

Mucosal Eosinophilia and Neutrophilia Are Not Associated With QOL or Olfactory Function in Chronic Rhinosinusitis

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Abstract

Background: Chronic rhinosinusitis (CRS) is often differentiated by histopathologic phenotypes (eosinophilic versus neutrophilic), which may impact disease severity measures and outcomes. As such, it has been suggested that counts of cellular elements be included as part of a standard pathological report following endoscopic sinus surgery (ESS).

Objectives: This cross-sectional study evaluated associations of mucosal eosinophilia and neutrophilia with measures of quality-of-life (QoL) and olfactory function.

Methods: Patients with medically refractory CRS completed the SNOT-22 survey and Brief Smell Identification Test (BSIT) at enrollment. In addition, baseline Lund-Mackay computed tomography (CT) and Lund-Kennedy endoscopy scores were collected. Ethmoid mucosa was biopsied during ESS and reviewed using microscopy to quantify densest infiltrate of eosinophils or neutrophils per high-powered-field (HPF). Eosinophilic CRS (eCRS) and neutrophilic CRS (nCRS), both with and without nasal polyposis (NP), were compared across SNOT-22 and BSIT scores.

Results: 77/168 patients demonstrated mucosal eosinophilia (eCRS) while a total of 42/168 patients demonstrated mucosal neutrophilia (nCRS). After adjusting for polyp status, 35/168 had eCRSsNP, 42/168 eCRSsNP, 75/168 non-eCRSsNP, 16/168 non-eCRSsNP. Additionally, 22/161 were noted to have nCRSsNP, 20/161 nCRSsNP, 84/161 non-nCRSsNP, and 35/161 non-nCRSsNP. A small subset of patients demonstrated both eosinophilia and neutrophilia: 14 CRSsNP and 7 CRSsNP. When evaluating average Lund-Mackay Scores (LMS), significant differences existed between non-eCRSsNP and eCRSsNP ($p = 0.006$). However, after controlling for nasal polyps, eosinophilia did not significantly associate with differences in the Lund-Kennedy Score. Neutrophilia did not significantly associate with any changes in LMS or LKS after controlling for NP. Eosinophilic and neutrophilic histopathologic subtypes did not significantly associate with differences in baseline SNOT-22 or BSIT measures after controlling for NP.

Conclusion: Neither the presence of mucosal eosinophilia nor mucosal neutrophilia demonstrated significant associations with SNOT-22 quality-of-life or BSIT olfactory function scores when controlling for comorbid nasal polyposis.

Keywords

sinusitis, chronic disease, outcome assessment (health care), quality of life, histology, inflammation, neutrophils, eosinophils

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Introduction

Chronic rhinosinusitis (CRS) encompasses a heterogeneous condition.¹ Initial attempts were made to subcategorize patients by phenotype, categorized by the presence or absence of nasal polyps (NP). Later attempts utilized endotype, based on type-1 or type-2 CD4+ T-helper lymphocyte inflammation (Th-1 or Th-2), which broadly subcategorized patients as those with Th-1 inflammation and tissue neutrophilia and those with Th-2 inflammation and tissue eosinophilia.¹ Most recently, cluster analysis of immune markers have further identified 10 clusters of CRS. Though these classifications have transitioned towards analysis of immune markers, there remains interest in whether mucosal histology influences clinical features and treatment outcomes of CRS.^{2,3} Some have even proposed that eosinophilia could serve as a guide for patient management.⁴⁻⁷

Previous studies have evaluated associations of tissue eosinophil density with disease severity, olfactory function, and patient-reported quality-of-life (QoL).⁸⁻¹⁰ Evaluations of neutrophilia have demonstrated some variations in disease severity, but the association between mucosal neutrophilia and clinical features of CRS has not been thoroughly explored.¹¹⁻¹³ The purpose of this study was to evaluate associations between sinonasal eosinophilia and neutrophilia with QoL and olfactory function. Our hypothesis was that higher density of mucosal eosinophilia and neutrophilia would adversely impact these clinical cofactors with eosinophilia having greater impact than neutrophilia.

Materials and Methods

Sample Population

Adult patients diagnosed with CRS per the American Academy of Otolaryngology criteria, who were recalcitrant to medical management, were prospectively enrolled as part of a clinical trial evaluating CRS outcomes.¹⁴ The Institutional Review Board (IRB) at Oregon Health & Science University approved this investigation (eIRB#7198). Adult patients voluntarily elected endoscopic sinus surgery (ESS) prior to providing informed consent for study participation in accordance with the Declaration of Helsinki.

