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Neutrophils are Underrecognized Contributors to Inflammatory Burden and Quality of Life in Chronic Rhinosinusitis

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55 ABBREVIATIONS

56 AERD: aspirin-exacerbated respiratory disease

57 AFRS: allergic fungal rhinosinusitis

58 AR: allergic rhinitis

59 BMI: body mass index
60 CRS: chronic rhinosinusitis
61 CRSsNP: chronic rhinosinusitis without polyps
62 CRSwNP: chronic rhinosinusitis with nasal polyps
63 CT: computed tomography
64 LTR, anti-leukotriene
65 NCS, nasal corticosteroid
66 SNOT-22: 22 item sinonasal outcome test
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77 To the Editor:

78 Substantial research and clinical interest has focused on the role of eosinophilic inflammation in
79 chronic rhinosinusitis (CRS) pathophysiology, largely due to its well-documented impacts on disease severity
80 and treatment outcomes¹⁻³. Conversely, neutrophilic inflammation has received comparably less attention, with
81 a majority of studies excluding tissue neutrophilia as a biological variable entirely.⁴ This dichotomy is
82 counterintuitive given the predominant role of neutrophilic inflammation in other airway diseases. In particular,
83 neutrophilic asthma phenotypes have been associated with greater disease severity and are often refractory to
84 many standard medical therapies.^{5,6} Given the shared pathophysiology between asthma and CRS, we sought to

characterize neutrophilic phenotypes of CRS and objectively assess the impact of neutrophilic inflammation on CRS disease severity and inflammatory burden.

To establish standards to define eosinophilic and neutrophilic CRS, cell counts were measured in sinonasal tissue biopsies from 30 patients undergoing sinus or skull base surgery for non-inflammatory pathology, all of whom had no clinical or radiographic history of CRS. Mean eosinophil and neutrophil counts were 1.2 cells/HPF and 1.0 cells/HPF, respectively, with eosinophilic and neutrophilic disease subsequently defined by adding two standard deviations to each mean value. This approach resulted in cut points of approximately 5 eosinophils/HPF and 4 neutrophils/HPF. Of note, the cut point for eosinophilic CRS defined using this method is similar to estimates published elsewhere using various approaches^{1,2}. Using a cohort of 164 prospectively enrolled CRS patients (Table S1), we next sought to determine whether granulocytic tissue infiltration is associated with baseline disease-specific quality of life scores. A range of cut points were used to determine whether 'high' or 'low' cell counts have an impact on baseline disease-specific quality of life, assessed using the validated 22-item sinonasal outcome test (SNOT-22) questionnaire. Eosinophil counts (range 5-200 cells/HPF) had no association with baseline SNOT-22 scores (Figure 1A, Table S2). Conversely, neutrophil counts (range 1-20 cells/HPF) had a significantly impacted on baseline SNOT-22 scores, with an optimal cut point of 4 neutrophils/HPF (mean SNOT-22 difference = 10.4 +/- 4.0, p=0.01). Of note, the 10.4 point difference in SNOT-22 scores between neutrophil 'high' and neutrophil 'low' subjects is greater than the minimal clinically important difference of 8.9 points that has previously been established for the SNOT-22 instrument⁷. Likewise, the symptom-based cut point of 4 neutrophils/HPF matched that defined above using a histologic approach. Collectively, these results suggest that disease-specific quality of life in CRS patients is preferentially impacted by neutrophilic rather than eosinophilic inflammation.

To more objectively evaluate CRS-associated granulocytic inflammation, we incorporated an asthma-based classification system⁸, with patients assigned to eosinophilic, neutrophilic, paucigranulocytic, or mixed granulocytic phenotypes based on our objective and pre-defined criteria (Figure 1A). Using cut points defined above, almost half of the study population (45%) met criteria for eosinophilic CRS (Table 1). The remaining patients were assigned to paucigranulocytic (20%), neutrophilic (14%), and mixed granulocytic (21%) groups. The groups varied significantly by polyp status (p<0.0001), with the eosinophilic (75%) and mixed granulocytic groups (74%) predictably having the highest proportion of patients with nasal polyps (Figure S1). Most other demographic and clinical factors were not significantly different between groups, including age, sex, race, BMI, smoking status, asthma, and medication usage. Baseline disease-specific quality of life assessed using the SNOT-22 questionnaire varied significantly between groups (p=0.03) (Table 1), with similar trends

116 noted for several SNOT-22 subdomain scores. Patients with mixed granulocytic CRS had the highest SNOT-22
117 scores, indicative of worse disease-specific quality of life and higher symptom burden. In order to exclude the
118 possible confounding effect of nasal polyps we analyzed SNOT-22 scores in CRSwNP separately, and again
119 found significant differences between groups ($p=0.03$) (Table S3). The groups also varied with respect to
120 baseline CT score ($p<0.0001$) and olfactory function ($p=0.04$), with the mixed granulocytic phenotype again
121 having the worst scores (Table 1).

