ORIGINAL ARTICLE

Progressive Mucosal Injury in Patients With Gastroesophageal Reflux Disease and Increasing Peripheral Blood Eosinophil Counts

Farzaneh Banki, MD; Patrick Flanagan, MS; Joerg Zehetner, MD; Analisa Armstrong, MS; Jeffrey A. Hagen, MD; Amit Joshi, MD; Raina Sinha, MD; Daniel Oh, MD; Arzu Oezcelik, MD; Steven R. DeMeester, MD; Emmanuele Abate, MD; John C. Lipham, MD; Parakrama Chandrasoma, MD; Peter F. Crookes, MD; Tom R. DeMeester, MD

Hypothesis: Peripheral blood eosinophil count increases with the degree of mucosal injury associated with gastroesophageal reflux disease (GERD).

Design: Retrospective review.

Setting: Single-institution tertiary hospital.

Patients: Two hundred ninety-five patients (215 men and 80 women; median age, 57 years [interquartile range (IQR), 46-66 years]). One hundred had GERD without intestinal metaplasia, 100 had GERD with intestinal metaplasia, 40 had GERD with dysplasia, and 55 had GERD with intramucosal carcinoma. Results of complete blood count with differential and serum chemistry studies were compared among the groups using a nonparametric test for trend.

Results: Patients with a higher degree of mucosal injury were older ($P < .001$). There were no differences between white blood count, percent neutrophil count, absolute neutrophil count, and hematocrit levels among the groups. Serum albumin level decreased as the degree of mucosal injury increased ($P = .04$) but lost significance when controlled for age ($P = .53$). Percent eosinophil counts were 2.0 (IQR, 1.3-2.8) in patients with GERD without intestinal metaplasia, 2.5 (IQR, 1.6-3.7) in GERD with intestinal metaplasia, 2.6 (IQR, 1.7-4.4) in GERD with dysplasia, and 2.7 (IQR, 1.5-4.3) in GERD with intramucosal carcinoma. This progressive increase in the percent eosinophil count was statistically significant ($P = .006$), remained significant after controlling for age ($P = .04$), and was also significant when measuring the absolute eosinophil count.

Conclusion: There is a progressive increase in the percent and absolute peripheral blood eosinophil count associated with progressive mucosal injury in patients with GERD.

Arch Surg. 2010;145(4):363-366

Author Affiliations: University of Southern California, Los Angeles. Dr Banki is now with the Department of Cardiothoracic and Vascular Surgery, The University of Texas Medical School at Houston, Houston.

THE PRESENCE OF INTRAepithelial eosinophils on esophageal biopsy was first noted to be a specific marker of esophagitis in children by Winter et al,[1] who correlated the presence of eosinophils with abnormal esophageal acid exposure on pH monitoring. Additional studies have been published confirming the presence of eosinophils in the mucosa of patients with gastroesophageal reflux disease (GERD), but no studies have evaluated the significance of the peripheral blood eosinophil count in patients with GERD. The aim of our study was to evaluate alterations in the peripheral blood eosinophil count in patients with GERD and assess the association between the peripheral blood eosinophil count and the degree of mucosal injury.

METHODS

The subjects of this study were chosen from a population of patients who were referred for evaluation and surgical treatment of GERD and its complications in the Thoracic and Foregut Division at the Department of Surgery of the University of Southern California. All patients underwent esophageal motility, pH study, and upper gastrointestinal endoscopy with biopsy. Laboratory studies included measurements of white blood cell count, neutrophil count, eosinophil count, albumin, and hematocrit.

Esophageal motility and pH studies were performed according to a standard protocol.[2] The pH probe was placed 5 cm above the manometrically determined upper border of the lower esophageal sphincter. Upper gastrointestinal endoscopy was performed according to a protocol for biopsies of the gastric antrum and body, gastroesophageal junction, and distal esophagus.[3] Patients were classified into 4 groups based on the degree of mucosal injury determined through biopsies taken from the gastroesophageal junction and distal esophagus.

