Tissue Eosinophilia in Chronic Sinusitis

Quantification Techniques

Neil Bhattacharyya, MD; Darshan K. Vyas, MD; Frank P. Fechner, MD; Richard E. Gliklich, MD; Ralph Metson, MD

Objective: To ascertain the reliability of a proposed method for quantifying tissue eosinophilia in sinus mucosa.

Design: Prospective cohort study.

Interventions and Outcome Measures: Pathology slides from patients undergoing endoscopic sinus surgery for chronic rhinosinusitis were independently assessed by 2 reviewers. Using a proposed systematic counting method, the degree of tissue eosinophilia was quantified. Disease severity was assessed by computed tomographic (CT) staging. Intrarater, interrater, and intrapatient reliability was determined using correlational reliability analysis. The degree of correlation between tissue eosinophilia and CT stage was determined.

Results: One hundred thirty-two slides from 65 patients were reviewed. The mean (SD) eosinophil density was 23.4 (37.2) eosinophils per high-power field. Only 12 patients (18%) had no eosinophils on histopathologic analysis. Strong intrarater ($r \geq 0.91$ for each rater, $P < .001$) and interrater reliability ($r \geq 0.82$ between raters, $P < .001$) was noted for the quantification method. A moderate degree of correlation was found between CT scan stage and degree of tissue eosinophilia (Spearman $r = 0.62$, $P < .001$).

Conclusions: The proposed method for quantifying tissue eosinophilia in sinus mucosa is reliable and valid. A relatively strong correlation exists between CT scan stage and tissue eosinophilia in chronic rhinosinusitis.


CHRONIC rhinosinusitis is an extremely common clinical problem, affecting as many as 30 million Americans. Despite recent progress in formalizing clinical diagnostic criteria, radiographic staging, and management protocols, much remains to be learned about the pathogenesis of this entity. Recent studies have identified a potential link between chronic rhinosinusitis and the inflammatory cells and mediators present in sinonasal mucosa. Several authors have identified hypereosinophilia within the sinonasal mucosa in chronic rhinosinusitis and linked this finding with the production of inflammatory mediators and subsequent tissue damage within the respiratory mucosa. In addition, the degree of tissue eosinophilia has been found to predict extensive disease as measured by computed tomographic (CT) scan stage. Other data suggest that eosinophilia may be predictive of success or failure with endoscopic sinus surgery.

Despite mounting evidence that the eosinophil has a significant role in the pathogenesis of chronic rhinosinusitis, conflicting data have emerged in the literature regarding its impact on the disease. Variability in the definition of tissue hypereosinophilia and quantification of eosinophilia may account for some of these contradictory findings. To better understand the role of the eosinophil in chronic rhinosinusitis, an accurate and reproducible method for quantifying tissue eosinophilia is required. Therefore, we sought to establish a statistically reliable and valid method for quantifying tissue eosinophilia in sinus mucosal specimens. Furthermore, we explored the relationship between tissue eosinophilia and extent of disease as measured by the CT scan stage of the chronic rhinosinusitis.

RESULTS

Of the initial 71 patients in the inception cohort, 6 patients had missing or unavailable pathology slides and were therefore excluded from the subsequent analysis. Twenty-three (35%) of the remaining 65
MATERIALS AND METHODS

An inception cohort of 71 consecutive patients undergoing surgical therapy for medically refractory chronic rhinosinusitis constituted the study population. All patients met clinical criteria established by the American Academy of Otolaryngology for the diagnosis of chronic rhinosinusitis; thorough medical management had failed for all. Preoperative CT scans were performed and staged according to a previously described system. A second group of 5 patients without a history of chronic rhinosinusitis undergoing endoscopic orbital decompression comprised a control or reference group.