Exclusion Criteria

Participants with diagnoses of ciliary dyskinesia, cystic fibrosis, allergic fungal sinusitis, odontogenic sinusitis, or antrochoanal polyposis were excluded due to potential disparity in symptom severity, QoL or variability in treatment approach. Patients receiving non-corticosteroid immunosuppressive therapies were

excluded to avoid confounding effects on the inflammatory milieu. At the time of study enrollment, it was standard practice for the primary surgeon to prescribe a preoperative course of corticosteroids. To address this potential confounding affect, the relationship between corticosteroid use and histopathologic eosinophil or neutrophil counts were also described.

Clinical Measures of Disease Severity

Sinonasal endoscopy and computed tomography (CT) were performed preoperatively and evaluated using Lund-Kennedy endoscopy scores (LKS) and Lund-Mackay CT scores (LMS). Higher total LKS scores (range: 0–20) or total LMS (range 0–24) reflect worse overall disease severity.^{15,16}

Endoscopic Sinus Surgery and Histopathology

The enrolling physician determined optimal surgical approach, performed under general anesthesia, using diagnostic findings from CT and endoscopic examinations. Ethmoid sinus mucosa was examined after surgery. Specimens were immersed in 10% neutral buffered formalin (NBF), fixed for at least 4 hours (hrs.), placed into plastic mesh cassettes in toto and again immersed in NBF for another 3–12hrs. Following a 120 second cold water rinse, the tissue cassette was immersed in an acid decalcification solution (Decal Stat, Tallman, New York) for 8hrs. and again rinsed with cold water for 120 seconds. Cassettes were then treated in a 12hr. cycle in an automated processor (Sakura VIP, Sakura Finetek USA, Torrance, California) prior to paraffin embedding (Sakura Tissue-Tec; Sakura Finetek). Histological sections of 4 µm were prepared.

Microscopy of each hematoxylin-eosin stained slide was conducted by a board-certified surgical pathologist (D.S.) using a binocular microscope (Leica DM2000; Leica Microsystems Inc., Bannockburn, Illinois) with a graduated reticle (×10, 10.0 mm. with 1.0 mm. divisions) at ×400 power magnification. Eosinophils and neutrophils were quantified on a continuous scale from the foci of densest cellular infiltrate.

Four phenotypic categorizations of CRS, eosinophilic chronic rhinosinusitis (eCRS) and neutrophilic chronic rhinosinusitis (nCRS), both with nasal polyposis (wNP) and without nasal polyposis (sNP), were considered the primary exposure variables of interest. Eosinophilic CRS was classified if tissue eosinophils were greater than > 10/HPF, and.^{8-10,17,18} Subjects without evidence of eosinophilia were designated non-eCRS. Neutrophilic CRS was classified if neutrophilic counts were > 4/HPF per previous studies of neutrophilic disease using specimens from non-inflammatory, healthy controls.^{19,20} Subjects without evidence of neutrophilia were

designated non-nCRS. The presence of eosinophilia and neutrophilia were considered separate, independent predictive factors while recognizing the potential to coalescence between both histopathologic markers.

Patient Outcome Measures

Study participants completed the 22-item SinoNasal Outcome Test (SNOT-22; score range: 0–110), to measure sinonasal symptom severity (2006, Washington University, St. Louis, Missouri).²¹ The SNOT-22 has identified 5 symptom sub-domains: rhinologic symptoms (score range: 0–30), extra-nasal rhinologic (score range: 0–15), ear/facial symptoms (score range: 0–25), psychological dysfunction (score range: 0–35), and sleep dysfunction (score range: 0–25).²² Higher scores reflect worse symptom severity.

Study participants also completed the Brief Smell Identification Test (BSIT, Sensonics International, Haddon Heights, NJ), a 12-item screener of olfactory function. Higher scores (score range: 0–12) reflect superior olfactory identification, while total scores ≥ 9 are interpreted as normal olfactory function.²³

Database Management and Statistical Analysis

Data collection was finalized using a secure, closed-environment database (Access; Microsoft Corporation, Redmond, Washington). Retrospective analyses of prospectively collected data was completed using SPSS statistical software (version 26.0, IBM Corporation, Armonk, New York) following basic prevalence study design. Scaled/continuous study data was evaluated both graphically and through a Shapiro-Wilk test for assumptions of normality and distribution. Two-tailed Spearman's rank correlation coefficients (R) were used to identify associations between scaled measures. Analysis of variance (ANOVA), with corresponding F-test statistics, was used to detect observed mean differences in clinical measure and patient outcomes between histopathologic subgroups. Globally significant ANOVAs ($p < 0.050$) were further evaluated across multiple, pairwise comparisons using Bonferroni corrections. Chi-square (χ^2) testing aided in comparing olfactory dysfunction prevalence between phenotypes while Mann-Whitney U testing compared mean counts/HPF of eosinophilia and neutrophilia in patients with and without preoperative corticosteroid use. Type-I error probabilities (p-values) are reported for all comparisons.

