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Identification of clinically relevant chronic rhinosinusitis endotypes using cluster analysis of mucus cytokines

To the Editor:

Chronic rhinosinusitis (CRS) pathophysiology has yet to be clearly defined, largely because the diagnosis itself likely represents a heterogeneous syndrome rather than a distinct

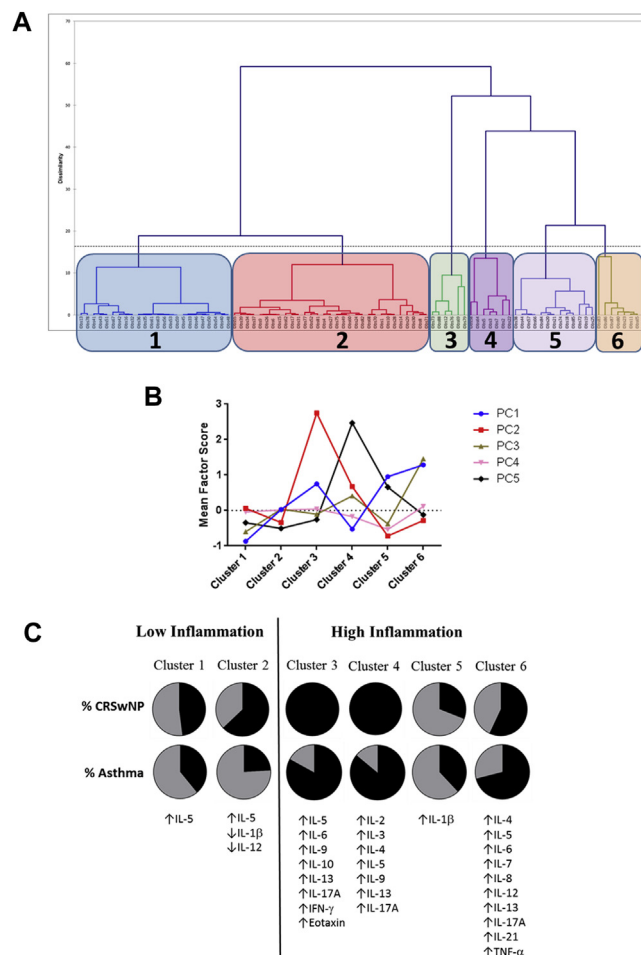


FIG 1. Identification of inflammatory endotypes using cluster analysis of CRS mucus. **A**, Dendrogram representing hierarchical cluster analysis of patients with CRS based on principal-component analysis of 18 mucus-derived biological variables. **B**, Mean factor scores for each of the 6 CRS clusters. **C**, Asthma/polyp prevalence and differences in mucus cytokine levels among each of the 6 CRS clusters, compared with healthy controls. PC, Principal component.

clinical entity. Several investigators have shown that CRS with nasal polyps (CRSwNP) and CRS without nasal polyps (CRSsNP) can both be linked with T_H1 -, T_H2 -, or T_H17 -associated inflammatory signatures,^{1,2} and there likewise appears to be a substantial geographic predisposition to select types of inflammatory burden.³ Recent attempts to identify inflammatory endotypes have presented a more nuanced approach to the classification of CRS, with Tomassen et al⁴ recently using surgically obtained tissue to identify potential CRS endotypes based entirely on inflammatory biomarkers. While such studies have preferentially used tissue-based assessment of inflammatory mediators, this approach may have some logistical limitations because it is inherently invasive and subject to variations in site-specific protein expression throughout the sinonasal cavity.^{5,6} Minimally invasive approaches to disease endotyping have been reported for asthma and other respiratory diseases, typically using analysis of sputum, mucus, or epithelial brushings.⁷⁻⁹ The aim of the current study was to define CRS endotypes in a US population entirely on the basis of analysis of mucus collected via a minimally invasive approach. Going a step further, we sought to additionally define the clinical relevance of putative

TABLE I. Differences in clinical and phenotypic characteristics among CRS endotypes

