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Patterns of Olfactory Dysfunction in Chronic Rhinosinusitis Identified by Hierarchical Cluster Analysis and Machine Learning Algorithms

Justin C. Morse, M.D.[1], **Meghan H. Shilts, M.S.**[2], **Kim A. Ely, M.D.**[3], **Ping Li, M.D.**[1], **Quanhu Sheng, Ph.D.**[4], **Li-Ching Huang, Ph.D.**[4], **Todd Wahnemuehler, M.D.**[1], **Naweed I. Chowdhury, M.D.**[1], **Rakesh K. Chandra, M.D.**[1], **Suman R. Das, Ph.D.**[2], and **Justin H. Turner, M.D. Ph.D.**[1]

[1]Department of Otolaryngology-Head and Neck Surgery, Vanderbilt University Medical Center; Nashville, TN 37232

[2]Department of Medicine; Vanderbilt University Medical Center; Nashville, TN 37232

[3]Department of Pathology, Microbiology, and Immunology; Vanderbilt University Medical Center; Nashville, TN 37232

[4]Department of Biostatistics; Vanderbilt University Medical Center; Nashville, TN 37232

Abstract

Introduction: Olfactory dysfunction is a common symptom of chronic rhinosinusitis (CRS). We previously identified several cytokines potentially linked to smell loss, potentially supporting an inflammatory etiology for CRS-association olfactory dysfunction. In the current study we sought to validate patterns of olfactory dysfunction in CRS using hierarchical cluster analysis, machine learning algorithms, and multivariate regression.

Methods: CRS patients undergoing functional endoscopic sinus surgery were administered the smell identification test (SIT) preoperatively. Mucus was collected from the middle meatus using an absorbent polyurethane sponge and 17 inflammatory mediators were assessed using a multiplexed flow cytometric bead assay. Hierarchal cluster analysis was performed to characterize inflammatory patterns and their association with SIT scores. The random forest approach was used to identify cytokines predictive of olfactory function.

Results: 110 patients were enrolled in the study. Hierarchical cluster analysis identified 5 distinct CRS clusters with statistically significant differences in SIT scores identified between individual clusters ($p<0.001$). A majority of anosmic patients were found in a single cluster, which was additionally characterized by nasal polyposis (100%) and a high incidence of allergic fungal rhinosinusitis (50%) and aspirin exacerbated respiratory disease (AERD) (33%). A random forest approach identified a strong association between olfaction and the cytokines IL-5 and IL-13.

Send Correspondence to: Justin H. Turner, M.D., Ph.D., Department of Otolaryngology-Head and Neck Surgery, Vanderbilt University Medical Center, 1215 21st Avenue South, Suite 7209, Nashville, TN 37232-8605, justin.h.turner@vanderbilt.edu.

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Multivariate modeling identified AERD, CT score, and IL-2 as the variables most predictive of olfactory function.

Conclusion: Olfactory dysfunction is associated with specific CRS endotypes that are characterized by severe nasal polyposis, tissue eosinophilia, and AERD. Mucus IL-2 levels, CT score, and AERD were independently associated with smell loss.

Keywords

anosmia; hyposmia; rhinosinusitis; endotype; mucus; cytokine; interleukin; cluster analysis; machine learning

BACKGROUND

Olfactory dysfunction is among the most common symptoms of CRS with a prevalence between 30–80%[1,2]. Unfortunately, the etiology of CRS-associated olfactory dysfunction remains poorly understood. Olfactory loss in CRS has previously been attributed to an inability of odorants to effectively reach the olfactory cleft, due either to structural abnormalities or presence of nasal polyps[3,4] Recent research has suggested that sinonasal inflammation may directly or indirectly affect olfactory neurons and olfactory function[5]. In animal models, certain cytokines have the ability to adversely affect olfactory neuron function, turnover, and regeneration[5–8]. An association between olfactory cleft cytokine levels and olfactory function has been partially validated in human tissue by several groups[9,10]. Recently, our group measured olfactory cleft mucus cytokine levels in CRS patients, and found that objective olfactory function was inversely related to several cytokines, including IL-2, IL-5, IL-6, IL-10, and IL-13. However, this study was limited by a small sample size and was not able to account for potential confounding factors.

In the current study we sought to validate patterns of olfactory dysfunction in CRS using multiple complementary statistical approaches. We previously hypothesized that mucus cytokine levels are reflective of olfactory inflammation and could be predictive of olfactory function[11]. This study seeks to expand upon this hypothesis by incorporating inflammatory, clinical, and demographic factors, with the ultimate goal of understanding constellations of disease features associated with olfactory dysfunction.

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 (ASAP) 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 were initially managed medically[12]. Patients who chose 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. Patients were excluded if they had received

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systemic steroids within 4 weeks of surgery; had diagnosis of cystic fibrosis, autoimmune, or granulomatous diseases; or were receiving immune-directed monoclonal antibodies. Diagnosis of allergic rhinitis and asthma was recorded. Allergic rhinitis was diagnosed based on positive skin prick testing and/or prior physician diagnosis and clinical history suggestive of seasonal variation of atopic symptoms with improvement following use of topical nasal steroid or oral antihistamines. Asthma was diagnosed based on a positive methacholine challenge or consistent pulmonary function studies, or by prior diagnosis by a pulmonologist. All patients underwent a high resolution CT scan of the paranasal sinuses within 3 months of surgery. Each scan was evaluated by two physicians who were blinded to subject identifiers and diagnosis. A standard Lund Mackay scoring system was used to assess overall extent of CRS. Subjects enrolled in the study also completed the 40-item Smell Identification Test (SIT) immediately prior to surgery, which has been previously validated for olfaction assessment.[13]. Normative SIT scores were extracted from the Smell Identification Test Administration Manual (Sensonics International; Haddon Heights, NJ). Raw scores were then adjusted for patient age and gender by subtracting the mean normative age- and sex-appropriate SIT score from the total SIT score for each subject[10]. A negative adjusted SIT score represents reduced sense of smell compared to the mean for that subject's age and gender.