122 Finally, we evaluated differences in inflammatory burden between granulocytic phenotypes using both
123 histologic and immunologic criteria. The severity of tissue inflammation varied significantly between groups
124 ($p=0.002$) and was greatest in the mixed granulocytic phenotype (Table 1). Immunologic signatures were
125 assessed in all patients by measuring mucus levels of 17 different cytokines (Table S4). The groups varied with
126 respect to levels of IL-1 β ($p=0.0004$), IL-5 ($p<0.0001$), IL-6 ($p=0.0004$), IL-8 ($p=0.0004$), and IL-13
127 ($p<0.0001$). Compared to a healthy control population, neutrophilic CRS was characterized by elevated levels
128 of IL-1 β ($p=0.008$) and IL-8 ($p=0.0009$), while eosinophilic CRS was characterized by elevated levels of IL-5
129 ($p<0.0001$) and IL-13 ($p=0.004$) (Figure 1B). The mixed granulocytic phenotype generally had higher cytokine
130 levels than eosinophilic CRS, suggesting greater overall inflammatory burden in these patients. These two
131 groups could be distinguished primarily by very high levels of IL-6 in the mixed granulocytic phenotype
132 compared to the eosinophilic phenotype (449.7 pg/mL vs. 132.1 pg/mL, $p=0.02$). Similar cytokine patterns
133 were observed when patients were evaluated according to polyp status (Figures S2 and S3).

134 Overall, our findings suggest that neutrophils are an important, yet underrecognized driver of
135 inflammation, disease severity, and quality of life in CRS. The lack of correlation between tissue eosinophilia
136 and SNOT-22 scores in our study is supported by previous research^{1,9}, and suggests that the presumption of
137 greater disease severity in eosinophilic CRS may instead be due to a higher frequency of nasal polyps which
138 frequently drive CT and endoscopy scores. Patients with eosinophilic vs. neutrophilic CRS have similar overall
139 inflammatory burden with a dominance of Th2-associated and pro-inflammatory/innate immunity-associated
140 cytokines, respectively. Our data suggests that the mixed granulocytic phenotype is the most severe form of
141 CRS and that both eosinophilic and neutrophilic tissue infiltration are significant drivers of inflammatory
142 burden in these patients. Further investigation into mechanisms of neutrophil recruitment and changes to innate
143 immune function is needed in order to develop personalized treatments targeting neutrophilic inflammation in
144 CRS. In addition, respective impacts of neutrophilic and eosinophilic inflammation on treatment outcomes
145 needs to be further explored. Based on our findings, we would advocate for more objective categorization of

146 CRS granulocytic phenotypes for future clinical and translational research as many patients may ultimately
147 require therapeutics that target both neutrophilic and eosinophilic pathways.

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218 TABLE LEGEND

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220 **Table 1. Differences in demographic, clinical and histologic features of CRS phenotypes.** Post-hoc
221 analysis of characteristics were examined for each of the four phenotypes (paucigranulocytic, neutrophilic,
222 eosinophilic, mixed granulocytic). Values are presented as either the mean +/- standard deviation or median
223 with interquartile range, depending on the normalcy of the data. Differences between groups were assessed
224 using the Kruskal-Wallis test or Chi-square analysis. Boldface indicates p-value < 0.05. AERD, aspirin-
225 exacerbated respiratory disease; AFRS, allergic fungal rhinosinusitis; BMI, body mass index; CT, computed
226 tomography; NCS, nasal corticosteroid; LTR, anti-leukotriene; SIT, smell identification test; SNOT-22,
227 sinonasal outcome test-22.

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229 **FIGURE LEGEND**

230 **Figure 1. Inflammatory burden in patients with CRS based on granulocyte-based phenotyping. (A)**

231 Identification of granulocyte cut points and mean differences in SNOT-22 scores (left panel). Assignment of

232 patients to granulocyte-based CRS phenotypes and corresponding granulocyte counts in CRS tissue (right

233 panel). (B) CRS phenotypes have varying levels of inflammatory burden as assessed by mucus cytokine levels.

234 Error bars represent medians with interquartile range for each cytokine.

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Table 1.

