Clinical Examination of Tissue Eosinophilia in Patients with Chronic Rhinosinusitis and Nasal Polyposis

Sarah A. Gitomer, MD¹, Cynthia R. Fountain, MD², Todd T. Kingdom, MD³, Anne E. Getz, MD³, Stefan H. Sillau, PhD⁴, Rohit K. Katial, MD⁵, and Vijay R. Ramakrishnan, MD³

No sponsorships or competing interests have been disclosed for this article.

Abstract

Objective. (1) Describe clinical and histopathologic findings in patients with chronic rhinosinusitis with nasal polyps (CRSwNP). (2) Determine if tissue and serum eosinophilia predicts disease severity in CRSwNP.

Study Design. Case series with chart review.

Setting. Academic hospital specializing in respiratory and allergic disease.

Subjects. Patients with CRSwNP treated from 2008 to 2010.

Methods. Clinical data were collected; sinus computed tomography (CT) scans were scored according to the Lund-Mackay system; and surgical specimens were evaluated for degree of tissue eosinophilia. Statistical analysis was performed to compare eosinophilia with indicators of disease severity.

Results. Seventy CRSwNP patients were included, with a mean Lund-Mackay score of 16.7; 62.1% of patients had severe asthma, and 62.9% were aspirin sensitive. Elevated tissue eosinophil level did not correlate with medication usage, olfactory symptoms, or Lund-Mackay scores, nor did it correlate with presence of asthma or aspirin-sensitivity (P = .09). Patients with mild asthma had significantly more tissue eosinophils versus patients with severe asthma, possibly because of the high amount of chronic corticosteroid use in severe asthmatics. There was no correlation between tissue and serum eosinophil counts (P = .97), but there was a significant positive correlation between CT score and peripheral eosinophil level (P < .05).

Conclusions. Higher serum eosinophil levels may indicate more extensive mucosal disease as measured on CT scan. Neither serum nor tissue eosinophilia predicted disease severity in our retrospective analysis of CRSwNP patients, and serum eosinophil level did not serve as a marker of tissue eosinophilia.

Keywords
aspirin exacerbated respiratory disease, chronic sinusitis, asthma, allergy, nasal polyps

Received November 2, 2015; revised January 13, 2016; accepted February 17, 2016.

Sinusitis is one of the most common medical problems in the United States, and it consists of a range of disease phenotypes defined by etiology and chronicity. Sinusitis can be subdivided according to clinical examination into CRS with nasal polyposis (CRSwNP) and that without. The approximate incidence of nasal polyposis in the adult population is 0.627 patients per thousand per year, and as many as 20% of CRS patients have nasal polyposis. Based on histologic analysis, CRSwNP and CRS without nasal polyposis appear to be unique clinical entities. Many general and specific inflammatory alterations have been described in nasal polyps; for instance, tissue from CRSwNP patients has significantly more eosinophilic infiltration than CRS without nasal polyposis tissue. However, there are some CRSwNP patients with predominantly neutrophilic infiltrate, and neutrophilic and eosinophilic inflammation are 2 distinct physiologic phenotypes with divergent clinical courses and therapeutic outcomes. Patients with both nasal polyposis and eosinophilic inflammation have been shown to have more severe rhinosinusitis, as defined by computed tomography (CT) score and nasal

1Department of Otolaryngology–Head and Neck Surgery, Baylor College of Medicine, Houston, Texas, USA
2Department of Obstetrics and Gynecology, Duke University School of Medicine, Durham, North Carolina, USA
3Department of Otolaryngology, University of Colorado School of Medicine, Aurora, Colorado, USA
4University of Colorado School of Medicine, Aurora, Colorado, USA
5National Jewish Health, Division of Allergy and Immunology, Denver, Colorado, USA