The study groups consisted of patients with GERD without intestinal metaplasia, GERD with intestinal metaplasia, GERD with dysplasia (low-grade dysplasia and high-grade dysplasia), and GERD with intramucosal carci-

Table. Comparison of Demographic Information and Laboratory Values in the 4 Study Groups With GERD Based on the Degree of Mucosal Injury

Characteristic	Median (Interquartile Range)				*P* Value
	Without IM (n=100)	With IM (n=100)	With Dysplasia (n=40)	With IMC (n=55)	
Age, y	47 (37-60)	57 (46-64)	59 (55-70)	66 (59-72)	<.001
Hematocrit %	43.4 (39.5-46.0)	42.0 (39.2-44.7)	41.8 (38.9-44.5)	42.2 (38.4-44.7)	.15
Albumin, g/dL	4.3 (4.2-4.5)	4.3 (4.1-4.6)	4.3 (4.1-4.5)	4.2 (4.0-4.4)	.53[a]
WBC, /μL	6500 (5500-7700)	6400 (5400-7600)	5900 (5000-7600)	6600 (5100-7600)	.37
Absolute neutrophil count, /μL	3900 (2900-5100)	3900 (3100-4800)	3700 (3000-5000)	3700 (3000-5300)	.93
Percent neutrophil count	60.1 (54.2-66.8)	60.8 (56.2-68.1)	64.1 (55.4-67.8)	61.8 (58.3-69.5)	.14
Absolute eosinophil count, /μL	130 (80-180)	160 (110-260)	170 (100-270)	160 (100-290)	.04[a]

Abbreviations: GERD, gastroesophageal reflux disease; IM, intestinal metaplasia; IMC, intramucosal carcinoma; WBC, white blood cell count.
SI conversion factors: To convert albumin to grams per liter, multiply by 10; eosinophil, neutrophil, and WBC to $\times 10^9$/L, multiply by 0.001.
[a] Adjusted for age.

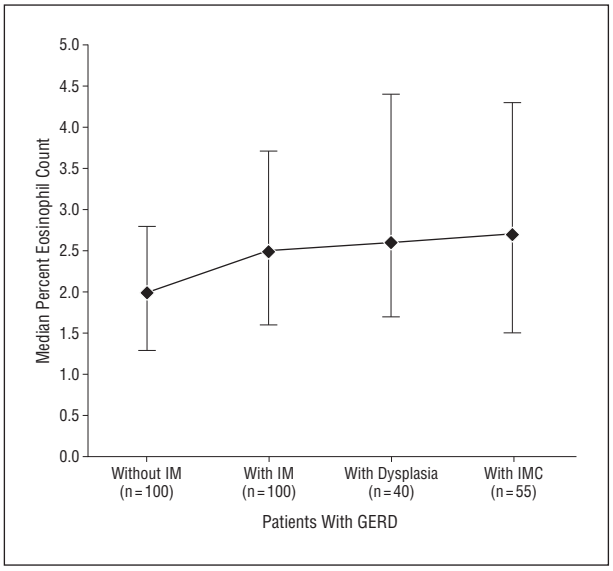

Figure. Association between percent eosinophil in the peripheral blood and the degree of mucosal injury in 4 patient groups. Dysplasia included low- and high-grade disease. Values are adjusted for age (*P*=.006, unadjusted for age; *P*=.04, adjusted for age). Error bars indicate interquartile range; GERD, gastroesophageal reflux disease; IM, intestinal metaplasia; IMC, intramucosal carcinoma.

noma. The presence of eosinophils in biopsy specimens from the gastroesophageal junction and distal esophagus was noted.

Blood samples were obtained from peripheral veins on the morning of the preoperative visit. Normal values for our laboratory were percent eosinophil count of 0.8 to 5.0, absolute eosinophil count of less than 200/μL, percent neutrophil count of 72.2 to 75.5, absolute neutrophil count of 1400 to 6500/μL, white blood cell count of 3800 to 10 800/μL, hematocrit level of 35.0% to 46.0%, and albumin level of 3.5 to 5.2 g/dL. To convert eosinophil, neutrophil, and white blood cell counts to $\times 10^9$/L, multiply by 0.001.