Characteristic	Cluster 1 (n = 31)	Cluster 2 (n = 24)	Cluster 3 (n = 6)	Cluster 4 (n = 7)	Cluster 5 (n = 13)	Cluster 6 (n = 7)	P value
Age (y)	49.5 ± 12.9	44.3 ± 9.9	55.7 ± 17.6	47.3 ± 13.6	52.7 ± 17.0	49.3 ± 10.5	.33
Sex, n (% female)	19 (61)	8 (33)	2 (33)	1 (14)	5 (38)	5 (71)	.08
Race, n (% white)	30 (97)	20 (83)	6 (100)	5 (71)	13 (100)	7 (100)	.08
Current smoker, n (%)	4 (13)	2 (8)	0 (0)	0 (0)	0 (0)	0 (0)	.51
BMI (kg/m ²)	30.7 ± 5.2	29.8 ± 7.6	28.4 ± 6.4	30.9 ± 2.9	28.3 ± 7.7	27.2 ± 4.6	.60
Nasal polyps, n (%)	15 (48)	15 (63)	6 (100)	7 (100)	4 (31)	4 (57)	.01
Asthma, n (%)	12 (39)	8 (24)	5 (83)	6 (86)	5 (38)	5 (71)	.03
Allergic rhinitis, n (%)	16 (52)	17 (71)	3 (50)	1 (14)	8 (62)	6 (86)	.31
AERD, n (%)	4 (13)	3 (13)	2 (33)	3 (43)	0 (0)	0 (0)	.07
AFRS, n (%)	1 (3)	5 (21)	1 (17)	2 (29)	1 (8)	0 (0)	.19
Taking NCS, n (%)	26 (84)	20 (83)	4 (67)	6 (86)	9 (69)	6 (86)	.79
Taking LTR, n (%)	5 (16)	10 (42)	4 (33)	3 (43)	0 (0)	3 (43)	.04
SNOT-22 score	45.0 ± 18.4	47.7 ± 11.5	51.3 ± 36.3	59.0 ± 17.3	42.0 ± 21.3	47.2 ± 25.9	.80
CT score	14.5 (11.0-19.0)	15.0 (11.5-20.8)	22.0 (19.3-23.3)	18.0 (17.5-23.5)	16.0 (11.0-17.8)	13.0 (10.0-15.5)	.02
SIT score	-7.0 (-24.5 to -3.0)	-4.0 (-21.0 to -3.0)	-29.0 (-31.0 to -24.0)	-16.0 (-26.6 to -0.8)	-2.5 (-19.8 to -1.0)	-7.0 (-17.5 to 2.0)	.03
Prior surgery, n (%)	9 (29)	8 (33)	4 (66)	5 (71)	9 (69)	7 (100)	.002
No. of prior surgeries	0.0 (0.0-1.0)	0.0 (0.0-1.0)	1.5 (0.0-3.8)	2.0 (1.0-3.0)	1.0 (0.0-2.0)	1.0 (1.0-1.0)	.004
Tissue eosinophils/ hpf	19.0 (1.0-50.0)	52.5 (15.0-100.0)	86.5 (41.8-213.8)	50.0 (37.8-100.0)	5.0 (0.5-43.5)	35.0 (1.0-100.0)	.01

AERD, Aspirin-exacerbated respiratory disease; AFRS, allergic fungal rhinosinusitis; BMI, body mass index; CT, computed tomography; NCS, nasal corticosteroid; LTR, antileukotriene; SIT, Smell Identification Test.

Post hoc analysis of clinical and phenotypic characteristics was done for each of the 6 identified CRS endotypes. Values are presented as either the mean ± SD or median with interquartile range, depending on the normalcy of the data. Differences between groups were assessed using the Kruskal-Wallis test or χ^2 analysis. Boldface indicates $P < .05$.

endotypes by assessing phenotypic characteristics, metrics of disease severity, quality of life, and surgery outcomes in these populations.

A total of 90 patients with CRS undergoing endoscopic sinus surgery were prospectively enrolled in the study (see Table E1 in this article's Online Repository at www.jacionline.org). Mucus was collected using an absorbent polyurethane sponge and we subsequently assayed 18 different mucus inflammatory mediators that reflect T_H1/T_H2/T_H17-associated inflammation. Because *a priori* selection of biological variables risks biasing data based on preconceived hypotheses, we attempted to include all cytokines that could be practically analyzed using a single platform, only excluding growth factors and most chemokines. Association between immunologic variables was assessed using principal-component analysis, with a 5-factor solution explaining 71.3% of the data variance (see Fig E1, A, in this article's Online Repository at www.jacionline.org). The first component (PC1) included IL-1 β , IL-6, IL-8, TNF- α , and eotaxin, while the second was composed of IL-5, IL-9, and IL-13 (see Table E2 in this article's Online Repository at www.jacionline.org). The remaining factors were composed of IL-2, IL-4, IL-12, and IL-21 (PC3), IFN- γ and IL-10 (PC4), and IL-3 and IL-17A (PC5). Hierarchical cluster analysis was then performed using the factor scores calculated for each patient (Fig 1, A). This suggested an optimum of 5 or 6 clusters, with mathematical

modeling and visual evaluation of the dendrogram validating a 6-cluster model (Fig E1, B). The mean factor scores for each of the 6 clusters are shown in Fig 1, B. Clusters were compared against each other and all were significantly different ($P < .001$) on all the factors, except for factor 4. Individual cytokines were all significantly different in at least 1 cluster (see Table E3 in this article's Online Repository at www.jacionline.org).