Corresponding Author:
Vijay R. Ramakrishnan, MD, Department of Otolaryngology, University of Colorado School of Medicine, 12631 E 17th Ave B-205, Aurora, CO 80045, USA.
Email: vijay.ramakrishnan@ucdenver.edu
endoscopy, than patients with nasal polyps or tissue eosinophilia alone. A subset of patients with nasal polyposis, aspirin sensitivity, and asthma (aspirin-exacerbated respiratory disease) has more severe CRS symptoms and more severe eosinophilic infiltrate than CRS patients who do not have aspirin sensitivity. In patients with more severe forms of disease, such as extensive CRSwNP, there is no accepted predictor of disease severity or response to treatment, although levels of blood and tissue eosinophilia have been suggested as possible simple biomarkers. In examination of all CRS patients, degree of tissue eosinophilia has been correlated with symptom severity, clinical markers of disease, disease outcomes, and relapse after surgery. However, no study has yet examined the CRSwNP population independently to determine if tissue eosinophilia correlates with eosinophilia has been correlated with symptom severity, clinical markers of disease severity in the CRSwNP population. This study examines the hypothesis that tissue eosinophilia correlates with disease outcomes with a continuous variable of tissue eosinophil count. Number of tissue eosinophils was also divided into 2 categories (≤10 or >10/HPF), as this level has been described as the limit for tissue eosinophilia, whereas a standard laboratory cutoff of 0.45 × 10⁹ cells/L was used for serum measurements. To compare asthmatics with non-asthmatics, those with and without aspirin sensitivity, those taking and not taking each recorded medication, and variables with 2 groups of continuous data, t tests were used. Analysis of variance was used to compare asthma subgroups. Linear regression models were made to compare number of previous operations or steroid courses with level of tissue eosinophilia. Multiple regression analysis was used to compare asthma subgroups with tissue eosinophilia, taking topical steroid use into account. A receiver operating characteristic curve was generated to evaluate the utility of serum eosinophil count as a marker of tissue eosinophilia.

**Methods**

**Chart Review**

A 2-year (2008-2010) retrospective chart review was approved by the National Jewish Health Institutional Review Board (HS-2672). The medical records database was queried for ICD-9 codes corresponding to CRS and nasal polyps (471.9 and 473.9). Patients were included if chart review confirmed the diagnosis of CRS by 2007 American Academy of Otolaryngology—Head and Neck Surgery Foundation criteria and documented polyps on nasal endoscopy. Patients with allergic fungal sinusitis, tumors, cystic fibrosis, or immunodeficiency were excluded from the study. Demographic information was collected from patient charts, including presence and severity of asthma, history of aspirin sensitivity, absolute peripheral eosinophil counts, and presence of dysosmia/anosmia. Severity of asthma was documented as intermittent, mild persistent, moderate persistent, and severe persistent, according to the National Asthma Education and Prevention Program clinical practice guidelines. Sinus CT scans were scored with the Lund-Mackay system by a researcher blinded to the remaining data. If a patient had multiple scans, the highest score was used for statistical analysis. Further clinical data were recorded, including the use of steroid-saline nasal rinses (budesonide inhalation suspension, 0.5 mg/2 mL), leukotriene modifiers, chronic oral corticosteroids, and number of previous sinus operations. Steroid bursts were defined as courses lasting <4 weeks, and chronic corticosteroid use was defined as any oral course beyond the scope of a single corticosteroid burst.

**Eosinophil Histology**

Standard hematoxylin and eosin–stained sinonasal tissue processed by the Department of Pathology for those patients undergoing surgery was reviewed in a blinded fashion to evaluate tissue eosinophilia via a method previously described in the literature. Slides were examined for the areas of densest cellular infiltrate to consistently classify eosinophil counts based on the areas of greatest inflammation. The absolute number of eosinophils per high-power field (HPF) was counted and recorded; the procedure was repeated in triplicate over 3 sites for each specimen. The average of these 3 values was used for data analysis.

**Statistics**

For continuous variables—including Lund-Mackay CT scores, absolute number of serum eosinophils, number of annual steroid bursts, and number of operations—a simple regression with Pearson correlation was used to compare disease outcomes with a continuous variable of tissue eosinophil count. Number of tissue eosinophils was also divided into 2 categories (≤10 or >10/HPF), as this level has been described as the limit for tissue eosinophilia, whereas a standard laboratory cutoff of 0.45 × 10⁹ cells/L was used for serum measurements. To compare asthmatics with non-asthmatics, those with and without aspirin sensitivity, those taking and not taking each recorded medication, and variables with 2 groups of continuous data, t tests were used. Analysis of variance was used to compare asthma subgroups. Linear regression models were made to compare number of previous operations or steroid courses with level of tissue eosinophilia. Multiple regression analysis was used to compare asthma subgroups with tissue eosinophilia, taking topical steroid use into account. A receiver operating characteristic curve was generated to evaluate the utility of serum eosinophil count as a marker of tissue eosinophilia.

