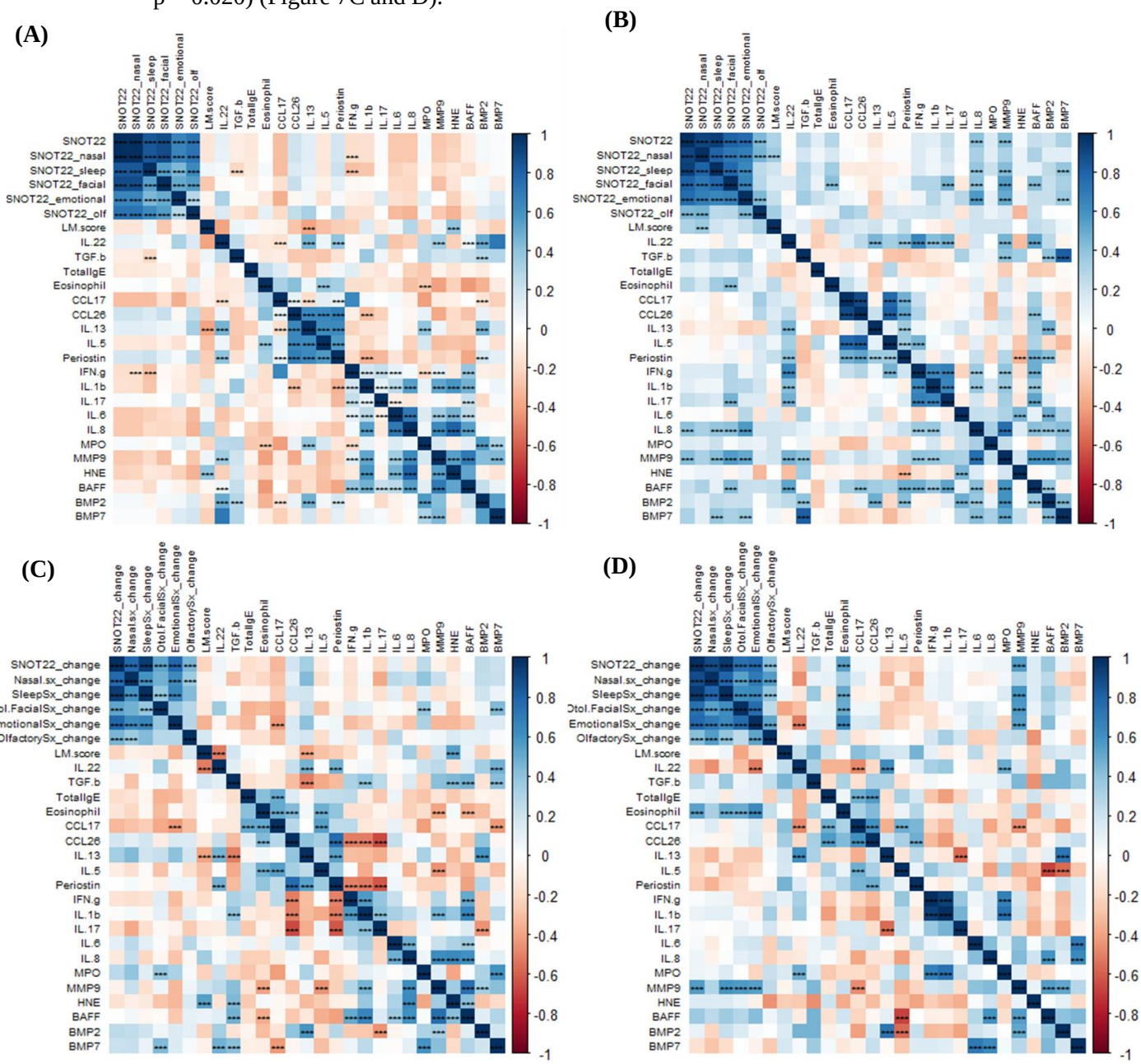

Figure 7. Correlation analysis of symptoms with cytokines in type 2 and non-type 2 CRS shown as correlation matrix. **(A)** CRSwNP: baseline symptoms and cytokines **(B)** CRSsNP: baseline symptoms and cytokines **(C)** CRSwNP: change in symptoms and cytokines **(D)** CRSsNP: change in symptoms and cytokines

Discussion

In this study, we investigated the association of tissue cytokine levels with baseline symptoms, symptom improvement at 1 year after surgery according to CRS endotypes. Generally, loss of smell is associated with CRSwNP, which is mainly comprised of type 2 endotype in the Western population [5-9]. In the EPOS 2020 guideline, olfactory loss is one of the criteria used to determine using biological treatment targeted at type 2 inflammation and assess its response [10]. 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 the use in CRS. The patients of type 2 CRS in our study showed clinical characteristics similar to what is previously known.