Results

Study Population

A total of 174 participants met inclusion criteria between April 2011 and December 2014. The final study

population consisted of 168 subjects for eosinophil evaluation and 161 subjects for neutrophil evaluation after exclusion due to cystic fibrosis ($n = 4$), allergic fungal sinusitis ($n = 1$), immunomodulator therapy ($n = 1$), poor neutrophil slide/stain quality ($n = 7$). Demographic and health characteristics of the final cohort are described in Table 1. Preoperative oral corticosteroids (≥ 5 -day course) were prescribed for 117/168 (70%) patients, while 36 (21%) patients did not receive preoperative steroid therapy due to patient comorbidities or corticosteroid intolerance. Chart review could not confirm corticosteroid therapy for the remaining 15 (9%) subjects.

Histopathologic Findings

Mucosal eosinophilia was noted in 77/168 (46%) and mucosal neutrophilia in 42/161 (26%) of participants. Average eosinophil counts were 71.5 (SD ± 128.9) (range: 0–610); average neutrophil counts were 6.8 (SD ± 21.2 ; range: 0–170). There were 35/168 (21%) subjects with eCRSsNP, 42/168 (25%) with eCRSwNP, 75/168 without eosinophilia or NP (non-eCRSsNP; 45%) and 16/168 without eosinophilia but with NP (non-eCRSwNP; 10%). There were 22/161 (14%) subjects with nCRSsNP, 20/161 (12%) with nCRSwNP, 84/161 (52%) without neutrophilia or NP (non-nCRSsNP), and 35/161 (22%) without neutrophilia but with NP (non-nCRSwNP). Twenty-one subjects presented with both eosinophilia and neutrophilia, 14/161 (8%) with NP and 7/161 (4%) without NP.

Clinical Measures of Disease Between Histopathologic Categories

Measures of disease severity were compared between any two CRS phenotypes with or without eosinophilia (Table 2). After adjustment to family-wise error rates for multiple pairwise comparisons of mean LKS, no significant differences existed between non-eCRSsNP and eCRSsNP ($p > 0.999$) or non-eCRSwNP and eCRSwNP ($p = 0.738$). When evaluating pairwise comparisons for average LMS, significant differences existed between non-eCRSsNP and eCRSsNP ($p = 0.006$). However, no significant difference in observed mean LMS was found between non-eCRSwNP and eCRSwNP ($p = 0.574$).

Measures of disease severity were also compared between any two independent CRS phenotypes with or without neutrophilia (Table 3). After adjustment for pairwise comparisons of average LKS, no significant differences existed in adjusted mean LKS between non-nCRSsNP and nCRSsNP ($p > 0.999$) or between non-nCRSwNP and nCRSwNP ($p > 0.999$). Similarly, no significant differences for LMS were noted between

Table 1. Preoperative Demographic Factors, Health Characteristics, and Outcome Measures of Final Study Cohort (n=168).

Characteristics	Mean $\pm$ SD	Range [LL, UL]	N (%)
Age (years)	50.7 $\pm$ 14.4	[20, 82]	—
Male	—	—	80 (48%)
Female	—	—	88 (52%)
White /Caucasian (race)	—	—	161 (96%)
African American (race)	—	—	2 (1%)
Asian (race)	—	—	3 (2%)
Hispanic/Latino (ethnicity)	—	—	5 (3%)
Nasal polyposis	—	—	58 (35%)
Septal deviation	—	—	94 (56%)
Turbinate hypertrophy	—	—	47 (28%)
Mucocele	—	—	3 (2%)
Asthma	—	—	50 (30%)
Aspirin sensitivity	—	—	10 (6%)
Allergic rhinitis	—	—	64 (38%)
Depression	—	—	33 (20%)
Tobacco use/smoker	—	—	10 (6%)
Alcohol use	—	—	87 (52%)
Corticosteroid dependency	—	—	3 (2%)
Diabetes mellitus (type I/II)	—	—	7 (4%)
GERD	—	—	4 (2%)
Lund–Kennedy staging score	6.7 $\pm$ 3.5	[0, 16]	—
Lund–Mackay staging score	12.5 $\pm$ 6.4	[0, 24]	—
Patient outcome measures			
SNOT-22 total score	56.3 $\pm$ 19.2	[0, 102]	—
Rhinologic domain	16.5 $\pm$ 6.4	[0, 28]	—
Extra-nasal rhinologic domain	8.2 $\pm$ 3.4	[0, 15]	—
Ear/facial domain	10.2 $\pm$ 5.3	[0, 25]	—
Psychological dysfunction domain	17.3 $\pm$ 7.7	[0, 35]	—
Sleep dysfunction domain	15.3 $\pm$ 6.2	[0, 25]	—
BSIT total score (n=156)	9.1 $\pm$ 3.0	[1, 12]	—
Olfactory dysfunction (BSIT $\leq$ 8)	—	—	45 (29%)

SD, standard deviation; LL, lower limit; UL, upper limit; N, sample size; GERD, gastroesophageal reflux disease; CT, computed tomography imaging; SNOT-22, 22-item SinoNasal Outcome Test survey; BSIT, Brief Smell Identification Test.