**Results**

Seventy patients with CRSwNP who underwent endoscopic sinus surgery at our institution were included in this study. The demographic characteristics of our cohort are reported in Table 1. In general, these patients had severe disease, with the majority reporting olfactory dysfunction, demonstrating a mean Lund-Mackay score of 16.7 (out of 24), and with >60% of patients having severe asthma. In accordance with disease severity evident in this cohort, a high percentage of patients (21%) were chronically utilizing daily oral corticosteroids, as defined by >4 weeks of use, and our patients had undergone a mean of 2.6 prior sinus operations.

Fifty-six patients (80%) demonstrated tissue eosinophilia, and 27 (51.9%) had serum eosinophilia. Disease severity—as measured by self-reported anosmia, medication use, radiographic grading, prior surgery, and asthma categorization—did not correlate with tissue eosinophilia (>10 eosinophils/HPF). Absolute number of tissue eosinophils was not significantly correlated with presence of subjective olfactory loss (P = .91) or Lund-Mackay scores (P = .25). There was no significant difference in level of mucosal eosinophilia between patients with and without aspirin sensitivity (P = .09) or between patients with and without asthma. However, there was a significant difference when tissue eosinophil levels were compared among all subgroups of asthma severity (Figure 1; P = .02). In subgroup
analysis with Tukey-Kramer adjustment, there was a significantly higher number of tissue eosinophils in the mild asthmatic group versus the severe asthmatic group ($P = .03$).

When subgroups were examined according to treatments utilized (medical or surgical), there was no significant difference among cohorts (Table 2, Figure 2). Patients using steroid-saline nasal irrigations ($P = .93$) or taking leukotriene modifiers ($P = .69$) or concurrent oral corticosteroids ($P = .25$) did not have significantly different tissue eosinophil counts when compared with patients not taking these medications in univariate and logistic regression analysis. There was no correlation between tissue eosinophilia and number of previous sinus operations ($P = .43$).

Blood eosinophil levels have traditionally been considered a marker of tissue eosinophilic inflammation but are less than ideal, as migration and activation may result in notable tissue effects even at low serum levels.$^{29}$ In this study, there was no correlation found between absolute number of tissue eosinophils and serum eosinophil levels ($P = .97$). Even when patient samples were divided into categories—high tissue eosinophilia ($>10$/HPF) and low tissue eosinophilia ($\leq 10$/HPF)—there was not a significant relationship between tissue and blood eosinophil level. To further examine the potential use of serum eosinophils as a marker of tissue eosinophils, we generated a receiver operating characteristic curve; the area under the curve calculation of 0.59 ($P = .4$) confirms the lack of utility of this as a diagnostic test.

**Discussion**

We present the first study analyzing the potential correlation between clinical disease severity and histologic tissue eosinophil levels in patients with CRSwNP. We present a unique patient population with severe refractory CRS from a tertiary care respiratory hospital. Within this patient population, we did not find evidence that histologic evaluation of tissue eosinophil level correlated with disease severity, as measured by presence of olfactory symptoms, treatment therapies utilized, sinus CT scan grading, or asthma categorization.

We compared elements of clinical and radiographic disease severity in patients with CRSwNP with both tissue and blood eosinophil levels. In our patient population, a majority (80%) of patients had tissue eosinophilic inflammation, as defined by high tissue eosinophilia ($>10$/HPF), similar to prior studies showing that CRSwNP sinonasal tissue often

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**Table 1.** Patient Population Characteristics and Average Levels of Eosinophilic Inflammation ($N = 70$).