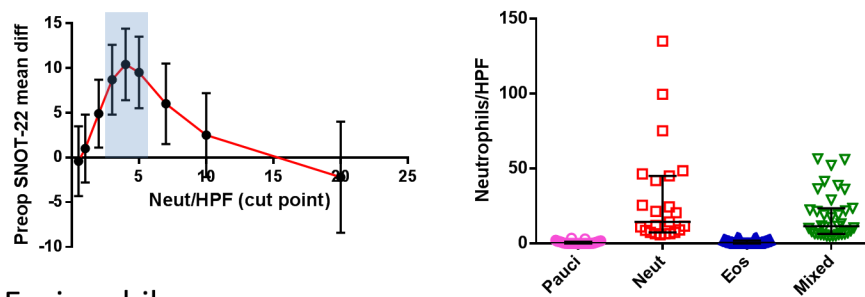
	Paucigranulocytic	Neutrophilic	Eosinophilic	Mixed Granulocytic	p value
No.	33	23	73	35	
Age (years)	50.3 +/- 14.0	54.1 +/- 16.6	48.1 +/- 13.5	47.5 +/- 13.6	0.26
Sex, no. (% female)	17 (52)	7 (30)	33 (45)	15 (43)	0.47
Race, no. (% white)	29 (88)	20 (87)	60 (82)	30 (86)	0.87
Current smoker, no. (%)	2 (6)	2 (9)	5 (7)	1 (3)	0.80
BMI (kg/m2)	30.5 +/- 11.2	30.4 +/- 6.7	30.5 +/- 6.0	28.6 +/- 6.4	0.68
Nasal polyps, no. (%)	6 (15)	11 (48)	55 (75)	26 (74)	<0.0001
Asthma, no. (%)	9 (27)	9 (39)	26 (36)	18 (51)	0.21
Allergic Rhinitis, no. (%)	19 (58)	12 (52)	45 (62)	26 (74)	0.20
AERD, no. (%)	0 (0)	1 (4)	10 (14)	5 (14)	0.09
AFRS, no. (%)	1 (3)	1 (4)	12 (16)	7 (20)	0.08
NCS, no. (%)	25 (86)	19 (83)	59 (81)	28 (80)	0.92
LTR, no. (%)	4 (12)	5 (22)	21 (29)	11 (31)	0.22
SNOT-22 score	43.0 +/- 19.5	47.0 +/- 20.3	41.2 +/- 18.5	56.2 +/- 21.7	0.03
Rhinologic	11.8 +/- 5.0	12.4 +/- 5.8	11.8 +/- 5.2	14.7 +/- 4.7	0.18
Extranasal	7.9 +/- 3.6	8.7 +/- 2.9	7.0 +/- 3.6	8.0 +/- 3.3	0.36
Ear/Facial	8.2 +/- 4.8	8.0 +/- 5.1	7.4 +/- 5.2	11.0 +/- 5.3	0.06

Psychological	11.5 +/- 8.8	12.7 +/- 6.9	11.2 +/- 7.9	17.1 +/- 9.0	0.05
Sleep	10.4 +/- 7.0	12.4 +/- 6.4	10.4 +/- 6.7	14.1 +/- 7.0	0.17
CT score	11.0 (8.0-14.0)	15.0 (7.5-19.0)	16.5 (13.0-20.5)	17.0 (14.0-21.0)	<0.0001
SIT score	-3.0 (-7.0--1.0)	-9.0 (-19.5--1.5)	-13.0 (-26.0--3.0)	-14.0 (-25.5--4.0)	0.04
Mucopurulence, no. (%)	11 (33)	14 (61)	12 (16)	9 (26)	0.0005
Prior surgery, no. (%)	8 (24)	8 (35)	29 (40)	17 (49)	0.21
Eosinophils/HPF (mean)	0.0 (0.0-1.7)	0.7 (0-1.7)	92.0 (47.0-221.0)	83.0 (37.0-130.0)	<0.0001
Eosinophils/HPF (max)	0 (0-4)	1 (0-4)	134 (83-305)	134 (58-208)	<0.0001
Eosinophilic aggregates, no. (%)	0 (0)	0 (0)	32 (44)	9 (26)	<0.0001
Neutrophils/HPF (mean)	0.3 (0.0-1.2)	14.5 (7.4-45.0)	0.4 (0.0-1.9)	11.6 (6.4-23.7)	<0.0001
Neutrophils/HPF (max)	1 (0-3)	31 (15-78)	1 (0-4)	23 (11-46)	<0.0001
Inflammation score, no. (%)					0.002
0	1 (3)	0 (0)	0 (0)	0 (0)	
1	30 (91)	12 (52)	44(60)	11 (31)	
2	2 (6)	9 (39)	23 (32)	14 (40)	
3	0 (0)	2 (9)	7 (14)	8 (23)	
Subepithelial edema, no. (%)					0.0003
0	5 (15)	0 (0)	3 (4)	1 (3)	
1	26 (79)	18 (78)	54 (74)	14 (29)	
2	2 (6)	5 (22)	16 (22)	16 (46)	
3	0 (0)	0 (0)	7 (10)	4 (11)	