Table 2. Comparison of Average Disease Severity Measures Between Histopathologic Phenotypes With and Without Eosinophilia.

Clinical Measures of Disease Severity:	Non-eCRSsNP	eCRSsNP	Non-eCRSwNP	eCRSwNP	F-Test Statistic	p-Value
	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD		
Lund-Kennedy staging score	4.6 $\pm$ 2.4	5.3 $\pm$ 2.21	9.3 $\pm$ 2.5	10.4 $\pm$ 2.8	60.48	<0.001
	4.6 $\pm$ 2.4	5.3 $\pm$ 2.21	—	—		>0.999
	4.6 $\pm$ 2.4	—	9.3 $\pm$ 2.5	—		<0.001
	4.6 $\pm$ 2.4	—	—	10.4 $\pm$ 2.8		<0.001
	—	5.3 $\pm$ 2.21	5.3 $\pm$ 2.21	—		<0.001
	—	5.3 $\pm$ 2.21	—	10.4 $\pm$ 2.8		<0.001
	—	—	5.3 $\pm$ 2.21	10.4 $\pm$ 2.8		0.738
	—	—	—	—		—
Lund-Mackay staging score	8.8 $\pm$ 5.4	12.2 $\pm$ 4.1	15.6 $\pm$ 6.1	18.1 $\pm$ 4.7	32.90	<0.001
	8.8 $\pm$ 5.4	12.2 $\pm$ 4.1	—	—		0.006
	8.8 $\pm$ 5.4	—	15.6 $\pm$ 6.1	—		<0.001
	8.8 $\pm$ 5.4	—	—	18.1 $\pm$ 4.7		<0.001
	—	12.2 $\pm$ 4.1	15.6 $\pm$ 6.1	—		0.166
	—	12.2 $\pm$ 4.1	—	18.1 $\pm$ 4.7		<0.001
	—	—	15.6 $\pm$ 6.1	18.1 $\pm$ 4.7		0.574
	—	—	—	—		—

SD, standard deviation; CT, computed tomography; non-eCRSsNP, chronic rhinosinusitis without eosinophilia or nasal polyposis; non-eCRSwNP, chronic rhinosinusitis with nasal polyposis without eosinophilia; eCRSsNP, eosinophilic chronic rhinosinusitis without nasal polyposis; eCRSwNP, eosinophilic chronic rhinosinusitis with nasal polyposis. F-test statistics are associated with omnibus findings across all pairwise comparisons using analysis of variance.

Table 3. Comparison of Average Disease Severity Measures Between Histopathologic Phenotypes With and Without Neutrophilia.

Clinical Measures of Disease Severity:	Non-nCRSsNP	nCRSsNP	Non-nCRSwNP	nCRSwNP	F-Test Statistic	p-Value
	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD		
Lund-Kennedy staging score	4.8 $\pm$ 2.4	5.1 $\pm$ 2.0	9.9 $\pm$ 2.7	10.7 $\pm$ 2.7	56.34	<0.001
	4.8 $\pm$ 2.4	5.1 $\pm$ 2.0	–	–		>0.999
	4.8 $\pm$ 2.4	–	9.9 $\pm$ 2.7	–		<0.001
	4.8 $\pm$ 2.4	–	–	10.7 $\pm$ 2.7		<0.001
	–	5.1 $\pm$ 2.0	9.9 $\pm$ 2.7	–		<0.001
	–	5.1 $\pm$ 2.0	–	10.7 $\pm$ 2.7		<0.001
	–	–	9.9 $\pm$ 2.7	10.7 $\pm$ 2.7		>0.999
	–	–	–	–		<0.001
Lund-Mackay staging score	9.5 $\pm$ 5.2	11.0 $\pm$ 5.1	16.3 $\pm$ 5.2	19.0 $\pm$ 4.9	26.96	<0.001
	9.5 $\pm$ 5.2	11.0 $\pm$ 5.1	–	–		>0.999
	9.5 $\pm$ 5.2	–	11.0 $\pm$ 5.1	–		<0.001
	9.5 $\pm$ 5.2	–	–	19.0 $\pm$ 4.9		<0.001
	–	11.0 $\pm$ 5.1	11.0 $\pm$ 5.1	–		<0.001
	–	11.0 $\pm$ 5.1	–	19.0 $\pm$ 4.9		<0.001
	–	–	11.0 $\pm$ 5.1	19.0 $\pm$ 4.9		0.393
	–	–	–	–		

SD, standard deviation; CT, computed tomography; non-nCRSsNP, chronic rhinosinusitis without neutrophilia or nasal polyposis; non-nCRSwNP, chronic rhinosinusitis with nasal polyposis without neutrophilia; nCRSsNP, neutrophilia chronic rhinosinusitis without nasal polyposis; nCRSwNP, neutrophilic chronic rhinosinusitis with nasal polyposis. F-test statistics are associated with omnibus findings across all pairwise comparisons using analysis of variance.