In the PCA analysis according to endotypes of CRS, the symptoms that contributed the most to the dimension that differentiated between type 2 and non-type 2 CRS were of the otologic/facial pain symptom category. IL-17 was positively correlated with otologic/facial pain domain of baseline SNOT-22 score; MPO was positively correlated with the improvement of score in this domain in type 2 CRS patients. MMP-9 was positively correlated with improvement in otologic/facial pain symptom domain in non-type 2 CRS patients. Although some studies have investigated the association between endotype and clinical outcomes, to the best of our knowledge this is the first study to associate endotypes with clinical symptoms and further correlate them with cytokine profiles. The results of this study may provide clues to determining other endotypes through symptoms and consider new targets for therapy.

First, we confirmed that the clinical characteristics of type 2 CRS patients in our study were consistent with previously established results [4, 10, 21]. The atopic status, prevalence of asthma and blood eosinophil percentage were all increased in the type 2 CRS patients (Table 1). However, baseline olfactory symptoms were not worse in type 2 CRS patients. Also, in the PCA involving endotypes, the dimension that differentiated between type 2 and non-type 2 CRS explained only 7.70% of the variance and item 21, which indicates olfactory symptoms, contributed less than items 8 (“dizziness or vertigo”) and 10 (“facial pain or pressure”) to this dimension (Figure 2C, D). Furthermore, olfactory loss in type 2 CRS patients has been associated with various cytokines in previous studies [5, 7-9]. The correlation analysis in our study, however, revealed no cytokine that was significantly associated with baseline olfactory symptom in type 2 CRS.

Both the CRSwNP phenotype and type 2 endotype has been associated with olfactory dysfunction. In a study by Tomassen et al., clustering of CRS patients based on tissue cytokines resulted in 10 clusters where high IL-5 cluster was associated with nasal polyp phenotype and high asthma prevalence [22]. In a similar study in a Chinese population, the patients were clustered into 7 clusters, one of which was comprised of CRSwNP patients with high prevalence of allergic rhinitis and asthma and was associated with highest hyposmia scores [23]. In the PCA according to phenotypes, item 21 (“difficulty to feel ‘smells’ or ‘tastes’”) contributed the most to dimension 2 which explained 15.34% of the variance (Figure 5A, B), demonstrating olfactory symptom is correlated with CRSwNP. These results may suggest that the conductive type of olfactory loss due to nasal polyps contributed more greatly to olfactory symptoms in CRS patients more than

type 2 inflammation in this population.

Prevalence of type 2 endotype in this study population was compared to published studies. The proportion of patients with any type 2 endotype were 62% in CRSwNP patients, which was smaller compared to patient populations in western countries where the majority of patients exhibit type 2 endotypes [24]. Sixty-four percent of CRSsNP subjects exhibited any type 2 endotype whether single or mixed. This is comparable to the study by Tan et al., which reported that type 2 was also the most common endotype in CRSsNP [25], while a multi-center study in Europe, Asia and Oceania has reported differences in type 2 positivity according to different regions [26].

Otologic/facial pain symptom was associated with non-type 2 CRS in this study and was associated with neutrophilic markers such as IL-17, MPO and MMP-9 at the baseline scores or improvement after sugery. Symptoms are an important part of the definition of CRS [10]. Although symptoms may not always be reflective of disease severity assessed by objective measures [27], CRS symptoms can affect the quality of life of patients, and some symptoms can affect patients more than others [28]. Results of 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 of otologic and facial pain symptom domains (Figure 2C, D). Non-type 2 CRS patients experienced significant improvement in otologic and facial pain symptoms at 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 [29]. In the mentioned study, purulent discharge was associated with only the single type 3 endotype while

intraoperative pus was associated with all type 3 endotypes. Pus and purulent discharge are often associated with bacterial infection and type 3 immunity. Type 3 immunity is associated with protection against extracellular bacterial and promote neutrophil recruitment [30] Varying rates of facial pain or pressure as symptoms of sinusitis have been reported [31, 32]. Negative pressure caused by obstruction of the osteomeatal complex which is one of main pathophysiology of non-type 2 CRS have been suggested as possible causes of facial pain [33]. Various nociceptive neurons are distributed within the sinonasal mucosa [34] and neutrophilic cytokines may have a role in stimulating these neurons and eliciting pain. As the non-type 2 CRS patients experienced significantly more improvement in otologic/facial symptoms compared to type 2 CRS patients, it can be postulated that these symptoms can be significantly alleviated by surgery in non-type 2 CRS.