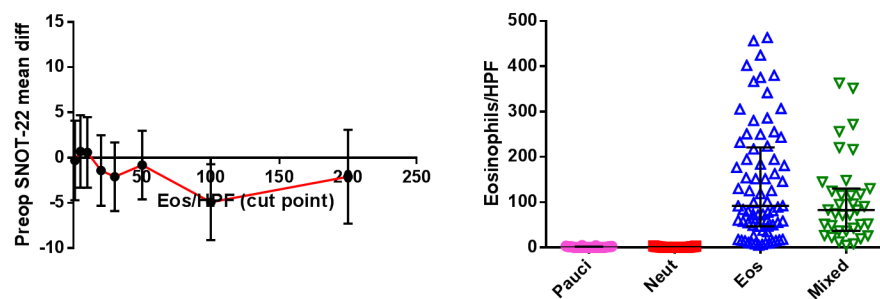
Papillary changes, no. (%)	1 (3)	1 (10)	3 (4)	4 (11)	0.38
Squamous metaplasia, no. (%)	4 (12)	3 (13)	10 (14)	3 (9)	0.90
Fibrosis, no. (%)	4 (12)	10 (44)	17 (23)	14 (40)	0.02
Charcot-Leyden crystals, no. (%)	2 (6)	2 (9)	15 (21)	10 (29)	0.06

A

Neutrophils

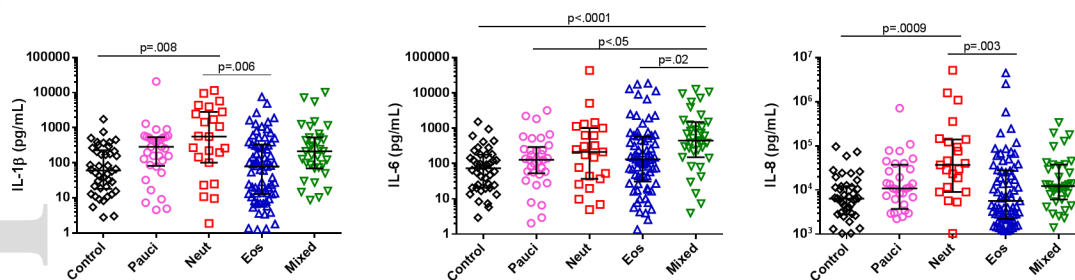


Eosinophils

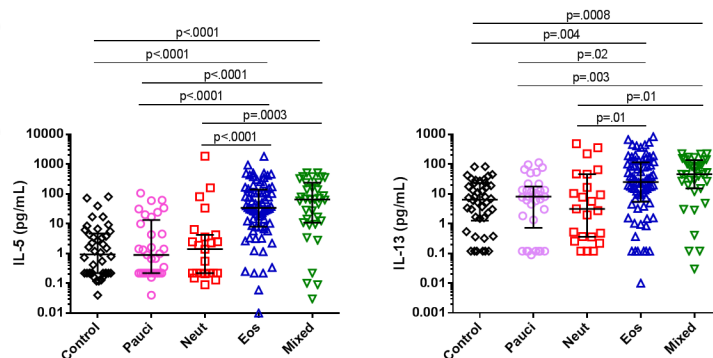


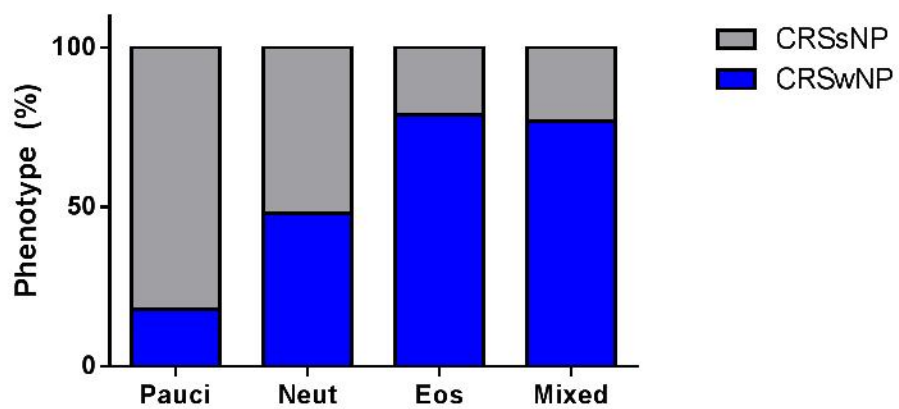
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Pro-inflammatory/Innate Immunity Cytokines