Table 4. Comparison of Average Patient Outcome Measures Between Histopathologic Phenotypes With and Without Eosinophilia.

Patient Outcome Measures:	Non-eCRSsNP	eCRSsNP	Non-eCRSwNP	eCRSwNP	F-Test Statistic	p-Value
	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD		
SNOT-22 total score	56.7 $\pm$ 18.2	52.9 $\pm$ 20.5	58.9 $\pm$ 23.3	57.3 $\pm$ 18.4	0.51	0.675
Rhinologic domain	15.3 $\pm$ 6.2	14.5 $\pm$ 6.3	18.9 $\pm$ 5.9	19.5 $\pm$ 5.8	6.43	< 0.001
Extra-nasal rhinologic domain	8.5 $\pm$ 3.3	7.4 $\pm$ 3.8	8.7 $\pm$ 3.3	8.0 $\pm$ 3.2	0.93	0.426
Ear/facial domain	10.9 $\pm$ 5.1	10.1 $\pm$ 5.2	9.8 $\pm$ 6.7	9.3 $\pm$ 5.5	0.81	0.493
Psychological dysfunction domain	17.9 $\pm$ 7.7	16.9 $\pm$ 7.7	17.5 $\pm$ 9.3	16.6 $\pm$ 7.1	0.26	0.852
Sleep dysfunction domain	15.7 $\pm$ 6.1	14.4 $\pm$ 6.4	15.3 $\pm$ 7.5	15.5 $\pm$ 6.0	0.32	0.808
BSIT total score (n = 156)	10.2 $\pm$ 2.1	9.6 $\pm$ 2.0	7.7 $\pm$ 3.6	7.0 $\pm$ 3.7	12.85	< 0.001

SD, standard deviation; SNOT-22, 22-item SinoNasal Outcome Test survey; BSIT, Brief Smell Identification Test; F-test statistics are associated with omnibus findings across all pairwise comparisons using analysis of variance. Non-eCRSsNP, chronic rhinosinusitis without eosinophilia or nasal polyposis; non-eCRSwNP, chronic rhinosinusitis with nasal polyposis without eosinophilia; eCRSsNP, eosinophilic chronic rhinosinusitis without nasal polyposis; eCRSwNP, eosinophilic chronic rhinosinusitis with nasal polyposis.

After adjusting for multiple comparisons, significant differences in mean SNOT-22 rhinologic scores were noted between non-eCRSsNP and eCRSwNP ($p = 0.003$) as well as between eCRSsNP and eCRSwNP ($p = 0.003$). Average BSIT scores were significantly better for non-eCRSsNP when compared to non-eCRSwNP ($p = 0.008$) and eCRSwNP ($p < 0.001$) and for eCRSsNP compared to eCRSwNP ($p = 0.001$).

non-nCRSsNP and nCRSsNP ($p > 0.999$) or between non-nCRSwNP and nCRSwNP ($p = 0.393$).

Disease Measures Between Histopathologic Categories

Average measures were compared using omnibus testing to identify potential differences between any two CRS phenotypes with and without eosinophilia (Table 4) or neutrophilia (Table 5). After adjustment for multiple comparisons, no significant difference for any patient outcome measure was found between non-eCRSsNP and eCRSsNP ($p \geq 0.794$) or non-eCRSwNP and

eCRSwNP ($p > 0.999$). Similarly, no significant difference for any patient outcome measure was found between non-nCRSsNP and nCRSsNP ($p > 0.999$) or non-nCRSwNP and nCRSwNP ($p > 0.999$).

Comparisons of the prevalence of olfactory dysfunction across phenotypes with and without eosinophilia are described in Figure 1. No significant differences in prevalence were noted between non-eCRSsNP and eCRSsNP ($\chi^2 = 0.20$; $p = 0.659$) or between non-eCRSwNP and eCRSwNP ($\chi^2 = 0.002$; $p = 0.962$) phenotypes. Similarly, comparisons of the prevalence of olfactory dysfunction across phenotypes with and without neutrophilia are displayed in Figure 2. Again, no

Table 5. Comparison of Average Patient Outcome Measures Between Histopathologic Phenotypes With and Without Neutrophilia.