When compared with a control population, cluster 1 and cluster 2 were defined by low levels of most cytokines and were largely indistinguishable from healthy patients without CRS (Fig 1, C). Cluster 3 and cluster 4 both carried a predominantly T_H2 signature, with cluster 3 defined by high levels of IL-5, IL-6, IL-9, IL-10, IL-13, IL-17A, IFN- γ , and eotaxin. Cluster 4 also had elevated levels of IL-5, IL-9, and IL-13, though these were lower than in cluster 3. Cluster 4 additionally was distinguished by elevated IL-2, IL-3, and IL-4, and was noted to have higher levels of IL-17A than did cluster 3. Cluster 5 was characterized by very high levels of IL-1 β , while cluster 6 had elevated levels of IL-4, IL-5, IL-6, IL-7, IL-8, IL-12, IL-13, IL-17A, IL-21, and TNF- α . Cluster 5 was the only cluster in which IL-5 levels were not elevated compared with controls. Similar to recently characterized clusters in a European population,⁴ our results suggest that T_H2-associated inflammation is present in most patients with CRS, but dominant in only a small minority (clusters 3 and 4). One in 5 patients (cluster 5) presented with a T_H2-low,

proinflammatory signature. Interestingly, more than half the patients (clusters 1 and 2) were characterized by disease with low overall inflammatory burden that did not correspond to a distinctly T_H1 -, T_H2 -, or T_H17 -associated signature. Collectively, the inflammatory endotypes outlined in this study confirm that CRS is a heterogeneous inflammatory disease, and suggest that endotypes likely do not adhere to strict T-helper cell features.

Endotypes were then compared against phenotypic characteristics of the study population (Table 1). Cluster 3 and cluster 4 were both composed exclusively of patients with CRSwNP and had the highest levels of comorbid asthma, at 83% and 86%, respectively. These clusters were also characterized by tissue eosinophilia, worse objective measures of disease severity (22-item Sinonasal Outcome Test [SNOT-22], computed tomography, Smell Identification Test scores), and a high incidence of prior endoscopic sinus surgery. Cluster 1 and cluster 2 were characterized by mild disease, with low computed tomography scores and good olfactory function, and a low incidence of prior surgery. These groups were heterogeneous, with each composed of a fairly equal number of patients with CRSwNP (48% and 63%, respectively), and most did not have asthma. Cluster 5 was composed primarily of patients with CRSsNP (69%) and had a low incidence of comorbid asthma (38%). This cluster was characterized by mild-to-moderate disease, but had a fairly high incidence of prior endoscopic sinus surgery (69%). All patients in cluster 6 had prior endoscopic sinus surgery, but were heterogeneous in terms of polyp status.

Immunologic and phenotypic characteristics of clusters 3 to 6 were collectively suggestive of more severe disease. We explored this further by separating subjects into 2 groups, the first composed of clusters 1 and 2, and the second composed of clusters 3 to 6. This distinction was based on differences in overall inflammatory burden (Fig 1, C), and corresponded to differences in clinical characteristics and further analysis of the dendrogram (see Fig E2, A, in this article's Online Repository at www.jacionline.org). Subjects in clusters 3 to 6 had a greater likelihood of prior endoscopic sinus surgery than those in clusters 1 and 2 (75.8% vs 37.8%; $P = .001$) (Fig E2, B) and had a greater number of prior surgeries (median, 1.0 vs 0.0; $P < .0001$) (Fig E2, C). Because this association was largely based on retrospective data, we then analyzed postoperative changes in SNOT-22 scores for patients in each collective group. Postoperative SNOT-22 scores improved in clusters 1 and 2, and this benefit was maintained 1 year after surgery. In contrast, postoperative SNOT-22 scores in clusters 3 to 6 improved initially, but then returned almost to baseline at 1 year after surgery (Fig E2, D). At greater than 6 months follow-up, this corresponded to a significantly greater % improvement in postoperative SNOT-22 score for patients in clusters 1 and 2, compared with those in clusters 3 to 6 (mean, $52.0\% \pm 11.0\%$ vs $-44.2\% \pm 50.2\%$, respectively; $P = .04$) (Fig E2, E and F). Collectively, these data suggest that cluster analysis of mucus biomarkers can both define CRS inflammatory endotypes and potentially predict postoperative outcomes and need for revision surgery.