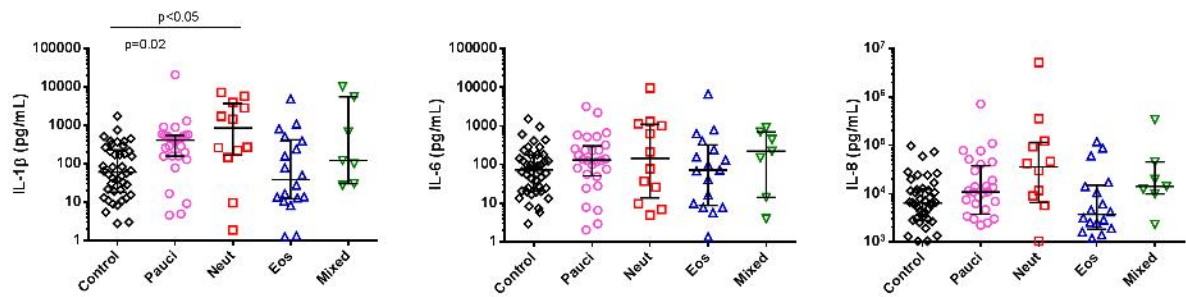


T-helper 2 Cytokines

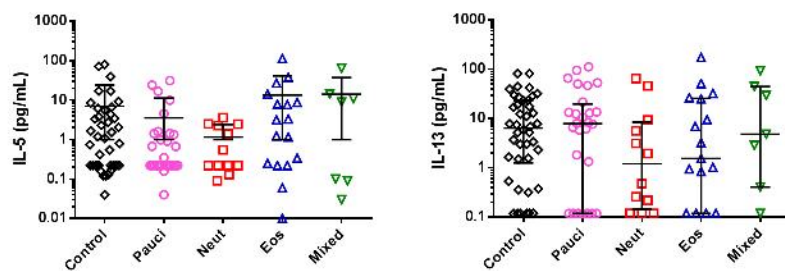




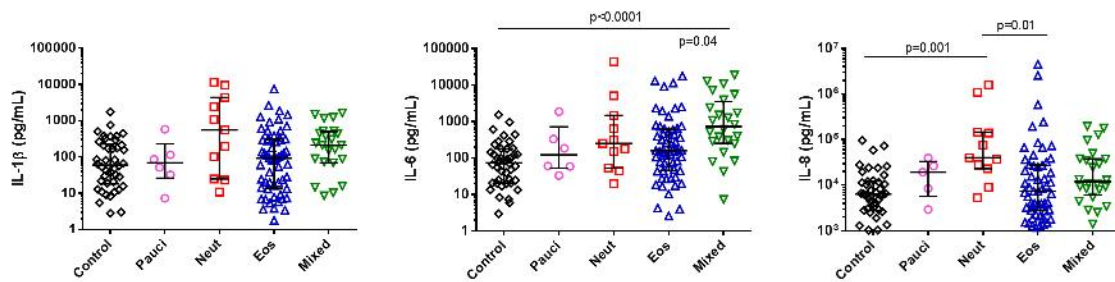
Pro-inflammatory/Innate Immunity Cytokines



T-helper 2 Cytokines



Pro-inflammatory/Innate Immunity Cytokines



T-helper 2 Cytokines

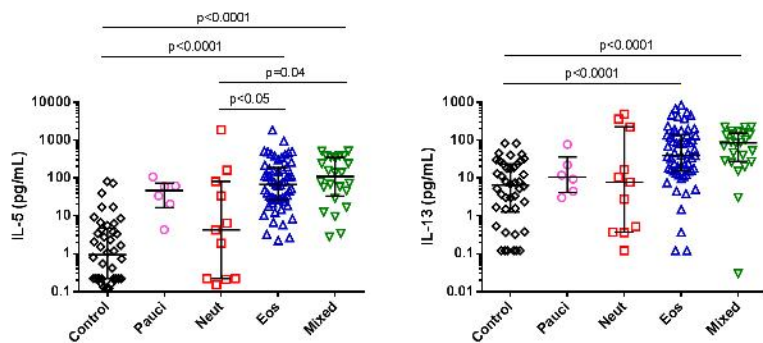


Table S1. Study Population and demographic characteristics for healthy controls and patients with CRS. Data are presented as frequencies (percentages), means +/- SD, or medians with interquartile range. Boldface indicates p-value < 0.05. AERD, Aspirin-exacerbated respiratory disease; AFRS, allergic fungal rhinosinusitis; BMI, body mass index; CT, computed tomography; NCS, nasal corticosteroid; SNOT-22, sinonasal outcome test; LTR, antileukotriene; SIT, Smell Identification Test.