Patient Outcome Measures:	Non-nCRSsNP	nCRSsNP	Non-nCRSwNP	nCRSwNP	F-Test Statistic	p-Value
	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD		
SNOT-22 total score	57.1 $\pm$ 19.2	50.6 $\pm$ 18.6	58.1 $\pm$ 19.8	56.6 $\pm$ 21.2	0.78	0.507
Rhinologic domain	15.2 $\pm$ 6.1	14.8 $\pm$ 6.8	19.8 $\pm$ 6.3	18.6 $\pm$ 5.2	6.02	0.001
Extra-nasal rhinologic domain	8.4 $\pm$ 3.3	7.5 $\pm$ 3.7	8.1 $\pm$ 3.2	8.2 $\pm$ 3.3	0.43	0.732
Ear/facial domain	10.9 $\pm$ 5.0	9.5 $\pm$ 5.4	9.5 $\pm$ 5.3	9.1 $\pm$ 6.5	1.17	0.323
Psychological dysfunction domain	18.6 $\pm$ 7.9	14.8 $\pm$ 6.6	17.4 $\pm$ 7.4	15.8 $\pm$ 8.8	1.79	0.151
Sleep dysfunction domain	15.3 $\pm$ 6.3	14.9 $\pm$ 6.3	15.1 $\pm$ 6.7	15.6 $\pm$ 6.0	0.04	0.990
BSIT total score (n = 156)	10.0 $\pm$ 1.9	10.1 $\pm$ 2.6	6.7 $\pm$ 3.6	7.7 $\pm$ 3.8	13.6	< 0.001

SD, standard deviation; SNOT-22, 22-item SinoNasal Outcome Test survey; BSIT, Brief Smell Identification Test; F-test statistics are associated with omnibus findings across all pairwise comparisons using analysis of variance. Non-nCRSsNP, chronic rhinosinusitis without neutrophilia or nasal polyposis; non-nCRSwNP, chronic rhinosinusitis with nasal polyposis without neutrophilia; nCRSsNP, neutrophilia chronic rhinosinusitis without nasal polyposis; nCRSwNP, neutrophilic chronic rhinosinusitis with nasal polyposis.

After adjustment, significant differences in mean SNOT-22 rhinologic domain scores were reported between non-nCRSsNP and non-nCRSwNP ($p = 0.001$) as well as between non-nCRSwNP and nCRSsNP ($p = 0.020$). Average BSIT scores were significantly better for non-nCRSsNP when compared to non-nCRSwNP ($p < 0.001$) and nCRSwNP ($p < 0.001$). Patients with non-nCRSwNP had significantly worse BSIT scores compared to nCRSsNP ($p < 0.001$) and patients with nCRSsNP had significantly better mean BSIT scores compared to patients with nCRSwNP ($p = 0.033$).

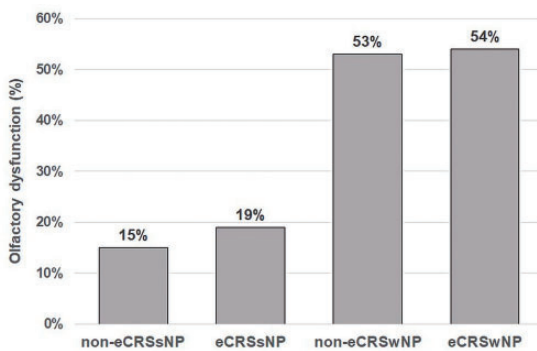


Figure 1. Comparison of olfactory dysfunction prevalence between histopathologic phenotypes with and without eosinophilia. Non-eCRSsNP, chronic rhinosinusitis without eosinophilia or nasal polyposis; non-eCRSwNP, chronic rhinosinusitis with nasal polyposis without eosinophilia; eCRSsNP, eosinophilic chronic rhinosinusitis without nasal polyposis; eCRSwNP, eosinophilic chronic rhinosinusitis with nasal polyposis.

significant differences were noted between non-nCRSsNP and nCRSsNP ($\chi^2 = 0.10$; $p = 0.752$) or non-nCRSwNP and nCRSwNP ($\chi^2 = 1.31$; $p = 0.253$).

Associations With Histopathology and Perioperative Steroid Use

Histopathology of patients receiving preoperative corticosteroids ($n = 117$) were compared against patient who did not ($n = 36$). The average eosinophils/HPF or neutrophils/HPF were not significantly different between corticosteroid use groups (78.3 ± 133.1 vs. 52.6 ± 114.0 eosinophils/HPF; $\Delta = 25.7$; 95% CI: -22.8 to 74.3 ; $p = 0.245$) (6.7 ± 20.1 vs. 3.4 ± 4.6 neutrophils/HPF; $\Delta = 3.3$; 95% CI: -3.5 to 10.1 ; $p = 0.339$). Preoperative