We recognize that there are some limitations to the current study that warrant discussion. First, the study only enrolled patients receiving endoscopic sinus surgery and may fail to capture a representative population of patients with CRS. It is likely that enrolling only surgical patients could bias the study toward those with greater disease severity. Second, though statistically adequate, the total number of subjects in the study

was somewhat small ($n = 90$), resulting in some clusters with fewer than 10 patients. Validation of the disease clusters identified in this study will most certainly require larger prospective cohorts that potentially incorporate patients treated both medically and surgically.

Our study is the first to report inflammatory CRS endotypes in a North American population based on cluster analysis of biological variables, and the first to use minimally invasive collection of sinonasal mucus for this purpose. Similar to tissue-based results from a European population,⁴ we identified diverse inflammatory endotypes that differ substantially with respect to phenotype and disease behavior. To further define the clinical relevance of clusters identified in the current study, we analyzed *post hoc* intercluster differences among common measures of CRS disease severity and quality of life. The ease by which mucus can be collected potentially allows for longitudinal studies that assess the stability of inflammatory signatures over time, as well as impacts of therapeutic interventions.

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METHODS

Study design and population

This study was approved by the Vanderbilt University Institutional Review Board. Patients presented to the Vanderbilt Asthma, Sinus, and Allergy Program and Otolaryngology Clinic at the Vanderbilt Bill Wilkerson Center. CRS was diagnosed according to the European Position Paper on Rhinosinusitis and Nasal Polyps and the International Consensus Statement on Allergy and Rhinology and therefore was initially managed medically.^{E1,E2} Patients with continued symptoms who elected to undergo endoscopic sinus surgery were prospectively enrolled. Only patients with diffuse, bilateral inflammatory CRS were included, and patients with odontogenic rhinosinusitis, fungus balls, and isolated osteomeatal complex obstruction were excluded. Control cases included patients undergoing pituitary or skull base surgery without a clinical or radiographic history of CRS. Patients were excluded if they had received systemic steroids within 4 weeks of surgery. Patients with cystic fibrosis, autoimmune or granulomatous diseases, or receiving immune-directed mAbs were excluded. The presence of concomitant allergic rhinitis and asthma was recorded. Allergic rhinitis was diagnosed on the basis of positive skin prick test result and/or prior physician diagnosis and clinical history suggestive of seasonal variation in atopic symptoms with improvement after use of topical nasal steroid or oral antihistamines. Asthma was diagnosed on the basis of a positive methacholine challenge or consistent pulmonary function studies, or by prior diagnosis by a pulmonologist. Patient-reported symptom severity was measured using the SNOT-22.^{E3} All patients underwent a high-resolution computed tomography scan of the paranasal sinuses within 3 months of surgery. Each scan was evaluated by 2 physicians who were blinded to subject identifiers and diagnosis. A standard Lund Mackay scoring system was used to assess the overall extent of CRS. Subjects enrolled in the study also completed the 40-item Smell Identification Test (SIT) immediately before surgery. The SIT has excellent sensitivity, correlates closely with scores attained via formal threshold testing, and has the advantage of being easily and quickly administered to subjects on the day of surgical intervention.^{E4} Raw scores were adjusted for patient age and sex by subtracting the mean normative age- and sex-appropriate SIT score from the total SIT score for each subject.^{E5} Thus, a negative-adjusted SIT score represents reduced sense of smell compared with the mean for that subject's age and sex. Normative SIT scores were extracted from the Smell Identification Test Administration Manual.^{E6}

Mucus collection and histopathologic evaluation of sinonasal tissue

At the beginning of surgery, 9 × 24 mm polyurethane sponges (Summit Medical; St Paul, Minn) were placed bilaterally into the middle meatus or ethmoid cavity of each subject under endoscopic guidance as previously reported.^{E7} This approach has been previously validated, and has advantages over other methods for mucus collection, including standardization between subjects and avoidance of specimen dilution.^{E8-E10} Each sponge was removed after 5 minutes, placed in a sterile microcentrifuge tube, and immediately processed. Sponges were placed into a microporous centrifugal filter device (MilliporeSigma; Billerica, Mass) and centrifuged at 14,000g for 10 minutes to elute mucus. Samples were then combined, gently vortexed, and again centrifuged for 5 minutes to remove any cellular debris. Supernatants were removed, placed into a new microcentrifuge tube, and frozen at −80°C for later analysis.