<table>
<thead>
<tr>
<th>Patient Characteristics</th>
<th>n (%) or Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Females</td>
<td>38 (54.3)</td>
</tr>
<tr>
<td>Age, y</td>
<td>46.3 ± 14.4</td>
</tr>
<tr>
<td>Disease severity</td>
<td></td>
</tr>
<tr>
<td>Asthmatics</td>
<td>65 (92.9)</td>
</tr>
<tr>
<td>Severe asthmatics</td>
<td>42 (62.1)</td>
</tr>
<tr>
<td>Aspirin-sensitive asthmatics</td>
<td>44 (62.9)</td>
</tr>
<tr>
<td>Lund-Mackay score</td>
<td>16.7 ± 4.6</td>
</tr>
<tr>
<td>Reporting olfactory disability</td>
<td>47 (67.1)</td>
</tr>
<tr>
<td>Treatments</td>
<td></td>
</tr>
<tr>
<td>Topical corticosteroid usage</td>
<td>44 (62.9)</td>
</tr>
<tr>
<td>Leukotriene modifier</td>
<td>51 (72.9)</td>
</tr>
<tr>
<td>Concurrent oral steroid use</td>
<td>15 (21.4)</td>
</tr>
<tr>
<td>Steroid bursts/year</td>
<td>2.62 ± 1.9</td>
</tr>
<tr>
<td>Operations</td>
<td>2.64 ± 1.9</td>
</tr>
<tr>
<td>Eosinophil levels</td>
<td></td>
</tr>
<tr>
<td>Tissue eosinophils/HPF</td>
<td>46.3 ± 14.4</td>
</tr>
<tr>
<td>High tissue eosinophils ($&gt;10$/HPF)</td>
<td>56 (80.0)</td>
</tr>
<tr>
<td>Blood eosinophils (K/µL)</td>
<td>0.55 ± 0.69</td>
</tr>
<tr>
<td>Serum eosinophilia</td>
<td>27 (51.9)</td>
</tr>
</tbody>
</table>

**Table 2.** Correlation between Tissue Eosinophil Level and Indicators of Disease.

<table>
<thead>
<tr>
<th>Patient Characteristics</th>
<th>$P$ Value</th>
<th>Odds Ratio or $r^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disease</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asthma (Y/N)</td>
<td>.093</td>
<td>OR = 2.94</td>
</tr>
<tr>
<td>Asthma severity</td>
<td>.023a</td>
<td>OR = 2.94</td>
</tr>
<tr>
<td>Mild vs severe asthma</td>
<td>.026</td>
<td>OR = 2.94</td>
</tr>
<tr>
<td>Aspirin sensitivity (Y/N)</td>
<td>.087</td>
<td></td>
</tr>
<tr>
<td>Lund-Mackay score</td>
<td>.25</td>
<td>$r^2 = 0.02$</td>
</tr>
<tr>
<td>Olfactory symptoms (Y/N)</td>
<td>.91</td>
<td>OR = 1.02</td>
</tr>
<tr>
<td>Treatments</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Topical corticosteroid usage</td>
<td>.78</td>
<td>OR = 0.93</td>
</tr>
<tr>
<td>Leukotriene modifier</td>
<td>.69</td>
<td>OR = 1.10</td>
</tr>
<tr>
<td>Concurrent oral steroid use</td>
<td>.25</td>
<td>OR = 0.73</td>
</tr>
<tr>
<td>No. of steroid bursts in prior year</td>
<td>.89</td>
<td>$r^2 = 0.0$</td>
</tr>
<tr>
<td>No. of previous surgeries</td>
<td>.43</td>
<td>$r^2 = 0.01$</td>
</tr>
<tr>
<td>Serum eosinophilia</td>
<td>.9705</td>
<td>$r^2 = 0.01$</td>
</tr>
</tbody>
</table>

Abbreviation: Y/N, yes/no.

*aP < .05.*

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**Figure 1.** Tissue eosinophil levels in asthmatics. Tissue eosinophils were similar in asthmatics versus nonasthmatics; however, patients with mild persistent asthma demonstrated significantly more tissue eosinophils than patients with severe persistent asthma.
exhibits tissue eosinophilia. Prior literature examining patients without nasal polyps or including all groups of CRS patients has shown a correlation between tissue eosinophil level and disease severity or prognosis. However, CRS represents a diverse spectrum of disease with variable pathogenesis and clinical course. In the select subset of CRS patients examined in our study, these general trends did not persist. The cohort of nasal polyposis patients presented here had severe CRS, as evidenced by their high CT score, multiple prior surgeries, prevalence of severe asthma, and extensive medication usage. However, in this cohort with severe clinical disease, there was no delineation of disease severity based on tissue eosinophilia, calling into question its use as a predictor of disease severity in polyp patients, at least in retrospective use.