The mucosal specimens obtained at the time of endoscopic sinus surgery were fixed in formalin embedded in paraffin. Standard 5-µm sections were stained with hematoxylin-eosin. Light microscopic examination of each slide was then conducted according to a structured protocol. Initially, a low-power survey of the entire slide was performed at ×20 and ×100 magnification. In this survey, sinus mucosa (excluding nasal mucosa) was identified by its lack of glandular elements and the presence of goblet cells (when identifiable). Subsequently, the segment of sinus mucosa containing the greatest degree of eosinophilic inflammation was identified and then examined under a total magnification of ×450, with a 10 × 10-mm reticulate present in the eyepiece. The total number of eosinophils present within this grid was determined as the eosinophil count per high-power field (HPF). Second and third eosinophil counts within this same area of diseased mucosa were performed by the same observer after removing the eyes from the microscope for 30 seconds and then recounting. The slide was then placed under low-power magnification and in a random position for a second observer to repeat the low-power survey, identification of the most diseased area, and eosinophil count and recounts procedure. Values were recorded for eosinophil counts, with each observer blinded to the counts of the other observer. For patients with more than one slide of sinus mucosa (ie, left and right sinus contents), all slides were processed in the same manner to look for variability within patients.

Statistical analysis was then performed to determine intrarater reliability, interrater reliability, and intrapatient consistency. Standard techniques for determination of Pearson correlation coefficients and statistical significance of these coefficients were used. Intrarater reliability was determined for counts 1, 2, and 3 for each slide of each patient, thus determining the counting error. Interrater agreement was determined by averaging the 3 eosinophil counts per slide for each observer and then statistically comparing these counts between observers. Finally, intrapatient variability was assessed by comparing the mean eosinophil count for all 6 counts (3 counts per observer for 2 observers) between slides for a given patient. These 3 reliability/variability assessments are diagrammatically represented in Figure 1. An average eosinophil density for each patient was computed by averaging the counts from both observers for all slides for that patient, resulting in a mean eosinophil count per HPF for a given patient.

Figure 1. Diagrammatic representation of eosinophil counts and recounts for a single patient. Small bidirectional arrows indicate comparisons for intrarater reliability; medium bidirectional arrows, comparisons for interrater reliability; and long bidirectional arrow, intrapatient variability.

Table

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Figure 2

Each patient had between 1 and 3 pathology slides of sinus mucosa prepared based on the sidedness of the surgical procedure (bilateral procedures resulted in at least 2 slides) and the amount of material present in the paraffin block. A total of 132 pathology slides were reviewed according to this protocol, resulting in a total of 396 data points per observer and 792 total data points.

For patients in the chronic rhinosinusitis group, the mean (SD) eosinophil density was 23.4 (37.2) eosinophils per HPF. Three hundred nine (39.0%) data points had eosinophil counts per HPF of 0. Twelve patients (18%) had no eosinophils on histopathologic analysis. For the control group, the mean eosinophil density in the sinus mucosa was 0.0 eosinophils per HPF.

Correlation coefficients and corresponding P values for the 3 reliability measurements are displayed in the Table. Strong intrarater and interrater reliabilities were noted for the counting method. Regarding intrapatient consistency of tissue eosinophilia, some variability was noted between slide 1 and slide 2, but the correlation between an individual patient’s slides was still strongly significant (r = 0.64, P < .001). A moderately strong degree of correlation was noted between the CT...
Increasing evidence has emerged that the eosinophil may have a central role in the pathogenesis of chronic rhinosinusitis. Several authors\(^5,9\) have found that tissue eosinophilia is more prevalent in the mucosa of patients with chronic rhinosinusitis than controls. Other studies have implicated the eosinophil as a source of immunomodulators and cellular by-products that influence the mucosal response to allergens and infection. Therefore, the eosinophil may not only have a role in the pathogenesis of chronic rhinosinusitis, but may also be a useful indicator of disease severity and prognosis for various forms of therapy. In an important study by Newman et al,\(^5\) tissue eosinophilia was found to strongly correlate with chronic rhinosinusitis disease severity on CT scan. Bhattacharyya and Fried\(^10\) found a statistically higher rate of peripheral blood eosinophilia in patients with chronic rhinosinusitis compared with controls. Other investigators have also found that tissue eosinophilia may predict surgical treatment outcome in patients with chronic rhinosinusitis.\(^6\)