Cytokine assays were performed using a multiplex cytokine bead assay (BD Biosciences; Franklin Lakes, NJ) according to the manufacturer's protocol. Briefly, 50 μL of mucus was incubated with 50 μL of mixed capture beads for each measured inflammatory mediator and incubated for 1 hour. Mixed detection reagent (50 μL) was then added to each sample and standard, and incubated for an additional 2 hours. After addition of 1 mL wash buffer, samples were centrifuged at 200g for 5 minutes and the supernatant was discarded. The beads were then resuspended in 300 μL wash buffer and analyzed on an LSR Fortessa flow cytometer (BD Biosciences; Franklin Lakes, NJ). Data were analyzed using BD FACS Array Software version 3.0 (BD Biosciences).

Sinonasal tissue was collected from the ethmoid bulla or posterior ethmoid sinus in all patients undergoing endoscopic sinus surgery for CRS. Tissue from healthy controls was collected from either the ethmoid sinus or the sphenoid recess. Histopathological evaluation of excised tissue was performed by a pathologist in a blinded fashion and the mean number of eosinophils counted over 5 randomly selected hpfs was recorded.

Statistics

Sample size for principal-component analysis and subsequent clustering was estimated by establishing a subject to variable ratio of 5:1 (90 subjects, 18 biological variables) as recommended by Gorsuch^{E11} and O'Rourke and Hatcher.^{E12} Adequacy of the sample size was verified *post hoc* by assessing variable communality (heavy loading of variables in retained components). Descriptive statistics and frequency distributions were examined for each biological variable and all were positively skewed. To normalize data for subsequent analysis, values were transformed by taking the natural logarithm, resulting in elimination or significant reduction of skewing for all variables. A principal-component factor analysis with varimax rotation was then performed on the transformed biological variables. Variables with a loading of more than 0.5 were retained. The appropriate number of factors was selected by analysis of the Scree plot, with a requirement that retained factors explain at least 70% of data variance, and that each factor have an eigenvalue of more than 1.0. The regression method was then used to calculate a factor score for each subject in each of the 5 factors. Hierarchical cluster analysis was performed using Ward's method on squared Euclidean distances using the 5 factor scores. The hierarchical structure of the data was visualized using a dendrogram. The appropriate number of clusters was selected by calculating within- and between-class variance for models that included between 2 and 15 clusters, with a goal of minimizing within-class variance and maximizing between-class variance. This was also verified by identifying the bend on the accompanying Scree plot.

Clusters were then retrospectively compared against the individual components used for analysis, and then against the individual biological variables themselves. Subsequently, clusters were compared against demographic and clinical data. For comparison between groups, normality of data was assessed using the D'Agostino-Pearson omnibus test. Variables with a normal distribution were compared using a Student *t* test or ANOVA, while nonparametric data were analyzed using the Mann-Whitney test or Kruskal-Wallis test followed by Dunn's test for multiple comparisons. Comparative data were presented as means ± SD or medians with interquartile range, respectively. A *P* value of .05 was considered statistically significant for all comparisons. Statistical analyses were performed with Prism 6 software (Graphpad; La Jolla, Calif), and principal-component and hierarchical cluster analysis were performed using SAS (SAS Institute Inc; Cary, NC) and XLSTAT (Addinsoft; New York, NY).

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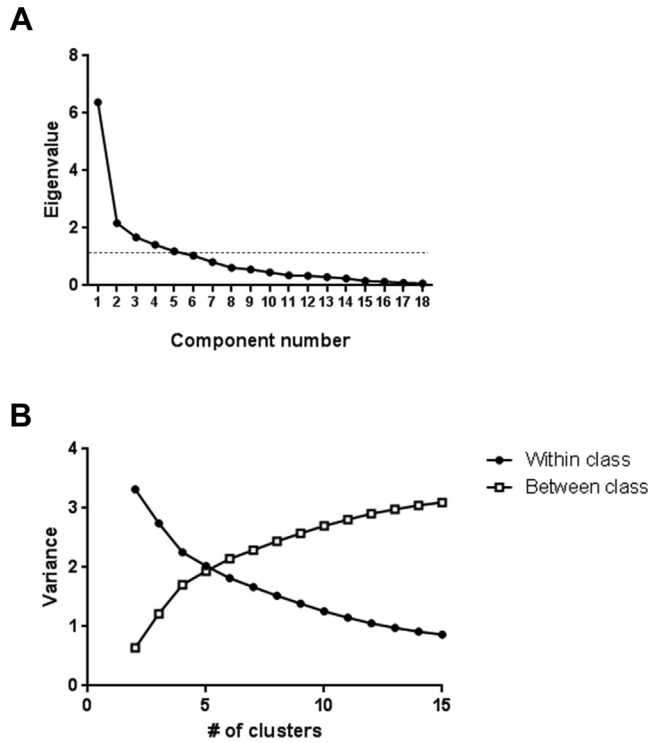


FIG E1. Validation of the number of principal components and clusters. **A**, Eigenvalues for calculated factors in a Scree plot. The optimum number of factors was estimated by identifying an approximate break point on the plotted curve and by eliminating factors with an eigenvalue of less than 1. **B**, The ideal number of CRS clusters was determined by comparing within- and between-class variance for models that included between 2 and 15 clusters, with a goal of minimizing within-class and maximizing between-class variance.