Mucus Collection and Histopathologic Evaluation of Sinonasal Tissue

At the beginning of surgery, 9 × 24mm polyurethane sponges (Summit Medical; St. Paul, MN) were placed into the middle meatus or ethmoid cavity of each subject under endoscopic guidance as previously reported[11]. This approach has advantages over other methods for mucus collection, including standardization between subjects and avoidance of specimen dilution. 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, MA) and centrifuged at 14,000 x g for 10 minutes to elute mucus. Samples were then 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 as previously described[14,15].

Sinonasal tissue was collected from the ethmoid bulla or ethmoid sinus in all patients undergoing endoscopic sinus surgery for CRS. Eosinophil and neutrophil counts were obtained from a dedicated, blinded histopathological evaluation of excised tissue by a pathologist and averaged over 5 randomly selected high power fields.

Statistics

Sample size for principal component analysis and subsequent clustering was estimated by establishing a subject to variable ratio of 5 (17 biological variables, 110 subjects) as recommended by Gorsuch and Hatcher[16,17]. 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. In order to normalize data for subsequent analysis, values

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were transformed by taking the square root, 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 > 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 > 1.0. The regression method was then used to calculate a factor score for each subject in each of the five factors. Hierarchical cluster analysis was performed using Ward's method on squared Euclidian distances using the five factor scores. The hierarchical structure of the data was visualized using a dendogram. The appropriate number of clusters (*k*) was selected using the Elbow method. Total within sum of squared error (SSE) was calculated for between 2 and 10 clusters and *k* was chosen at the break point where the SSE started to smooth. Cluster stability was verified using bootstrap analysis, with all clusters having a stability of at least 0.7 (indicating plausible structure and good overall cluster stability)[18].

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's t-test or analysis of variance, while nonparametric data was analyzed using the Mann-Whitney test or Kruskal-Wallis test. Comparative data was presented as medians with interquartile range. A p value of < 0.05 was considered statistically significant for all comparisons. Statistical analyses were performed with Prism 6 software (Graphpad; La Jolla, CA), and principal component and hierarchical cluster analysis were performed using R version 3.4 (The R Project for Statistical Computing, Vienna, Austria. http://www.R-project.org/).

The random forest algorithm was used to examine cytokines which were most predictive of SIT score. Analysis was performed in R using the randomForest package[19]. The training and validation sets each represented half of the samples, chosen at random without replacement. The number of trees generated was 100, 1,000, 10000, and 1,000,000 with 5 variables (i.e., cytokines) chosen at each split. The percent variance explained appeared to level off at ~8% between 100,000 and 1,000,000 trees generated, and therefore the number of trees was not further increased. Variable importance plots were examined for both the training and validation sets for each set of trees generated to verify that the variable importance ordering remained consistent.

Assessment of predictive variables on SIT scores including demographic characteristics and cytokines was assessed with univariate and multivariate regression modeling, performed using STATA (StataCorp. 2017. Stata Statistical Software: Release 15. College Station, TX: StataCorp LLC). In the multivariate model, all clinical factors with P < 0.2 in the univariate modeling were included in models for age- and sex-adjusted olfactory scores. Collinearity diagnostics were performed using the variance inflation factor and when applicable a correlation matrix of the model was utilized to identify variables with collinearity. If a variable was determined to be collinear it was dropped from the model and the model was

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reanalyzed to determine effect on other predictor coefficients. A value of $P < 0.05$ was deemed to be statistically significant.

RESULTS:

Study Population and Demographics

Patients included in the study were undergoing functional endoscopic sinus surgery for CRS and completed the validated smell identification test (SIT) immediately prior to their procedure. A total of 110 patients with olfactory testing were enrolled, all of whom are part of an ongoing prospective translational study that has been partially characterized elsewhere[11,14,15.] A majority of patients had nasal polyps (55%), with comorbid asthma and allergic rhinitis present in 42% and 67% of subjects, respectively (Table 1). Eleven patients had aspirin exacerbated respiratory disease (AERD), while 14 were diagnosed with allergic fungal rhinosinusitis (AFRS). Almost one-half of enrolled subjects had undergone prior endoscopic sinus surgery. Disease burden was significant, with a median CT score of 15.0. The median age- and sex-adjusted SIT score was −7.0 among all CRS patients, with significant differences based on polyp status. CRSwNP (CRS with nasal polyps) patients had a median adjusted SIT score of −20.0 (−5.0 - −26.5) compared to a median score of −3.0 (−1.0 - −7.0) among CRSsNP (CRS without nasal polyps) patients ($p < 0.0001$).

Olfactory Function in Inflammatory CRS Clusters

We previously characterized several inflammatory CRS endotypes using hierarchical cluster analysis of mucus cytokines[14]. In the process of validating these endotypes, we repeated cluster analysis in an updated cohort of 147 patients, 110 of whom had olfactory testing. Hierarchal cluster analysis identified 5 CRS clusters with unique inflammatory signatures (Figure 1). Demographic and clinical characteristics of each cluster are detailed in Table 2. Age- and sex adjusted SIT scores were significantly different between clusters ($p<0.001$) (Figure 2A). Patients with the worst olfactory function were primarily concentrated within a single cluster (cluster 4) (Figure 1). The median adjusted SIT score in this cluster was −25.0, indicative of total anosmia (Figure 2A)[13]. More than 80% of patients in this cluster had either AERD or AFRS, both of which varied significantly among all clusters ($p<0.001$) (Figure 2B). Cluster 4 was associated with a Th2-dominant signature, with elevated levels of IL-5 ($p<.001$) and IL-13 ($p<.001$) compared to other clusters (Figure 3).