	Healthy Control	CRS	p value
No.	42	164	
Age (years)	49.6 +/- 14.2	50.9 +/- 15.7	0.59
Sex, no. (% female)	30 (71)	72 (44)	0.002
Race, no. (% white)	31 (74)	139 (85)	0.11
Current smoker, no. (%)	1 (2)	9 (6)	0.69
BMI (kg/m2)	30.1 +/- 7.5	30.8 +/- 8.4	0.61
Nasal polyps, no. (%)	0 (0)	98 (60)	<0.0001
Asthma, no. (%)	1 (2)	62 (38)	<0.0001
Allergic Rhinitis, no. (%)	4 (10)	102 (63)	<0.0001
AERD, no. (%)	0 (0)	16 (10)	0.05
AFRS, no. (%)	0 (0)	21 (13)	0.009
NCS, no. (%)	1 (2)	132 (81)	<0.0001
LTR, no. (%)	2 (5)	41 (25)	0.003
SNOT-22 score	-	45.1 +/- 19.9	-
CT score	-	15.0 (11.0-20.0)	-
SIT score	-	-7.0 (-23.1--2.0)	-
Prior surgery, no. (%)	0 (0)	62 (38)	<0.0001

Table S2. Identification of ideal neutrophil and eosinophil cut points using baseline SNOT-22 scores. HPF, high powered field; SNOT-22, sinonasal outcome test. Boldface indicates p-value < 0.05.

Cut point	Baseline SNOT-22 (below cut point)	Baseline SNOT-22 (above cut point)	Difference (mean +/- SEM)	p value
Neutrophils/HPF				
≤0.5	45.5 +/- 2.7	45.0 +/- 2.6	-0.4 +/- 3.9	0.91
≤1	44.7 +/- 2.4	45.7 +/- 3.0	1.0 +/- 3.8	0.80
≤2	43.0 +/- 2.3	47.9 +/- 3.1	4.9 +/- 3.8	0.20
≤3	42.0 +/- 2.2	50.7 +/- 3.4	8.7 +/- 3.9	0.03
≤4	42.0 +/- 2.1	52.4 +/- 3.6	10.4 +/- 4.0	0.01
≤5	42.3 +/- 2.2	51.7 +/- 3.6	9.5 +/- 4.0	0.02
≤7	43.8 +/- 2.1	49.8 +/- 4.5	6.0 +/- 4.5	0.19
≤10	45.6 +/- 1.8	48.1 +/- 4.8	2.5 +/- 4.7	0.59
≤20	45.5 +/- 2.0	43.2 +/- 7.3	-2.2 +/- 6.2	0.72
Eosinophils/HPF				
≤1	45.2 +/- 3.6	44.9 +/- 2.3	-0.3 +/- 4.4	0.95
≤5	44.5 +/- 3.1	45.2 +/- 2.5	0.7 +/- 4.0	0.85
≤10	44.6 +/- 2.9	45.2 +/- 2.5	0.6 +/- 3.9	0.87
≤20	45.7 +/- 2.6	44.3 +/- 2.7	-1.4 +/- 3.9	0.71
≤30	46.1 +/- 2.6	44.0 +/- 2.8	-2.1 +/- 3.8	0.59
≤50	45.4 +/- 2.6	44.5 +/- 2.8	-0.8 +/- 3.8	0.83
≤100	46.4 +/- 2.2	41.5 +/- 3.6	-4.9 +/- 4.2	0.25
≤200	45.3 +/- 2.1	43.2 +/- 4.8	-2.1 +/- 5.2	0.68

Table S3. Differences in SNOT-22 total and subdomain scores among CRSwNP patients. Post-hoc analysis of SNOT-22 scores were examined for each of the four phenotypes (paucigranulocytic, neutrophilic, eosinophilic, mixed granulocytic). Values are presented as the mean +/- standard deviation. Differences between groups were assessed using a one-way ANOVA. Boldface indicates p-value < 0.05. SNOT-22, sinonasal outcome test-22.