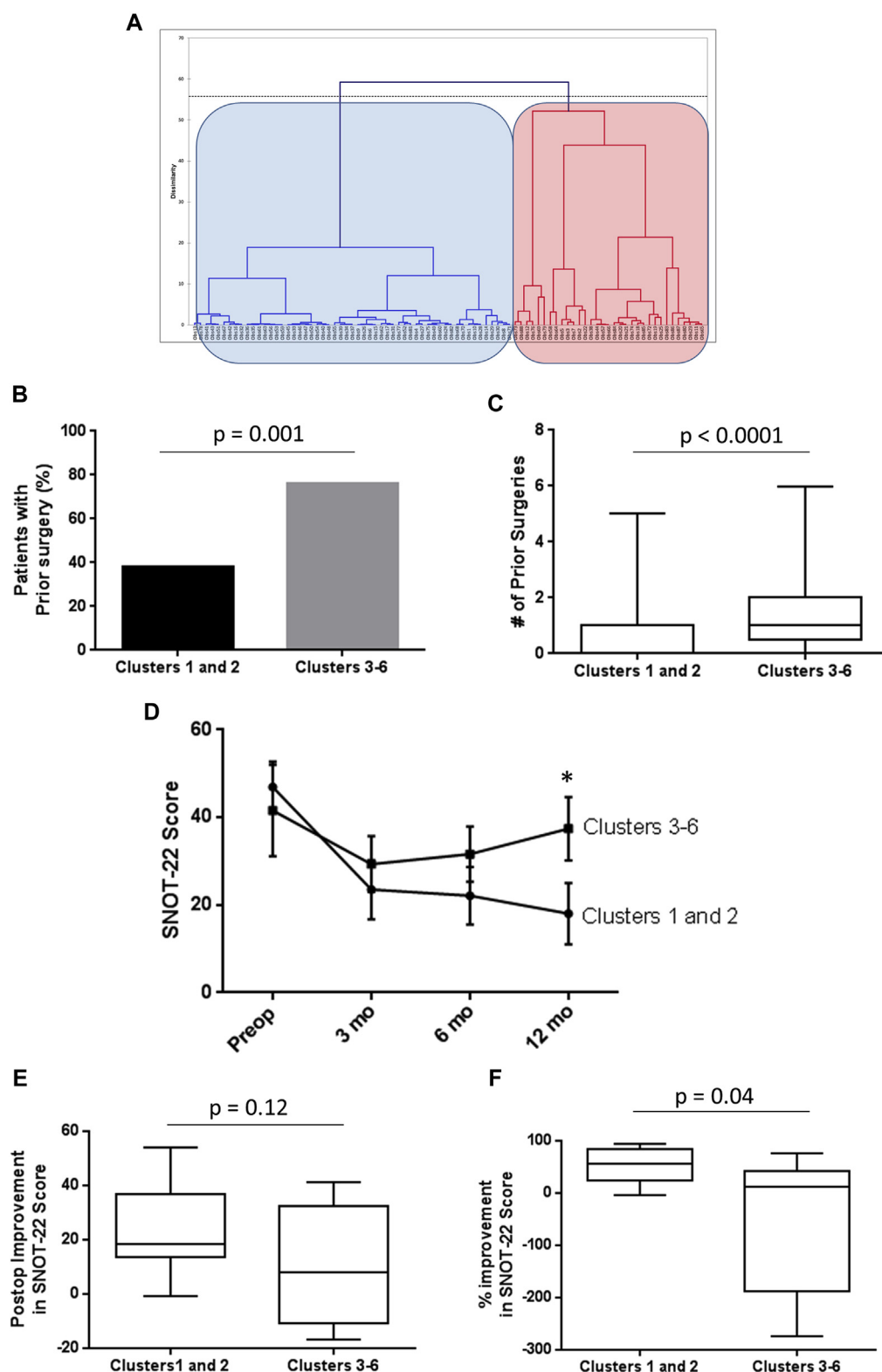


FIG E2. Postoperative improvement in sinonasal quality of life among high- and low-inflammation clusters. **A**, Clusters were combined by defining a higher “cutoff point” on the dendrogram. This corresponded to high- and low-inflammation clusters with distinct pathophysiology, as defined in Fig 3. Subjects in clusters 3 to 6 were more likely to have had prior endoscopic sinus surgery than subjects in clusters 1 and 2 (75.8% vs 37.8%; $P < .001$) (**B**) and had a greater number of prior procedures (median, 1.0 vs 0.0; $P < .0001$) (**C**). **D**, Postoperative sinonasal quality of life, as assessed by the SNOT-22 questionnaire, was plotted for all subjects with postoperative follow-up of at least 6 months. Comparison of total (**E**) and % improvement (**F**) in SNOT-22 scores was then done between each group. No significant difference in overall SNOT-22 improvement was identified between groups ($P = .12$); however, clusters 3 to 6 had a greater % improvement, compared with clusters 1 and 2 ($P = .04$). Data for SNOT-22 and frequency of prior sinus surgery are presented as box-whisker plots with the median and range for each group.

TABLE E1. Study population and demographic characteristics for healthy controls and patients with CRS

Characteristic	Healthy controls	CRS
No.	17	90
Age (y)	50.5 ± 13.1	48.5 ± 13.1
Sex, n (% female)	13 (76)	42 (47)
Race, n (% white)	13 (76)	80 (89)
Current smoker, n (%)	1 (6)	6 (7)
BMI (kg/m ²)	30.5 ± 7.0	29.7 ± 6.2
Nasal polyps, n (%)	0 (0)	53 (59)