Use of Machine Learning Algorithm to Identify Cytokines Impacting Olfactory Function

Hierarchical cluster analysis showed a close link between poor olfactory function and a single inflammatory CRS endotype, marked by elevated levels of Th2-associated cytokines. This result is consistent with previous reports that have associated decreased objective olfactory function with Th2 cytokines, including IL-5 and IL-13[10,15]. We sought to further validate these findings using a random forest model, which is an ensemble machine learning technique that fits decision trees using a random subset of features to predict the outcome of interest. The relative importance of predictors is then computed based on the mean increase in error and decrease in node purity when a variable of interest is excluded from the model. Interestingly, this approach also identified IL-5 and IL-13 as the cytokines most predictive of olfactory function in CRS(Figure 4)[19].

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Identification of Variables Affecting Olfactory Function Using Multivariate Regression

Our initial analysis and a small number of preceding studies have identified eosinophilic inflammation and Th2 cytokines as potential mediators of olfactory dysfunction in CRS[10,11,15]. Small sample sizes have limited the ability of prior studies to account for covariates and other potential confounding factors. We consequently incorporated a large number of demographic, clinical, and inflammatory factors to further analyze CRS-associated olfactory function in our large patient cohort. Univariate regression identified asthma status (p=0.016), polyp status (p <0.001), AERD (p <0.001), CT score (p <0.001), tissue eosinophilia (p=0.002), and prior surgery (p=0.011) as variables predictive of olfactory function. Cytokines associated with olfactory dysfunction included IL-2 (p=0.037), IL-5 (p=0.001), and IL-13 (p= <0.001) (Table 3). After multivariate analysis, only AERD (p=0.015), CT score (p=0.014), and IL-2 (p=0.005) remained as predictive variables (Table 4). IL-5 and IL-13, which were strongly associated with olfactory dysfunction after univariate analysis, demonstrated significant collinearity, and this was verified using a correlation matrix. Removal of either IL-5 or IL-13 from the model did not significantly affect the results or the strength of the model.

DISCUSSION

This study is the first to utilize hierarchal cluster analysis and machine learning algorithms to assess the relationship between inflammatory cytokines and CRS-associated olfactory dysfunction. It is likewise the first study with an adequate sample size for multivariate analysis of cytokines and other potential contributors to olfactory loss. These findings expand upon our group's previous work and potentially offer new insight into the potential role of cytokine-associated inflammation in olfactory loss[11].

Consistent with prior studies[3,20], our data suggests that polyp status alone is not a sufficient predictor of olfactory dysfunction. Most anosmic CRS patients were found in a single CRS disease cluster that was chiefly characterized by nasal polyps (100%), however, the majority of CRSwNP patients did not appear in this cluster. Rather, characteristics of this cluster were suggestive of a more severe form of CRSwNP, with many patients diagnosed with either AERD (33%) or AFRS (50%), and associated with a strong Th2-dominant inflammatory signature. We previously showed that olfactory cleft mucus cytokine levels correlate with olfactory function in CRSwNP patients, and this was particularly true for the Th2-associated cytokines IL-5 and IL-13[14]. This association was also seen in our random forest model, again suggesting that IL-5 and IL-13 were the strongest predictors of olfactory dysfunction in CRS. Surprisingly, our multivariate regression modelling did not identify either cytokine as independent predictors of olfactory dysfunction. This was largely due to collinearity of both IL-5 and IL-13 with other variables in the model, suggesting that these cytokines may be markers of more severe disease. This hypothesis is partially supported by a recent mouse study, which showed that allergic inflammation associated with elevated olfactory epithelium Th2 cytokines reduces the number of immature olfactory neurons, but does not affect the number of mature olfactory neurons or olfactory function[21].

Though our study did not confirm IL-5 and IL-13 as independent effectors of olfactory loss in CRS, we did identify a potential role for IL-2. Our previous study likewise identified this

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cytokine as being closely correlated with olfactory function[11]. IL-2 is a non-specific T-cell effector that regulates immunity and tolerance, but its role in chronic rhinosinusitis is poorly defined. A recent study showed that IL-2 may be associated with elevated IgD levels and presence of pathogenic bacteria in CRSsNP patients[22]. Potential functional relationships between IL-2 and the olfactory epithelium will require further investigation and confirmation.

Our study identified AERD as being independently associated with olfactory dysfunction in CRS. The pathophysiology of smell dysfunction in AERD is unclear, however, recent studies have started to identify factors that differentiate AERD from other CRSwNP patients[23]. Both the innate and adaptive immune system have roles in AERD pathophysiology and severity[24]. Both AERD and CRSwNP are associated with eosinophilic tissue inflammation, though studies have generally not shown significant differences in the number of tissue eosinophils in each group. Conversely, the eosinophil degranulation product, eosinophil cationic protein (ECP), is elevated in AERD patients compared to CRSwNP patients. This would suggest that eosinophils may be more highly activated in AERD[25,26]. Elevation of Th2-associated cytokines has been reported in both CRSwNP and AERD, yet a specific difference in inflammatory signatures has not been clearly defined[27]. Of note, hierarchical cluster analysis in this and prior studies from our group, do suggest that AERD may be associated with a specific inflammatory CRS endotype[14]. The relationship between AERD and olfaction is even less clear. Gudziol et al demonstrated that AERD patients have worse olfaction at baseline which subsequently improved after aspirin desensitization, however, no current studies to our knowledge have evaluated inflammatory profiles and olfaction in these patients[23]. Furthermore, smell testing has not been found to predictive of AERD[23]. Multiple studies have demonstrated greater disease severity among patients with AERD, based both on endoscopy[28] and CT scores[29]. This would suggest that reduced olfactory identification scores in AERD may be multifactorial, and likely due to collective differences in disease severity, polyp burden, and inflammatory signatures[30].

