Identification of Clinically Relevant Chronic Rhinosinusitis Endotypes using Cluster Analysis of Mucus Cytokines

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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 clinical entity. Several investigators have shown that CRS with (CRSwNP) and without polyps (CRSsNP) can both be linked with either Th1-, Th2, or Th17-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 al. recently using surgically-obtained tissue to identify potential CRS endotypes based entirely on inflammatory biomarkers[4]. While such studies have preferentially used tissue-based assessment of inflammatory mediators, this approach may have some logistical limitations as 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 employing analysis of sputum, mucus, or epithelial brushings[7–9]. The aim of the current study was to define CRS endotypes in a U.S. population based entirely on analysis of mucus collected via a minimally invasive approach. Going one step further, we sought to additionally define the

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

90 CRS patients undergoing endoscopic sinus surgery were prospectively enrolled in the study (Table E1). Mucus was collected using an absorbent polyurethane sponge and we subsequently assayed 18 different mucus inflammatory mediators that reflect Th1/Th2/ Th17-associated inflammation. Since *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 five factor solution explaining 71.3% of the data variance (Figure E1A). The first component (PC1) included IL-1β, IL-6, IL-8, TNF-α, and eotaxin, while the second was composed of IL-5, IL-9, and IL-13 (Table E2). 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 (Figure 1A). This suggested an optimum of 5 or 6 clusters, with mathematical modeling and visual evaluation of the dendogram validating a 6 cluster model (Figure E1B). The mean factor scores for each of the 6 clusters are shown in Figure 1B. Clusters were compared against each other and all were significantly different (p<0.001) on all of the factors, except for factor 4. Individual cytokines were all significantly different in at least one cluster (Table E3).

When compared to a control population, cluster 1 and cluster 2 were defined by low levels of most cytokines and were largely indistinguishable from healthy non-CRS patients (Figure 1C). Cluster 3 and Cluster 4 both carried a predominantly Th2 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 Cluster 3. Cluster 5 was characterized by very high levels of IL-1β, while Cluster 6 had elevated 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 to controls. Similar to recently characterized clusters in a European population[4], our results suggest that Th2-associated inflammation is present in a majority of CRS patients, but dominant in only a small minority (Clusters 3 and 4). One in five patients (Cluster 5) presented with a Th2-low, pro-inflammatory signature. Interestingly, more than half of patients (Clusters 1 and 2) were characterized by disease with low overall inflammatory burden, that did not correspond to a distinctly Th1-, Th2- or Th17-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 CRSwNP patients 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 (SNOT-22, CT, SIT scores) and a high incidence of prior endoscopic sinus surgery. Cluster 1

and Cluster 2 were characterized by mild disease, with low CT 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 CRSwNP patients (48% and 63%, respectively), and a majority were non-asthmatic. Cluster 5 was composed primarily of CRSsNP patients (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 3–6 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 3–6. This distinction was based on differences in overall inflammatory burden (Figure 1C), and corresponded to differences in clinical characteristics and further analysis of the dendogram (Figure E2A). Subjects in Clusters 3–6 had a greater likelihood of prior endoscopic sinus surgery than those in Clusters 1 and 2 (75.8% vs. 37.8%; p = 0.001) (Figure E2B) and had a greater number of prior surgeries (median, 1.0 vs. 0.0, p < 0.0001) (Figure E2C). Since 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 one year after surgery. In contrast, postoperative SNOT-22 scores in Clusters 3–6 improved initially, but then returned almost to baseline at one year after surgery (Figure E2D). 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 to those in clusters 3–6 (mean 52.0% ± 11.0 vs. −44.2% ± 50.2%, respectively; p=0.04) (Figure E2E, 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 CRS patients. 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 utilize 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. In order to further define the clinical relevance of clusters identified in the current study, we analyzed *post hoc* inter-cluster 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

ABBREVIATIONS

AERD	aspirin-exacerbated respiratory disease
AFRS	allergic fungal rhinosinusitis
CRSsNP	chronic rhinosinusitis without nasal polyps
CRSwNP	chronic rhinosinusitis with nasal polyps
SIT	smell identification test
SNOT-22	22 item sinonasal outcome test