Asthma, n (%)	0 (0)	42 (47)
Allergic rhinitis, n (%)	1 (6)	56 (62)
AERD, n (%)	0 (0)	12 (13)
AFRS, n (%)	0 (0)	10 (11)
Taking NCS, n (%)	1 (6)	71 (79)
Taking LTR, n (%)	0 (0)	25 (28)
SNOT-22 score	—	47.1 ± 18.7
CT score	1.0 (0.0-3.6)	16.0 (11.0-20.0)
SIT score	−4.0 (−7.0 to −1.0)	−7.0 (−24.5 to −3.0)
Prior surgery, n (%)	0 (0)	43 (48)

AERD, Aspirin-exacerbated respiratory disease; *AFRS*, allergic fungal rhinosinusitis; *BMI*, body mass index; *CT*, computed tomography; *NCS*, nasal corticosteroid; *LTR*, antileukotriene; *SIT*, Smell Identification Test.

Data are presented as frequencies (percentages), means ± SD, or medians with interquartile range.

TABLE E2. Loading of biological variables after principal-component analysis

Variable	Rotated factor				
	1	2	3	4	5
IL-1 β	0.883				
IL-8	0.833				
IL-6	0.718				
TNF- α	0.631				
Eotaxin	0.604				
IL-7					
IL-5		0.943			
IL-13		0.912			
IL-9		0.655			
IL-2			0.76		
IL-21			0.681		
IL-4			0.553		
IL-12			0.551		
IFN- γ				0.926	
IL-10				0.894	
IL-3					0.823
IL-17					0.505
RANTES					0.504

Values for all biological variables were transformed to achieve normalcy and then analyzed with principal-component analysis with varimax rotation. Variables with a loading of >0.5 were retained. Data for the first 5 factors are shown on the basis of analysis of the plotted Eigenvalues for each component. The 5-factor solution explained 71.3% of the collective data variance.

TABLE E3. Differences in mucus cytokines levels between the 6 identified CRS endotypes