Eosinophilic inflammation in allergic mouse models has previously been shown to have adverse effects on the olfactory epithelium[31], and human studies suggest similar findings[10,32]. While the exact mechanisms of eosinophil-associated olfactory loss remains unclear, it is well established that eosinophilia is correlated with a Th2 inflammatory profile[33]. Both local neurotoxicity secondary to release of eosinophilic granule proteins[34] and eosinophil-associated cytokine effects[35] have been postulated as possible mechanisms of eosinophil-associated olfactory loss. Interestingly, while tissue eosinophilia was associated with olfactory loss in our univariate model, multivariate analysis failed to support this link. Rather, our data suggests that eosinophilia may instead simply be indicative of more severe disease, with olfactory dysfunction being one of many indicators of disease severity.

Our study does have some limitations that should be acknowledged. Firstly, this study assessed smell function using the semi-objective smell identification test, rather than using formal and more quantitative assessment tools. While the SIT is a well-established method for assessment of smell function that is highly correlated to threshold testing, it remains possible that some differences in olfactory function could have been overlooked in this study. This possibility is partially supported by a small number of recent studies. For

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example, Lavin et al. found that Charcot-Leyden crystal protein gene expression in superior turbinate tissue was associated with olfactory thresholds, but not olfactory identification[32]. Conversely, Schlosser et al. found that elevated olfactory cleft IL-5 levels were associated with worse identification scores, but did not affect thresholds or discrimination[2]. The relationships identified in the current study will ultimately need to be validated using objective and quantitative olfactory testing. Second, it is possible that mucus cytokine levels may show temporal variations, particularly as relates to CRS and comorbid disease exacerbations. While we have attempted to limit the impact of this potential problem by assessing olfactory function and cytokine levels on the same day, subsequent studies that assess temporal variations in individual cytokines and any potential effects on olfactory function may help to clarify this issue.

To our knowledge, the current study is the largest to date to evaluate potential associations between sinonasal inflammation and olfaction in CRS patients. Strengths of the study include its prospective design, evaluation of a wide array of cytokines and inflammatory mediators, and use of multiple complementary statistical approaches. This study continues to underscore the limitations of phenotypic categorization of CRS, and further suggests that olfactory loss may be more closely associated with endotypic, rather than phenotypic differences.

CONCLUSION

Anosmia in CRS is associated with a Th2-driven inflammatory CRS endotype enriched with AERD and AFRS patients. Previously reported associations between the Th2-associated cytokines IL-5 and IL-13 and olfactory function were not confirmed after multivariate analysis, whereas IL-2 was the only cytokine independently associated with smell dysfunction in CRS. Additionally, disease characteristics that included radiographic severity and presence of AERD were also independently associated with olfactory dysfunction. These results suggest that a combination of inflammatory, clinical, and demographic factors likely contribute to olfactory loss in CRS patients.

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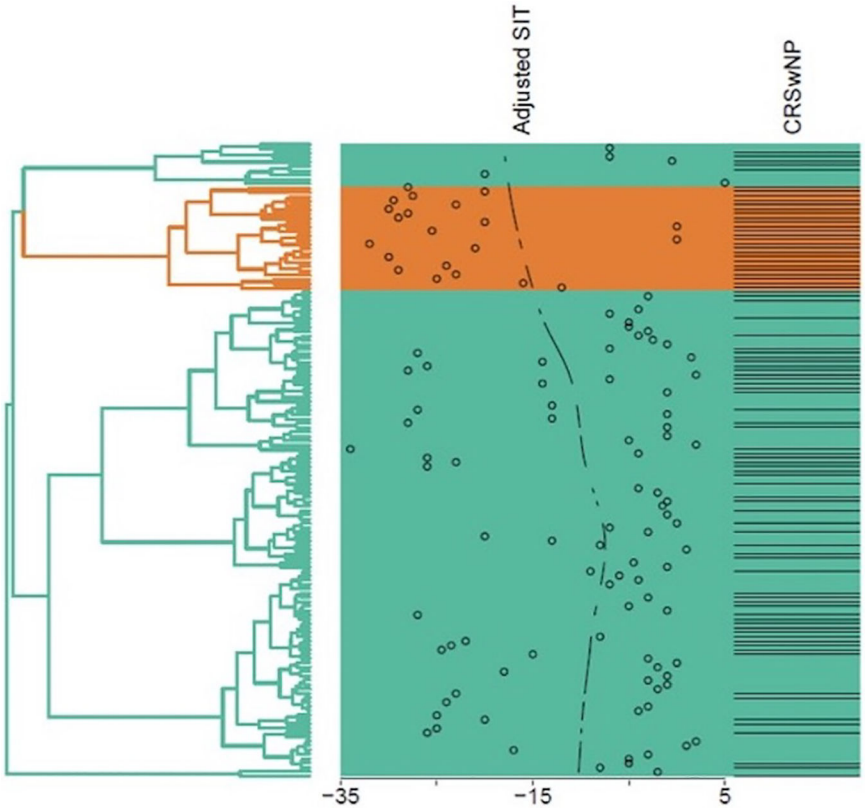

Figure 1: Dendrogram representing hierarchical cluster analysis of CRS patients and relationship with olfactory function.