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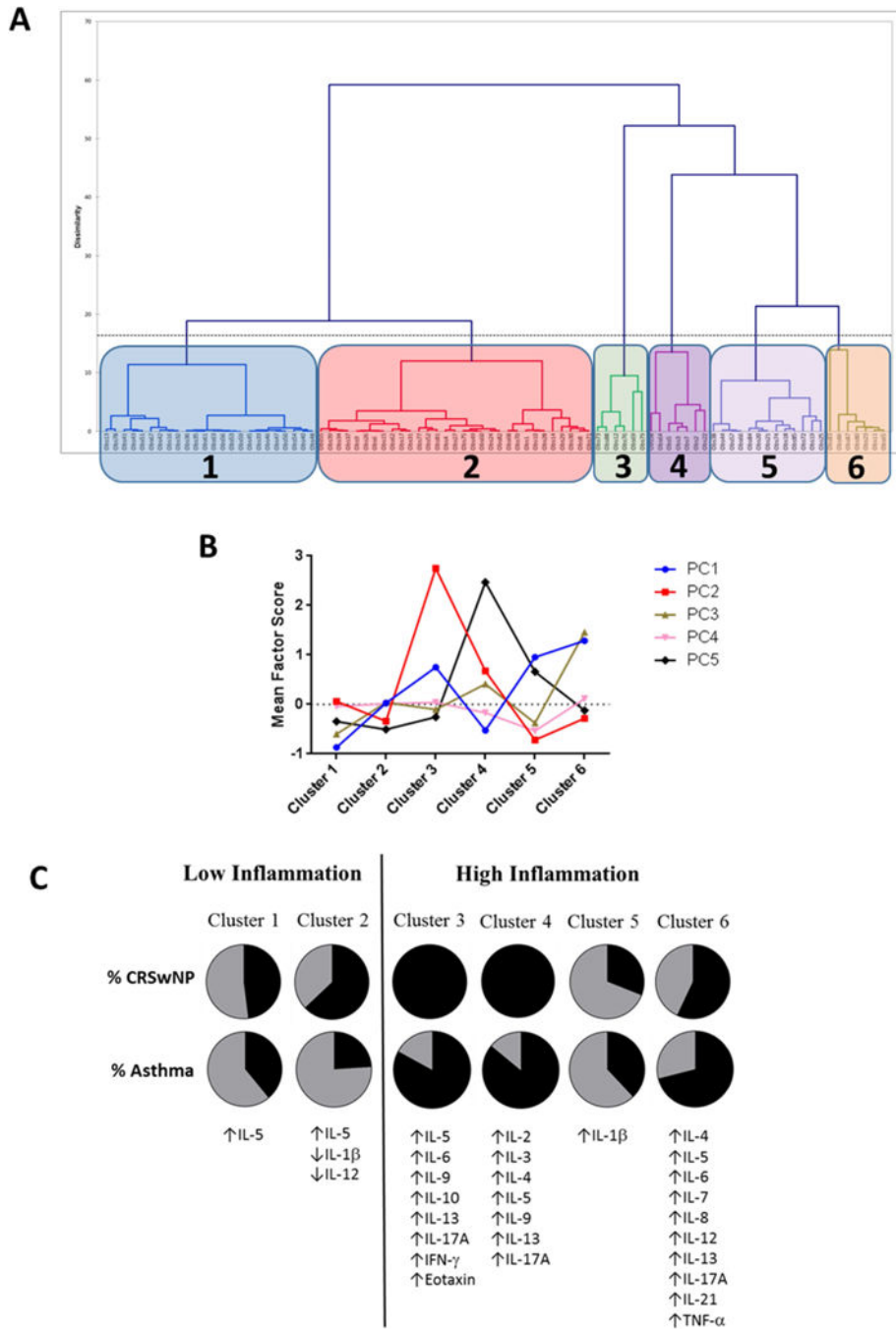
CAPSULE SUMMARY

CRS endotypes with prognostic potential can be identified via cluster analysis of mucus cytokine levels. These clusters correspond to distinct inflammatory signatures and differ based on phenotypic characteristics, disease severity, and surgical outcomes.

Figure 1. Identification of inflammatory endotypes using cluster analysis of CRS mucus
(A) Dendogram representing hierarchical cluster analysis of CRS patients 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 to healthy controls. CRSwNP, chronic rhinosinusitis with nasal polyps; PC, principal component.

Table 1

Differences in clinical and phenotypic characteristics among CRS endotypes

Post-hoc analysis of clinical and phenotypic characteristics were examined for each of the 6 identified CRS endotypes. Values are presented as either the mean +/− standard deviation or median with interquartile range, depending on the normalcy of the data. Differences between groups were assessed using the Kruskal-Wallis test or Chi-square analysis. **BOLD, p < 0.05**. AERD, aspirin-exacerbated respiratory disease; AFRS, allergic fungal rhinosinusitis; BMI, body mass index; CT, computed tomography; NCS, nasal corticosteroid; LTR, anti-leukotriene; SIT, smell identification test; SNOT-22, sinonasal outcome test-22.

	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	Cluster 6	p-value
No.	31	24	6	7	13	7	
Age (years)	49.5 +/− 12.9	44.3 +/− 9.9	55.7 +/− 17.6	47.3 +/− 13.6	52.7 +/− 17.0	49.3 +/− 10.5	0.33
Sex, no. (% female)	19 (61)	8 (33)	2 (33)	1 (14)	5 (38)	5 (71)	0.08
Race, no. (% white)	30 (97)	20 (83)	6 (100)	5 (71)	13 (100)	7 (100)	0.08
Current smoker, no. (%)	4 (13)	2 (8)	0 (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	0.60
Nasal polyps, no. (%)	15 (48)	15 (63)	6 (100)	7 (100)	4 (31)	4 (57)	**0.01**
Asthma, no. (%)	12 (39)	8 (24)	5 (83)	6 (86)	5 (38)	5 (71)	**0.03**
Allergic Rhinitis, no. (%)	16 (52)	17 (71)	3 (50)	1 (14)	8 (62)	6 (86)	0.31
AERD, no. (%)	4 (13)	3 (13)	2 (33)	3 (43)	0 (0)	0 (0)	0.07
AFRS, no. (%)	1 (3)	5 (21)	1 (17)	2 (29)	1 (8)	0 (0)	0.19
Taking NCS, no. (%)	26 (84)	20 (83)	4 (67)	6 (86)	9 (69)	6 (86)	0.79
Taking LTR, no. (%)	5 (16)	10 (42)	4 (33)	3 (43)	0 (0)	3 (43)	**0.04**
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	0.80
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)	**0.02**
SIT score	−7.0 (−24.5–3.0)	−4.0 (−21.0–3.0)	−29.0 (−31.0–24.0)	−16.0 (−26.6–0.8)	−2.5 (−19.8–1.0)	−7.0 (−17.5–2.0)	**0.03**
Prior surgery, no. (%)	9 (29)	8 (33)	4 (66)	5 (71)	9 (69)	7 (100)	**0.002**
# of prior surgeries	0.0 (0.0–1.0)	0.0 (0.0–1.0)	1.5 (1.0–3.8)	2.0 (1.0–3.0)	1.0 (0.0–2.0)	1.0 (1.0–1.0)	**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)	**0.01**