Cytokine	Control (n = 17)	Cluster 1 (n = 31)	Cluster 2 (n = 24)	Cluster 3 (n = 6)	Cluster 4 (n = 7)	Cluster 5 (n = 13)	Cluster 6 (n = 7)	P value
IL-1 β	42.1 (13.3-211.7)	101.9 (27.6-289.8)	7.1 (1.5-12.5)*	323.8 (80.2-1491.0)	24.9 (15.8-139.1)	549.1 (171.6-6211.0)†	521.6 (287.3-2845.0)	<.0001
IL-2	0.0 (0.0-11.1)	2.9 (0.0-12.8)	0.0 (0.0-1.6)	19.9 (4.0-36.1)	17.8 (11.3-46.9)†	0.0 (0.0-7.1)	8.8 (2.1-68.1)	.0001
IL-3	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.4 (0.0-1.2)	1.7 (1.4-13.0)†	0.0 (0.0-0.0)	0.0 (0.0-0.1)	<.0001
IL-4	0.08 (0.0-1.30)	0.0 (0.0-1.4)	0.0 (0.0-0.0)	2.5 (0.9-3.1)	4.2 (1.4-4.4)†	0.1 (0.0-2.3)	4.0 (1.9-5.2)†	<.0001
IL-5	0.12 (0.01-0.77)	3.2 (0.1-27.4)†	9.3 (1.6-60.8)†	507.1 (325.4-1843.0)†	160.6 (40.0-240.5)†	1.2 (0.1-10.4)	9.0 (2.5-18.6)†	<.0001
IL-6	59.8 (13.4-190.3)	116.3 (41.0-285.9)	9.8 (4.4-48.0)	1701.0 (1116.0-5092.0)†	205.8 (42.6-659.7)	119.9 (47.6-1138.0)	733.7 (265.5-1076.0)†	<.0001
IL-7	3.7 (1.6-9.8)	5.4 (1.6-11.3)	1.7 (0.4-3.0)	14.8 (12.7-18.9)	9.2 (2.9-19.9)	7.4 (2.2-20.5)	23.7 (12.6-47.6)†	<.0001
IL-8	3,067 (1,610-9,078)	5,461 (2,838-9,885)	1,337 (861-2,428)	7,838 (3,546-132,655)	3,264 (1,543-15,683)	12,840 (4,757-38,650)	13,001 (10,322-335,288)†	<.0001
IL-9	0.0 (0.0-1.6)	0.0 (0.0-0.0)	0.0 (0.0-0.0)	23.2 (8.6-64.6)†	16.6 (0.9-63.4)†	0.1 (0.0-3.1)	4.0 (1.1-16.9)	<.0001
IL-10	2.6 (0.1-6.3)	4.0 (0.9-8.2)	0.8 (0.0-3.1)	27.1 (15.0-63.2)†	10.6 (6.9-17.5)	1.2 (0.0-15.6)	9.9 (6.6-23.3)	<.0001
IL-12	43.5 (20.3-117.7)	72.3 (12.4-113.5)	3.4 (0.0-4.9)*	112.7 (10.4-325.7)	52.2 (20.2-87.2)	45.2 (2.8-121.2)	355.6 (188.7-681.0)†	<.0001
IL-13	1.6 (0.0-6.7)	3.0 (0.0-22.0)	7.7 (0.0-37.5)	468.6 (346.4-712.9)†	86.6 (36.8-358.8)†	2.9 (0.6-7.8)	52.4 (24.6-93.5)†	<.0001
IL-17A	0.0 (0.0-0.0)	0.0 (0.0-0.8)	0.0 (0.0-0.2)	4.0 (3.2-8.3)†	9.2 (4.9-14.5)†	2.1 (0.0-8.1)	4.2 (1.8-17.9)†	<.0001
IL-21	59.6 (0.0-155.3)	19.5 (0.0-118.9)	7.0 (0.0-12.0)	71.6 (0.0-305.2)	69.0 (9.9-88.2)	0.0 (0.0-4.8)	243.9 (102.0-653.9)†	.0001
TNF- α	5.1 (1.9-7.1)	5.0 (2.8-11.6)	0.2 (0.0-2.0)	59.7 (7.4-156.0)	8.3 (6.2-19.0)	9.7 (0.1-12.9)	15.6 (10.2-174.5)†	<.0001
IFN- γ	0.0 (0.0-1.4)	0.0 (0.0-2.7)	0.0 (0.0-0.8)	4.1 (2.6-10.6)†	2.1 (1.0-3.1)	0.0 (0.0-1.1)	2.1 (0.6-7.6)	.001
Eotaxin	17.8 (12.4-48.2)	14.3 (5.6-32.1)	7.4 (2.8-17.7)	112.7 (76.5-303.5)†	9.7 (7.6-11.0)	25.1 (5.8-54.8)	49.1 (28.3-107.6)	.0001
RANTES	1,848 (654-2,686)	4,355 (756-6,423)	3,107 (764-8,058)	2,128 (937-2,590)	331 (142-6,902)	120 (22-895)	3,207 (1,171-6,118)	.002

Median cytokine levels of control subjects and each of the 6 CRS clusters are shown for all 18 assayed biological variables. Significant differences among CRS endotypes and between each CRS endotype and the control group were identified using the Kruskal-Wallis test. Differences among the endotypes are represented by the listed *P* values, with a *P* value of <.05 considered statistically significant. *Post hoc* analysis of differences between each cluster and healthy controls were then performed. Data are represented as medians with interquartile range. Boldface indicates *P* < .05.

*Clusters with a lower median cytokine level compared with controls are annotated.

†Clusters with a higher median cytokine level compared with controls are annotated.