Hierarchical cluster analysis was performed using Ward's method on squared Euclidian distances using 17 cytokines and inflammatory mediators as biological variables. SIT score is recorded in the right panel for individual study subjects and as a continuous mean. Cluster 4, with the lowest SIT scores, is highlighted.

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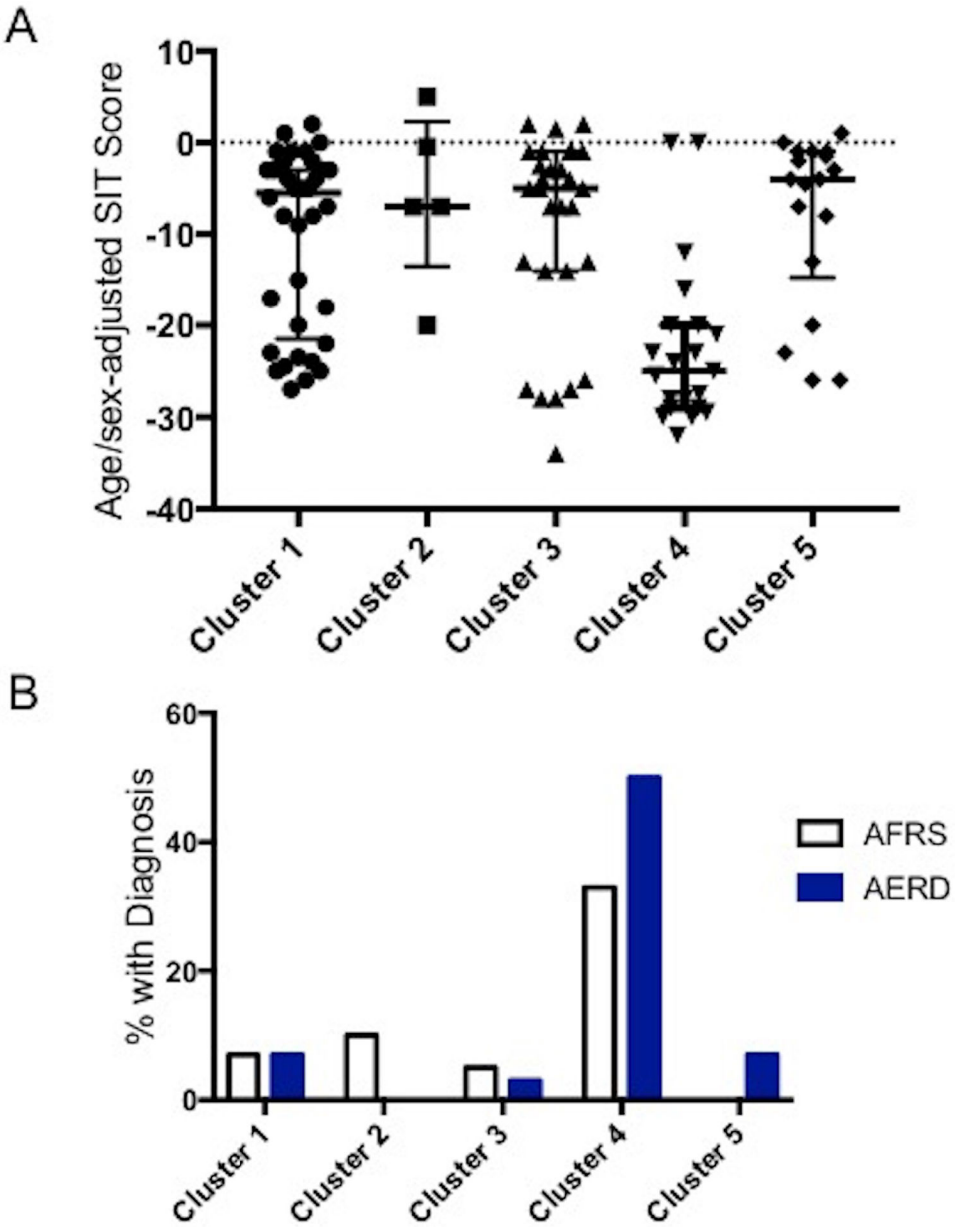

Figure 2: Smell Identification Scores Among Inflammatory CRS Clusters.

(A) SIT scores for individual patients in each cluster are presented as a scatter plot. Bars represent the median and interquartile range. Cluster 4 demonstrates significantly worse SIT scores compared to the other clusters($p<0.001$). (B) Cluster 4 was associated with significantly higher prevalence of both AERD and AFRS compared to other clusters ($p<0.001$).