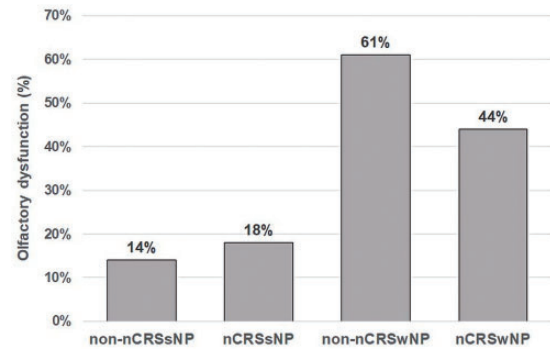


Figure 2. Comparison of olfactory dysfunction prevalence between histopathologic phenotypes with and without neutrophilia. Non-nCRSsNP, chronic rhinosinusitis without neutrophilia or nasal polyposis; non-nCRSwNP, chronic rhinosinusitis with nasal polyposis without neutrophilia; nCRSsNP, neutrophilia chronic rhinosinusitis without nasal polyposis; nCRSwNP, neutrophilic chronic rhinosinusitis with nasal polyposis.

SNOT-22 survey responses averaged slightly worse for patients completing preoperative steroid therapy, however were only statistically significant for the sleep dysfunction domain ($p = 0.049$; Figure 3). No significant differences in the prevalence of subjects receiving preoperative corticosteroid were found based on eosinophilic phenotypes including: non-eCRSsNP (71%) compared to eCRSsNP (68%; $\chi^2 = 0.09$; $p = 0.761$) and non-eCRSwNP (92%) compared to eCRSwNP (90%; $\chi^2 = 0.09$; $p = 0.767$). Similar frequency of preoperative steroids were given to non-nCRSsNP (69%) compared to nCRSsNP (70%; $\chi^2 = 0.01$; $p = 0.947$) and non-nCRSwNP (88%) compared to nCRSwNP (94%; $\chi^2 = 0.45$; $p = 0.504$).

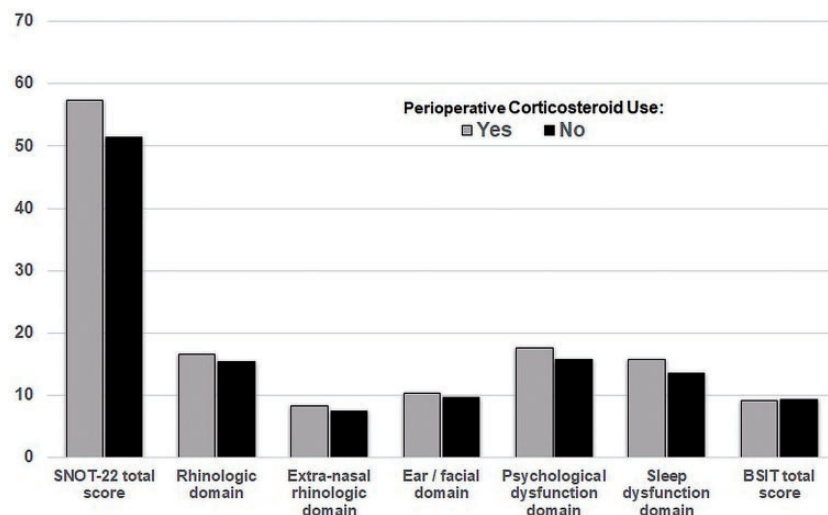


Figure 3. Comparisons of preoperative patient outcome measures between patients with and without perioperative steroid use. SNOT-22, 22-item SinoNasal Outcome Test; BSIT, Brief Smell Identification Test.

Discussion

This investigation examined relationships between both mucosal eosinophilia and neutrophilia with disease severity, QoL, and olfaction. Patients with eCRSsNP demonstrated significantly worse LMS than non-eCRSsNP. Otherwise, we could not demonstrate that mucosal eosinophilia or neutrophilia associated with worse disease severity, olfaction, or QoL in this population. This suggests that mucosal eosinophilia or neutrophilia may not be ideal for subcategorization of patients with CRS.

Chronic rhinosinusitis has traditionally been classified by clinical phenotype focused on NP or inflammatory endotype.^{10,17,24} Most recently, immunologic cluster analysis has demonstrated distinct clustering around IL-5, eosinophilic cationic protein, albumin, and IgE. Within those clusters, neutrophilic clusters generally comprise CRSsNP while eosinophilic clusters included both CRSwNP and CRSsNP.²

Despite a shift to cytokine-based endotyping, mucosal histology may play a role in disease management, since histologic findings are readily available, whereas most immunologic data remains relegated to the research domain.^{5,19} Our cohort demonstrated eosinophilia in 46% of patients, comprised of 21% eCRSsNP and 25% eCRSwNP. Neutrophilia was found in 26% of our cohort, affecting 12% of patients nCRSwNP and 13% of CRSsNP, which was higher than in prior studies performed by our group.⁸ Though both the present study and prior study participants originated from the same geographic areas, the two populations represent independent groups identified during different enrollment periods.