Biologic 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 need to be further studied. Monoclonal antibodies targeted at IL-17 have already been approved for treatment in psoriasis [35, 36]. As biologics targeted at type 2 inflammation have been effective in improving olfaction [37], those targeted at IL-17 or other neutrophilic markers may have an effect on relieving facial pain symptoms.

One limitation of this study is that the number of patients enrolled in this study did not allow for analysis according to all of the endotypes including mixed endotypes. Another possible limitation is that the olfactory symptoms were determined through a single item on the SNOT-22 questionnaire without psychophysical olfactory tests. There are mixed results on whether subjective

reporting of olfactory function correlates with psychophysical test results [38, 39]; however the subjective symptoms of patients may be more reflective of effects on their quality of life. As for the otologic and facial pain symptoms, there is possibility that not every patient's symptom was caused by sinusitis but other etiologies such as migraine. Furthermore, it was not possible for all patients to receive the same treatment and this may have affected the symptoms at 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. To overcome these limitations, larger studies with prospective design are warranted to investigate in more detail the possible differences according to mixed endotypes and to corroborate our findings.

Conclusion

In conclusion, we suggest that otologic and facial pain symptoms may be indicative of the non-type 2 endotypes and help in distinguishing them from type 2 CRS. Neutrophilic inflammation may be culprits in eliciting these symptoms. Further investigation on how neutrophilic cytokines manifest as these symptoms is warranted. Cytokines related to neutrophilic inflammation should be considered as targets for therapy and this may help relieve intractable otologic and facial pain symptoms in CRS patients.

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Abstract

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

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Chronic rhinosinusitis (CRS) has been classically classified according to phenotypes: CRS with nasal polyps (CRSwNP) and CRS without nasal polyps (CRSsNP). Recently, the importance of recognizing inflammatory endotypes have been highlighted especially with the advent of biologic treatments. The association of clinical symptoms with phenotypes and endotypes may be helpful in deciding treatment plans. Through this study, we investigated the relationship of pre- and post-operative symptom changes with endotypes or phenotypes of CRS and the cytokines that are related to these clinical presentations. Patients undergoing routine functional endoscopic sinus surgery were enrolled and nasal polyp and ethmoid mucosa were obtained. Endotype classification for type 2 CRS was defined as having interleukin (IL)-5 positivity, while type 1 and type 3 were defined as having interferon (IFN)-γ positivity and IL-17 positivity, respectively. Clinical symptom score was evaluated pre- and post-operatively by the 22-item Sinonasal Outcome Test (SNOT-22) and the four symptom subdomains: sleep

symptoms, nasal symptoms, otologic and facial symptoms and emotional function. Symptoms were compared between groups and principle component analysis was performed. Correlation between symptoms, change in symptoms after 1 year with cytokines were analyzed. A total of 102 subjects including 69 CRSwNP patients and 33 CRSsNP patients were analyzed. Type 2, 3 mixed endotypes were the most frequent in both CRSwNP and CRSsNP patients. Symptoms of the otologic/facial pain symptom category contributed most to the dimension that differentiated between type 2 and non-type 2 endotypes. Non-type 2 CRS patients exhibited significantly more symptom improvement in the facial symptom domains one year after surgery. We suggest that otologic and facial pain symptoms such as facial pain or pressure may be indicative of the non-type 2 endotypes and help in distinguishing them from type 2 CRS. Furthermore, cytokines of neutrophilic inflammation such as IL-17, MMP-9, and MPO were significantly correlated with otologic and facial pain symptoms. How these cytokines contribute to the development of these symptoms remains to be investigated, and may be targets for future therapy in CRS.

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Keywords : Chronic rhinosinusitis, endotyes, phenotypes, clinical symptoms, cytokines

Student Number : 2021-29678