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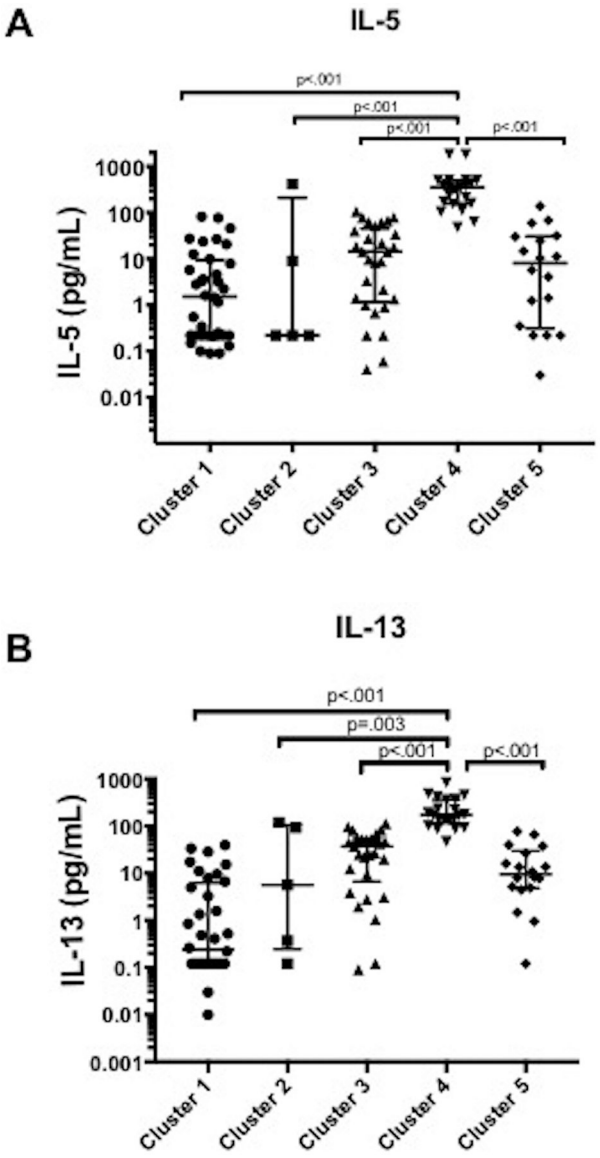

Figure 3: Cluster 4 is associated with elevated Th2 cytokines and anosmia.

Mucus cytokine levels for individual patients in each cluster are presented as a scatter plot. Bars represent the median and interquartile range. Cluster 4 demonstrates significantly elevated IL-5 (A) (p<.001) and IL-13 (B) (p<.001) compared to the other clusters.

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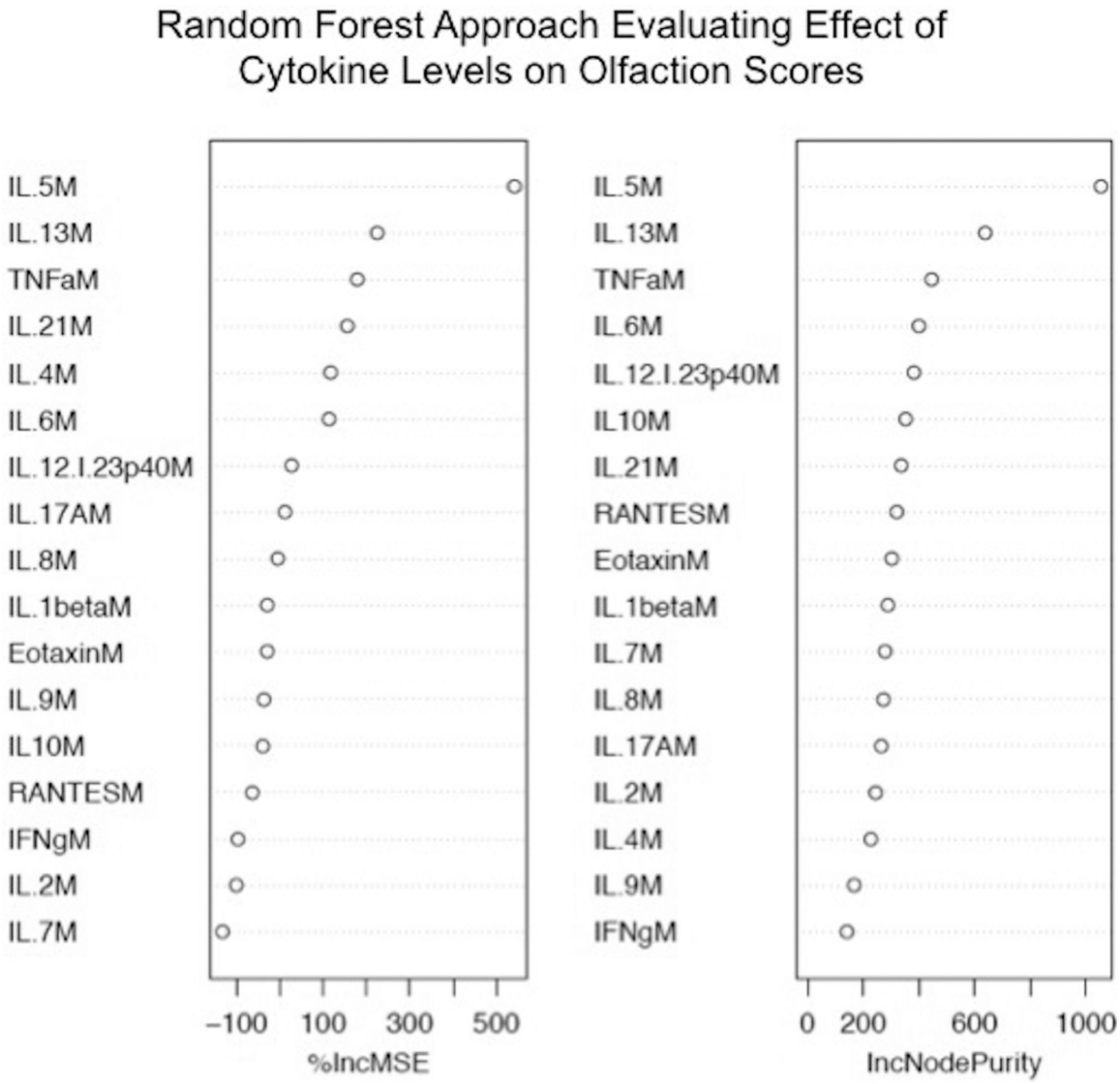

Figure 4: Cytokines Predictive of Olfactory Function Using an Ensemble Learning Method.
Each cytokine or inflammatory mediator is ranked based on their relative impact on decision tree construction. Results are presented as the % increase in mean square error (%IncMSE) and the increase in node purity (IncNodePurity), both representative of the impacts of each variable on the overall decision model.