Our cohort of patients with severe respiratory disease frequently used oral steroids, consistent with practice guidelines for both CRS and asthma. We found that patients with mild asthma had significantly elevated levels of tissue eosinophils when compared with patients with severe asthma, which was an unexpected finding that requires further consideration given the known role of the eosinophil in CRSwNP and asthma. This unanticipated finding may be related to the frequent use of corticosteroids in the management of severe inflammatory disease, as tissue eosinophil levels have been demonstrated to decrease during and after treatment with systemic or topical steroids in sinonasal and respiratory tissue. In addition, the absolute tissue eosinophil number may not be the ideal measurement, as tissue effects are mediated by cell trafficking and eosinophil degranulation and may occur in the setting of normal eosinophil numbers. It is also possible that more of our severe asthma patients fall into a noneosinophilic asthma phenotype, confounding the comparison to less severe asthmatics. Our patient cohort underwent endoscopic sinus surgery only after failure of medical therapy, including topical and sometimes oral steroids. This makes interpretation of absolute tissue eosinophil levels difficult in patients receiving maximal medical management for CRS or asthma, including possible oral, inhaled, and nasal steroids at different time points prior to tissue sampling. Although we controlled for nasal steroid irrigation use in our linear regression model and found a persistent significant difference in eosinophil level between mild and severe asthmatics, our power was not large enough to examine patients receiving each form of glucocorticoid therapy independently to determine if this difference would persist when taking into account all forms of steroids.

Most interesting, we did not find a correlation between tissue and blood eosinophilia, even when analyzed among nonsteroid users. This is similar to previous published investigation on sputum eosinophilia in asthmatics, which has shown that blood eosinophil level is not necessarily predictive of sputum eosinophil level; however, this is currently an area of debate in the literature. There are other advanced concepts of individualized steroid activity beyond simple administration, including numerous proposed mechanisms of steroid resistance, action on eosinophil margination and tissue infiltration, and eosinophil activation, which can influence this comparison.

We acknowledge that our conclusions apply to a select patient cohort with severe CRSwNP and that its retrospective design limits the widespread utility of the results. A retrospective study was necessary to include an adequate number of patients to initially investigate these questions, but further prospective investigation is warranted given the findings and potential importance of the topic. Furthermore, we were not able to eliminate either topical or systemic steroid use as a confounder of tissue eosinophilia, as CRSwNP patients generally undergo standard-of-care medical therapies (including steroids) at some point in the preoperative management. Just as in severe asthma, described earlier, patients with severe CRSwNP manifestations more frequently utilize corticosteroid therapy, and as a result, those with more severe disease may have related tissue effects. This could partially explain why these patients with severe disease did not have a correlation between tissue eosinophil levels and degree of disease, but further investigation is required.

**Conclusion**

In our analysis of CRSwNP patients, we failed to demonstrate a significant correlation between tissue eosinophilia and serum eosinophilia or disease severity. Corticosteroids
play a large role in treating this disease and carry the potential to influence tissue inflammation, making interpretation of tissue eosinophil levels challenging. In the “real world” clinical setting, it is difficult to infer prognostic information for CRSwNP patients from levels of tissue eosinophilia, and future prospective studies that thoroughly account for corticosteroid are indicated to definitively answer this question.

Author Contributions
Sarah A Gitomer, design of the project, acquisition, analysis and interpretation of the data, drafting and revising the work; Cynthia R. Fountain, acquisition of the data, drafting and revising the work; Todd T. Kingdom, conception, design of project, data analysis and interpretation; critical revision of the manuscript; Anne E. Getz, data analysis and interpretation; critical revision of the manuscript; Stefan H. Silbau, data analysis and interpretation; critical revision of the manuscript; Rohit K. Katal, conception, design of project, data analysis and interpretation; critical revision of the manuscript; Vijay R. Ramakrishnan, conception, design of project, data analysis and interpretation; critical revision of the manuscript.

Disclosures
Competing interests: None.
Sponsorships: None.
Funding source: None.

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