Results of complete blood cell count with differential and serum chemistry studies were compared among the groups using a nonparametric test for trend. Preliminary statistical analysis indicated that there was an association between progressive mucosal injury and age. As a result, comparisons were adjusted for age when required by computing residuals from a simple linear regression model. The presence of intraepithelial eosinophils in patients with and without intestinal metaplasia was compared using the χ^2 test. All statistical analyses were done using the Stata statistical software program. Statistical power calculations indicated that a sample size of 100 patients per group would be necessary to detect significant differences in peripheral blood eosinophil count in patients with GERD. The study was approved by the institutional board review at the University of Southern California.

RESULTS

The study population consisted of 295 patients: 215 men and 80 women, with a median age of 57 years (interquartile range [IQR], 46-66 years). One hundred patients with GERD and no intestinal metaplasia and 100 with GERD and intestinal metaplasia were randomly identified for inclusion. There were 40 patients with GERD and dysplasia and 55 with GERD and intramucosal carcinoma who met the inclusion criteria.

Demographic information and laboratory values in the 4 patient groups are shown in the **Table**. Patients with a higher degree of mucosal injury were older (*P*<.001). There were no differences between white blood count, percent neutrophil count, absolute neutrophil count, or hematocrit levels among the groups. Serum albumin decreased as the degree of mucosal injury increased (*P*=.04) but lost significance when controlled for age (*P*=.53). The absolute eosinophil count progressively increased with increase in mucosal injury (*P*=.01) and remained significant after controlling for age (*P*=.04). The percent eosinophil count in the 4 patient groups are shown in the **Figure**. The percent eosinophil count rose significantly with increase in mucosal injury (*P*=.006) and remained significant after controlling for age (*P*=.04).

Intraepithelial eosinophils were present in the distal esophagus or the gastroesophageal junction in 35% of patients with GERD without intestinal metaplasia and 42% in GERD with intestinal metaplasia (*P*=.34). When dysplasia or intramucosal carcinoma were present, intraepithelial eosinophils on pathology specimens were not assessed. There was no difference in the frequency of asthma or asthma-like symptoms among the groups (13 of 100 [13%] patients with GERD without intramucosal carcinoma, 13 of 100 [13%] with GERD and intramucosal carcinoma, 4 of 40 [10%] with GERD and dysplasia, and 2 of 55 [3.6%] with GERD and intramucosal carcinoma; *P*=.34).

COMMENT

The current study shows that an increase in the peripheral blood eosinophil count occurs in progressive stages of GERD. This suggests that eosinophils may have a unique purpose in the pathophysiology of this disease. Despite remaining within the normal range, a gradual and independent increase in the absolute eosinophil count and percent eosinophil count was observed as the degree of mucosal injury increased. This finding may have several clinical implications, and understanding this observation requires an appreciation of the presence and function of eosinophils in normal tissues.

Eosinophils reside mainly in tissue. It is estimated that for every 1 eosinophil in the peripheral blood, there are 100 eosinophils in the tissue.[4] Eosinophil infiltration of the tissue usually occurs independently of other blood leukocytes.[5] Eosinophils are normally seen in the gastrointestinal tract, spleen, and lymphatic and thymic tissues. The most predominant population of eosinophils is in the gastrointestinal tract,[6] where they are located in the lamina propria of the stomach, small intestine, cecum, and colon.[7] In contrast to the other segments of the gastrointestinal tract, eosinophils are uncommon in the esophageal mucosa, and their presence is a marker of pathology,[7] such as of reflux esophagitis, parasitic infections, systemic eosinophilic conditions,[8] and eosinophilic esophagitis.[9]

Accumulation of eosinophils in the tissue occurs in several other diseases not related to the esophagus,[10] such as bronchial asthma.[11] In this condition, the peripheral blood eosinophilia is shown to be correlated with the degree of bronchial hyperactivity.[12]