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Table 1

Study Population and Demographics.

Values are presented as either the mean ± standard deviation, or median with interquartile range. **BOLD, p < 0.05** CRS = chronic rhinosinusitis; CRSsNP = chronic rhinosinusitis without nasal polyps; CRSwNP = chronic rhinosinusitis with nasal polyps; yr = year; SNOT- 22 = sino-nasal outcomes test; CT = computed tomography; SIT score = smell identification test score; tissue eos/HPF = tissue eosinophils per high power field; AERD = aspirin exacerbated respiratory disease; AFRS = allergic fungal rhinosinusitis

	All CRS	CRSsNP	CRSwNP	P Value
No. (%)	110	49 (45)	61(55)	*
Age, yr	48.15±13.37	49.39±13.86	47.15±12.98	0.472
Sex, no. male (%)	59 (54)	24 (49)	35 (57)	0.443
Asthma, no. (%)	46 (42)	12 (24)	34 (56)	**0.007**
Allergic rhinitis, no. (%)	74 (67)	27 (55)	47 (77)	**0.024**
SNOT-22 score	44.0 (29.0–58.0)	48.0 (33.1–57.5)	43.0 (28.0–61.0)	0.678
CT score	15.0 (11.0–20.0)	12.0 (8.5–14.5)	18.0 (14.3–22.0)	**<0.001**
SIT score	−7.0 (−2.0 – −23.1)	−3.0 (−1.0 – −7.0)	−20.0 (−5.0 – −26.5)	**<0.001**
Prior surgery, no. (%)	41 (37)	12 (24)	29 (48)	**0.017**
Tissue eos/HPF	25.7 (1.7–124)	2.0 (0.0–25.0)	80.6 (17.0–226.5)	**<0.001**
AERD	11 (10)	0 (0)	11 (20)	**<0.001**
AFRS	14 (13)	0 (0)	14 (25)	**<0.001**

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Table 2: Characteristics of CRS Clusters.

Values are presented as either the mean ± standard deviation, or median with interquartile range. yr = year; BMI = Body Mass Index; kg = kilogram; m^2= $meter^2$; AERD = aspirin exacerbated respiratory disease; AFRS = allergic fungal rhinosinusitis; SNOT- 22 = sino-nasal outcomes test; CT = computed tomography; SIT score = smell identification test score; tissue eos/HPF = tissue eosinophils per high power field **BOLD, p < 0.05**

	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	p-value
No.	46	10	37	24	27	
Age (yr)	47.5 (41.3–57.5)	66.0 (56.8–70.1)	46.0 (39.0–58.0)	46.0 (38.5–53.0)	53.0 (39.0–60.5)	**0.040**
Sex, no. (% female)	23 (50)	5 (50)	22 (59)	8 (33)	6 (22)	**0.030**
Race, no. (% white)	39 (85)	9 (90)	33 (89)	19 (79)	24 (89)	0.970
Current smoker, no. (%)	5 (11)	0 (0)	1 (3)	0 (0)	0 (0)	0.090
BMI (kg/m2)	28.7 (24.0–34.6)	28.3 (24.6–31.7)	30.6 (26.2–33.4)	29.0 (26.5–32.3)	29.3 (26.7–34.6)	0.910
Nasal polyps, no. (%)	22 (48)	5 (50)	20 (54)	24 (100)	15 (56)	**<0.001**
Asthma, no. (%)	14 (30)	4 (40)	16 (43)	19 (79)	24 (89)	**<0.001**
Allergic Rhinitis, no. (%)	30 (65)	5 (50)	21 (57)	17 (71)	20 (74)	0.490
AERD, no. (%)	3 (7)	1 (10)	2 (5)	8 (33)	0 (0)	**<0.001**
AFRS, no. (%)	3 (7)	0 (10)	1 (3)	12 (50)	2 (7)	**<0.001**
SNOT-22 score	45.6 +/− 16.2	45.8 +/− 29.0	41.9 +/− 16.4	46.1 +/− 22.1	50.2 +/− 23.1	0.750
CT score	14.5 (11.1–17.8)	14.0 (11.0–18.5)	13.0 (11.0–16.0)	22.0 (20.0–23.0)	14.0 (11.0–16.0)	**<0.001**
SIT score	−5.5 (−20.5–−3.0)	−7.0 (−7.0–−0.5)	−5.0 (−14.0–−1.0)	−25.0 (−29.0–−20.0)	−4.0 (−11.8–−1.1)	**<0.001**
Prior surgery, no. (%)	15 (33)	5 (50)	14 (38)	14 (58)	7 (26)	0.130
Tissue eos/HPF	27.5 (2.2–100.0)	10.0 (5.5–35.5)	30.0 (3.0–75.0)	91.5 (50.0–100.0)	18.0 (0.5–62.5)	**0.001**

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Table 3

Olfactory Assessment of All CRS Patients (Age-Sex Adjusted SIT)-Univariate Model.