	Paucigranulocytic	Neutrophilic	Eosinophilic	Mixed Granulocytic	p value
No.	6	11	55	26	
SNOT-22 score	29.3 +/- 16.7	50.6 +/- 22.4	41.0 +/- 18.8	55.0 +/- 21.4	0.03
Rhinologic	9.2 +/- 5.9	13.3 +/- 5.3	12.4 +/- 5.4	14.3 +/- 4.7	0.24
Extranasal	5.0 +/- 3.7	9.4 +/- 2.6	6.8 +/- 3.7	7.8 +/- 3.4	0.11
Ear/Facial	7.7 +/- 5.5	8.9 +/- 5.3	7.6 +/- 5.1	10.8 +/- 5.7	0.24
Psychological	5.7 +/- 8.9	13.5 +/- 7.8	10.8 +/- 7.8	16.8 +/- 8.8	0.02
Sleep	5.5 +/- 6.7	13.0 +/- 7.1	10.1 +/- 7.1	14.0 +/- 7.3	0.06

Table S4. Differences in mucus cytokine levels between the four CRS phenotypes and a healthy control population. Median cytokine levels of control subjects and each of the four CRS phenotypes are shown for all 17 assayed biological variables. Significant differences among histologic categories were identified using the Kruskal-Wallis test. Significant differences between individual phenotypes and the control group are marked with an asterisk ($p < 0.05$). Data are represented as medians with interquartile range. Boldface indicates p -value < 0.05 .

	Healthy Control	Paucigranulocytic	Neutrophilic	Eosinophilic	Mixed Granulocytic	p value
IL-1	60.2 (18.2-228.6)	287.3 (82.5-538.5)	557.0 (101.9-3970)*	78.0 (13.0-328.6)	211.7 (68.9-529.8)	0.0004
IL-2	2.9 (2.2-20.3)	2.2 (2.2-13.2)	2.2 (2.2-4.8)	4.8 (2.2-15.0)	9.5 (2.2-25.9)	0.06
IL-4	0.3 (0.3-1.3)	0.3 (0.3-0.5)	0.3 (0.3-2.3)	0.3-0.3-1.9)	0.6 (0.3-2.1)	0.21
IL-5	0.9 (0.2-4.7)	0.9 (0.2-13.4)	1.4 (0.2-4.2)	34.0 (8.0-140.1)*	64.9 (10.9-238.1)*	<0.0001
IL-6	72.9 (20.6-179.0)	127.3 (53.1-293.4)	212.2 (37.0-1014)	132.1 (30.8-578.6)	449.7 (150.2-1500)*	0.0004
IL-7	6.6 (2.5-29.3)	11.6 (3.5-25.2)	6.0 (1.5-24.1)	9.1 (2.5-19.0)	16.1 (4.1-26.5)	0.34
IL-8	6359 (2777-12303)	10814 (3721-37249)	36929 (8993-139714)*	5641 (2212-27854)	12316 (6088-37686)	0.0004
IL-9	0.6 (0.6-2.9)	0.6 (0.6-5.6)	0.6 (0.6-4.7)	2.0 (0.6-9.3)	3.0 (0.6-16.9)	0.08
IL-10	4.1 (0.4-14.5)	4.6 (0.6-12.8)	5.6 (1.9-20.2)	4.3 (1.3-13.8)	13.0 (3.2-31.3)	0.08
IL-12	48.3 (21.5-164.4)	72.3 (39.8-143.5)	32.9 (12.5-102.9)	31.2 (4.3-112.3)	70.7 (10.7-196.3)	0.13
IL-13	6.4 (1.3-23.4)	8.1 (0.7-17.8)	3.2 (0.4-46.1)	24.9 (5.5-115.1)*	46.0 (15.2-136.6)*	<0.0001
IL-17A	0.1 (0.1-1.5)	0.1 (0.1-0.8)	0.7 (0.1-4.6)	0.1 (0.1-2.3)	0.7 (0.1-4.7)	0.08
IL-21	150.9 (7.0-291.5)	158.0 (18.7-244.7)	30.9 (6.9-179.6)	45.3 (7.0-214.3)	155.3 (10.4-288.2)	0.12
TNF-α	4.7 (1.0-10.5)	8.7 (2.3-19.4)	9.9 (3.3-39.2)	5.7 (1.7-13.8)	10.2 (2.2-16.9)	0.09
IFN-γ	0.4 (0.4-2.1)	0.4 (0.4-0.7)	0.4 (0.4-3.0)	0.4 (0.4-2.5)	0.5 (0.4-3.1)	0.22
Eotaxin	18.2 (8.1-54.1)	19.1 (9.0-44.8)	18.3 (6.0-45.8)	14.1 (5.8-44.0)	26.4 (5.5-83.9)	0.68
RANTES	1515 (576-6233)	1062 (349-5547)	583 (172-3247)	824 (250-5770)	601 (145-4172)	0.30