Prior studies demonstrated that tissue eosinophilia correlates with worse LMS and LKS in CRS patients.^{18,25–27} Our study did demonstrate a similar

relationship, as well as weak correlation between neutrophil density and both worse LKS and LMS. However, after controlling for NP, the only remaining significant correlation was eCRSsNP demonstrating worse LMS than those with CRSsNP, which is consistent with some prior studies.^{8,28} Conversely, in a cohort of 99 patients, Kuhar et al. noted that eosinophilia correlated with increasing LMS scores in patients with CRSwNP, but not in patients with CRSsNP.²⁵ Overall, the presence of NP alone is likely the major factor driving worse measures of disease severity.

Chowdhury et al. noted that in patients with CRS, tissue eosinophilia correlated with improved extra-nasal subdomain scores of the SNOT-22. However, after pairwise corrections, significant correlations were excluded.²⁹ Other studies failed to demonstrate a relationship between tissue eosinophilia and SNOT-22 scores.^{18,19,25,26} The results of our study are consistent with these prior reports. Though past investigations have postulated that neutrophilia may be present in CRS, relationships between mucosal neutrophilia and patient presentation have not been thoroughly explored. Our investigation could not demonstrate any significant associations between neutrophil density/HPF and symptom severity. These findings contradict Succar et al., which showed that neutrophilia correlated with significantly worse SNOT-22 measures.¹⁹ Our inability to demonstrate any meaningful correlations between tissue inflammation and symptom severity may indicate that inflammatory profile, as measured by eosinophilia or neutrophilia, is insufficient to predict overall symptom severity.

Our study failed to demonstrate significant associations between tissue eosinophil or neutrophil density

with olfaction after controlling for NP. These results differ from prior studies by Soler et al. which noted worse olfaction in patients with eCRSwNP.⁹ The difference is likely due to methodologic variation in identifying olfactory dysfunction. Soler et al. utilized the Smell Identification Test (SIT), whereas this study utilized the abbreviated form BSIT. While the BSIT has been shown to strongly correlate with the SIT, it may underestimate the true prevalence of olfactory dysfunction in patients with CRS.³⁰ The lack of significant associations between histopathologic measures and olfactory dysfunction may indicate that these cells are not associated with the causal pathway for sensorineural olfactory dysfunction in CRS.

There are several strengths of this study. First, definitions of neutrophilia are highly variable across the literature and a limitation to external validity. Since neutrophilia is a defining characteristic for CRS subtyping, scientific consensus should guide standard definitions of neutrophilia. We defined nCRS using criteria provided by Succar et al. which examined the tissue of CRS patients and determined an optimal cut-point of 4 cells/HPF.¹⁹ This should create a basis for further evaluation and comparison in future research. Another strength of this study is a large sample size, with patients nearly evenly distributed across phenotypes of CRS. Despite these strengths, there are important caveats. First, this study sought to identify the independent associations of eosinophilia or neutrophilia, with or without NP, however ~13% of our patients were identified as having both eosinophilia and neutrophilia. This exploratory data should serve as the basis for future investigation designed to evaluate the effect of simultaneous eosinophilia and neutrophilia on CRS independent of NP. Secondly, comparisons of various stratified subgroups, particularly those participants with non-eCRSwNP (n = 16), may not be adequately powered to detect clinically important between-group differences, however due to the exploratory nature of this investigation, these findings may still be advantageous with future investigation.

Additionally, within this patient cohort ~70% received preoperative oral corticosteroids prior to tissue specimen collection as standard practice by the enrolling physician. Preoperative corticosteroid use may influence the histopathologic leukocytic infiltrate, resulting in with the suppression of eosinophil or neutrophil density at the time of tissue biopsy. While this may influence the presence and/or density of inflammatory cells in sinonasal mucosa, preoperative steroids were not variably prescribed based on disease severity or identification of NP. Moreover, no significant difference in eosinophil or neutrophil counts/HPF were noted between patients that did or did not receive preoperative steroid therapy. Prior studies have also demonstrated that oral corticosteroids do not impact tissue

histopathology.²⁵ Lastly, ethmoid mucosa was utilized for mucosal specimens rather than olfactory cleft mucosa. While sinonasal inflammation is present in CRS patients, it is possible that ethmoid mucosa may have differing inflammatory profiles relative to olfactory mucosa, confounding these results or increasing the potential for misclassification bias of phenotypes in this population.³¹

Conclusion

Neither mucosal eosinophilia nor neutrophilia were found to demonstrate significant associations with measures of disease severity, QoL or olfactory function independent of NP status. The role of mucosal eosinophil and neutrophil density in the clinical decision making for patients with CRS requires further study.

Declaration of Conflicting Interests

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