CRS = chronic rhinosinusitis; CRSsNP = chronic rhinosinusitis without nasal polyps; CRSwNP = chronic rhinosinusitis with nasal polyps; CT = computed tomography; SIT score = smell identification test score; tissue eos/HPF = tissue eosinophils per high power field; NCS = nasal corticosteroid medication use; anti-leukotriene = anti-leukotriene nasal medication use; AERD = aspirin exacerbated respiratory disease; AFRS = allergic fungal rhinosinusitis; IL = interleukin; RANTES = regulated on activation, normal T cell expressed and secreted; TNF = tumor necrosis factor **BOLD, P< .05**

	Univariate Regression		
Variables	**Unadjusted β**	**95% CI**	**p value**
Age	−0.023	−0.180 to 0.135	0.770
Sex	0.422	−3.762 to 4.606	0.842
Asthma	−5.095	−9.213 to −0.978	**0.016**
Allergic Rhinitis	0.909	−3.535 to 5.353	0.686
AERD	−15.162	−21.488 to −8.835	**<0.001**
Current Smoker	3.943	−6.048 to 13.933	0.436
NCS	4.504	−0.555 to 9.564	0.080
Anti-Leukotriene	−4.020	−8.694 to 0.654	0.091
Prior Surgery	−5.470	−9.658 to −1.283	**0.011**
Eos/HPF (mean)	−0.028	−0.045 to −0.010	**0.002**
Neu/HPF (mean)	0.033	−0.094 to 0.160	0.609
Culture(+) Purulence	−1.589	−6.174 to 2.995	0.493
CT Score	−1.132	−1.477 to −0.787	**<0.001**
Polyp Status/Phenotype	−10.669	−14.342 to −6.997	**<0.001**
AFRS	−7.761	−13.660 to −1.863	**0.010**
IL-1β	0.001	−0.000 to 0.001	0.183
IL-2	−0.012	−0.024 to −0.001	**0.037**
IL-4	−0.433	−1.212 to 0.345	0.272
IL-5	−0.013	−0.020 to −0.005	**0.001**
IL-6	0.000	−0.001 to 0.000	0.887
IL-7	−0.003	−0.095 to 0.089	0.950
IL-8	0.000	−5.170e-06 to 0.000	0.245
IL-9	−0.031	−0.104 to 0.041	0.396
IL10	0.002	−0.016 to 0.019	0.858
IL-12/I-23p40	0.003	−0.005 to 0.011	0.464
IL-13	−0.036	−0.051 to −0.020	**<0.001**
IL-17α	−0.209	−0.562 to 0.145	0.244
IL-21	0.002	−0.008 to 0.012	0.701
TNFα	0.005	−0.014 to 0.024	0.627
IFNγ	0.031	−0.038 to 0.101	0.374
Eotaxin	−0.030	−0.062 to 0.002	0.068
RANTES	0.000	−0.000 to 0.001	0.400

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Table 4

Objective Olfactory Assessment of All CRS Patients (Age-Sex Adjusted SIT)-Multivariate Model.

CRS = chronic rhinosinusitis; CRSsNP = chronic rhinosinusitis without nasal polyps; CRSwNP = chronic rhinosinusitis with nasal polyps; CT = computed tomography; SIT score = smell identification test score; tissue eos/HPF = tissue eosinophils per high power field; NCS = nasal corticosteroid medication use; anti-leukotriene = anti-leukotriene nasal medication use; AERD = aspirin exacerbated respiratory disease; AFRS = allergic fungal rhinosinusitis; IL = interleukin; RANTES = regulated on activation, normal T cell expressed and secreted; TNF = tumor necrosis factor **BOLD, P< .05**

	Multivariate Regression			*Collinearity Statistics*
Variables	**Adjusted β**	**95% CI**	**p value**	**VIF**
Age	Not Modeled	*	*	*
Sex	Not Modeled	*	*	*
Asthma	0.726	−3.366 to 4.818	0.725	1.47
Allergic Rhinitis	Not Modeled	*	*	*
AERD	−9.102	−16.385 to −1.820	**0.015**	1.79
Current Smoker	Not Modeled	*	*	*
NCS	1.942	−2.522 to 6.336	0.39	1.15
Anti-Leukotriene	1.913	−2.511 to 6.336	0.393	1.31
Prior Surgery	−1.623	−5.464 to 2.218	0.404	1.24
Eos/HPF (mean)	−0.007	−0.024 to 0.010	0.418	1.44
Neu/HPF (mean)	Not Modeled	*	*	*
Culture(+) Purulence	Not Modeled	*	*	*
CT Score	−0.569	−1.019 to −0.118	**0.014**	1.93
Polyp Status/Phenotype	−4.123	−8.844 to 0.598	0.086	1.98
AFRS	−1.959	−8.319 to 4.402	0.542	1.58
IL-1β	Not Modeled	*	*	*
IL-2	−0.014	−0.024 to −0.004	**0.005**	1.11
IL-4	Not Modeled	*	*	*
IL-5	−0.004	−0.017 to 0.009	0.547	4.51
IL-6	Not Modeled	*	*	*
IL-7	Not Modeled	*	*	*
IL-8	Not Modeled	*	*	*
IL-9	Not Modeled	*	*	*
IL10	Not Modeled	*	*	*
IL-12/I-23p40	Not Modeled	*	*	*
IL-13	0.004	−0.028 to 0.036	0.802	5.62
IL-17α	Not Modeled	*	*	*
IL-21	Not Modeled	*	*	*
TNFα	Not Modeled	*	*	*
IFNγ	Not Modeled	*	*	*
Eotaxin	−0.010	−0.040 to 0.021	0.525	1.38

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	Multivariate Regression			***Collinearity Statistics***
Variables	**Adjusted β**	**95% CI**	**p value**	**VIF**
RANTES	Not Modeled	*	*	*