SUPPLEMENTARY MATERIAL FOR ONLINE REPOSITORY

METHODS

Study Design and Population

This study was approved by the Vanderbilt University Institutional Review Board. Patients presenting to the Vanderbilt Asthma, Sinus, and Allergy Program (ASAP) and Otolaryngology clinic at the Vanderbilt Bill Wilkerson Center were enrolled into an ongoing prospective study evaluating inflammatory biomarkers and clinical CRS outcomes. Patients diagnosed with CRS who elected to undergo endoscopic sinus surgery were included in the study, with the study population having been partially characterized elsewhere¹⁻⁴. CRS was diagnosed according to the European Position Paper on Rhinosinusitis and Nasal Polyps and the International Consensus Statement on Allergy and Rhinology^{5,6}. All patients were initially managed medically and only patients with diffuse, bilateral inflammatory CRS were included. 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 who were receiving immune-directed monoclonal antibodies were excluded. The presence of concomitant 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. Patient reported symptom severity was measured utilizing the Sinonasal Outcome Test-22 (SNOT-22)⁷. All patients underwent a high resolution CT scan of the paranasal sinuses within 3 months of surgery and a standard Lund Mackay CT scoring system was used to assess overall extent of CRS. Each scan was evaluated by two physicians in a blinded fashion. Subjects

enrolled in the study also completed the 40-item Smell Identification Test (SIT) immediately prior to surgery. Raw scores were adjusted for patient age and gender by subtracting the mean normative age- and sex-appropriate SIT score from the total SIT score for each subject⁸. Thus a negative adjusted SIT score represents reduced sense of smell compared to the mean for that subject's age and gender. Normative SIT scores were extracted from the Smell Identification Test Administration Manual (Sensonics International; Haddon Heights, NJ).

Measurement of Mucus Cytokine Levels

At the beginning of surgery, 9 x 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 reported⁹. 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. Briefly, 50 µL of mucus was incubated with 50 µL of mixed capture beads for each measured inflammatory mediator and incubated for 1 hour. 50 µL of mixed detection reagent 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 200 x g 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; San Jose, CA). Data was analyzed using BD FCAP Array Software version 3.0.

Histopathologic Evaluation of Sinonasal Tissue

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 sphenoethmoid recess. Histopathological evaluation of hematoxylin and eosin stained tissue was performed by a pathologist in a blinded fashion and using a structured approach^{10,11}. This standardized evaluation included both quantitative measurement of tissue eosinophils and neutrophils, and assessment of observable features associated with tissue architecture, including edema, papillary changes, squamous metaplasia, and fibrosis. Neutrophils were identified by myeloperoxidase antibody staining. Briefly, paraffin sections were deparaffinized in graded ethanol baths followed by antigen retrieval with boiling sodium citrate buffer (pH 6.6) for 10 minutes. Tissue endogenous peroxidases were quenched with 0.3% hydrogen peroxide and slides were then incubated with 3% bovine serum albumin (BSA) in phosphate-buffered saline (PBS) to prevent nonspecific binding. Slides were then incubated with primary polyclonal rabbit anti-human myeloperoxidase antibody (Agilent; Santa Clara, CA) for one hour at room temperature, and then with a biotinylated goat-anti-rabbit secondary antibody (Vector Laboratories; Burlingame, CA) and 3,3'-diaminobenzidine tetrahydrochloride (DAB) substrate (Thermo Scientific, Waltham, MA). Sections were then counterstained with hematoxylin for 10 minutes, mounted, and subsequently analyzed. Cut points to define eosinophilic and neutrophilic CRS were defined based on comparisons to 'normal' sinonasal tissue from a healthy non-CRS control population (30 patients). The number of eosinophils and neutrophils were counted over 5 high power fields to determine mean values. Cut points were then defined by adding two standard deviations to each mean value. Defined cut points were not impacted by comorbid allergic rhinitis (2 patients) or asthma (1 patient) in the control population.

Statistics

Granulocyte-based CRS phenotypes were compared against demographic, histologic, immunologic, 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 followed by Dunn's test for multiple comparisons. Comparative data was presented as means +/- standard deviation or medians with interquartile range, respectively. 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).

FIGURES:

Figure S1. Frequency of nasal polyposis in granulocyte-based CRS phenotypes. The percentage of CRSsNP and CRSwNP patients are shown for each CRS phenotype. CRSsNP, chronic rhinosinusitis without nasal polyps; CRSwNP, chronic rhinosinusitis with nasal polyps.

Figure S2. Inflammatory burden in patients with CRS without nasal polyps using granulocyte-based phenotyping. Mucus cytokine levels among cellular phenotypes in CRS patients without nasal polyps. Error bars represent medians with interquartile range.

Figure S3. Inflammatory burden in patients with CRS with nasal polyps using granulocyte-based phenotyping. Mucus cytokine levels among cellular phenotypes in CRS patients with nasal polyps. Error bars represent medians with interquartile range.

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