To more fully explore the effect of tissue eosinophilia on chronic rhinosinusitis, a statistically valid and reliable method of eosinophil quantification within sinus mucosa is required. Histomorphometric counting methods have been used in other inflammatory disease states and in chronic rhinosinusitis. However, different authors have used varying methods for quantifying tissue eosinophilia, making comparisons among studies somewhat difficult. For example, several studies have sought to explore the relationship between mucosal eosinophilia and the severity of chronic rhinosinusitis as measured by CT scan stage. Goldwyn et al\(^11\) found poor correlation between tissue eosinophilia and CT scan severity (Pearson \(r=0.131\)), whereas Newman et al\(^5\) found a statistically significant correlation between them. Neither study provided statistical measures of reliability for their histologic eosinophil quantification method. Baroody et al\(^9\) also found poor correlation between CT severity and tissue eosinophilia (\(r=0.25\)) and noted that their eosinophil quantification method had strong interrater reliability (\(r=0.84\)). In that study, however, a marking pen was used to identify the area on the pathology slide to be counted or recounted by the second observer. Therefore, this measure of interrater reliability is biased by the pen marking, potentially inflating the reliability score. In fact, the lack of correlation between tissue eosinophilia and chronic rhinosinusitis severity may be due to variability in the counting method rather than a true lack of association.

For a histologic quantification method to be clinically useful, it must be relatively simple to perform and statistically reliable. We sought to establish a method that could be used in a standardized fashion to further explore the relationship between the eosinophil and chronic rhinosinusitis. It is clear from the data that the proposed method of tissue eosinophilia quantification is statistically reliable and sound. High intrarater and interrater correlation coefficients were noted for the proposed method with appropriate statistical significance. This method is simple, uses readily available histologic and microscopic techniques, and has proven reliability.

Using a statistically reliable eosinophil counting method, we found a relatively strong correlation between severity of chronic rhinosinusitis as measured by CT scan stage and degree of tissue eosinophilia (Figure 2). This relationship has come into question based on several studies.\(^5,9,11\) In our study, the relationship

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**Statistics for Reliability Analysis of the Eosinophil Counting Method**

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\(P<.001\) for all.

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**Figure 2.** Distribution of computed tomographic (CT) scan stage.

**Figure 3.** Mean eosinophil density vs computed tomographic (CT) scan stage. HPF indicates high-power field. Error bars indicate 95% confidence limits.
found between the CT scan stage and the degree of tissue eosinophilia was independent of the anatomic side of the patient that was sampled for tissue measurements.

Although we found our method to be statistically valid and reliable, problems may still arise with its use in studies of sinus eosinophilia. Variability in the thickness of the tissue fixed in the pathology slide requires that the observer "focus through" the depth of the histologic specimen using the fine-tuning focus control of the microscope. Some inherent variability may arise between observers based on this operator-dependent portion of the counting system. Our pathology laboratory consistently uses a 5-µm-thick slice for processing hematoxylin-eosin specimens. Consistency in the cut thickness of the pathology specimens is important for the reliability of this method.

Other methods of quantifying tissue eosinophilia have been developed that use immunologic markers for the eosinophil or its by-products.12,13 These methods have been primarily used in the study of the pathogenic impact of the eosinophil and chronic rhinosinusitis, but may also be used to quantify tissue eosinophilia. Although these counting techniques are considered reliable, they require additional processing, time, and expense. In addition, they usually require a suitable control to ensure that the staining or immunofluorescence technique is accurate. Our method requires no additional processing time or expense and can be applied in a retrospective fashion to previously obtained sinus tissue specimens. The use of this reliable technique may allow for better study of the potential impact of the eosinophil on the pathogenesis, staging, and treatment outcomes of chronic rhinosinusitis.

References