One of the first studies to suggest that there is an association between GERD and the eosinophil infiltration of the esophagus was written by Winter et al[1] in 1982. They reported that the presence of intraepithelial eosinophils in children correlated with increased esophageal acid exposure on pH monitoring. This association was further supported by Lee[13] in 1985, who showed that eosinophil infiltration may indicate prolonged or severe GERD. The report of excessive intraepithelial eosinophilia by Attwood et al[14] in 1993 introduced the possibility that esophageal eosinophilia may not solely be a reflux phenomenon. They introduced the concept of eosinophilic esophagitis as a separate entity manifested by the symptom of dysphagia rather than heartburn.

The exact mechanism of esophageal infiltration by eosinophils is unknown but is likely due to diverse stimuli that recruit eosinophils from the peripheral circulation into inflammatory foci, where they modulate the immune responses and regulate vascular permeability. These stimuli include nonspecific tissue injury, infections, allograft, allergens, and tumors.[15] Recent studies have suggested several potential mechanisms whereby GERD is a stimulus for the recruitment of the eosinophils into the esophageal mucosa. In one study, vascular endothelial cells were shown to express adhesion molecules like vascular cell adhesion molecule–1 that are recognized by ligands on the eosinophil cell surface.[16] In another study, increased acid exposure was shown to induce the expression of vascular cell adhesion molecule–1 in cultures of human esophageal microvascular endothelial cells.[17] Acid exposure has also been shown in animal studies to increase esophageal blood flow, thereby enhancing the delivery of eosinophils to the esophageal epithelium.[18] In human esophageal mucosa, acid exposure has been shown to release a platelet-activating factor, which has the capacity to attract and activate eosinophils.[19] Furthermore, esophageal acid exposure has been shown to induce the esophageal epithelium to release chemokines that attract eosinophils.[20] Two recently described chemokines, eotaxin-1 and eotaxin-2, are reported to be relatively specific for eosinophils.[21,22] Eosinophil infiltration of tissue frequently occurs independently of other blood leukocytes, suggesting that a specific mechanism exists for the extravasation of eosinophils into the tissues.

The maturation of eosinophils in the bone marrow and their release into the blood is dependent on cytokines, including IL-5 (interleukin 5),[23] IL-2,[24] and granulocyte macrophage and colony-stimulating factors.[25,26] Of these factors, IL-5 is known as an eosinophil-differentiation factor and appears to be directly and specifically involved in eosinopoiesis[23] and their release into the peripheral circulation.[27]

The elevation of eosinophil counts in the blood of patients with intramucosal carcinoma in our study corresponds to previous reports in which both peripheral blood and tissue eosinophil count are known to be elevated in other neoplasms. Iwasaki et al,[28] in a prospective study of 647 patients with gastric carcinoma, found that 157 (24%) patients showed eosinophil infiltration in the resected tumor. They identified a chemotactic factor (eosinophil chemotactic factor) in the tumor extracts with the capacity for marked eosinophil chemotaxis. In their study, the degree of eosinophil infiltration into tumors correlated well with the blood eosinophil count. Similarly, in colonic carcinoma, eosinophil infiltration of the tumor specimen has been reported.[29] In carcinoma of the lung, an eosinopoiesis factor has been isolated from tumor extracts as well as tumor cells from patients with lung cancer. In these patients, peripheral blood eosinophilia and tumor eosinophil infiltration are known to secrete an eosinopoietic polypeptide that stimulates bone marrow production of eosinophils followed by a rise in the peripheral blood eosinophil count.[30]

This study provides data confirming that eosinophils may have a particular role in patients with GERD and introduces the concept that an increase in the peripheral blood eosinophil count correlates with the degree of mucosal injury. The exact etiology of the increase in the eosinophil count and their selective infiltration in patients with GERD remains unknown. It is hypothesized that the degree of increase is related to the degree of acid- and bile-induced injury at the intraepithelial level. The intraepithelial injury initiates a cascade of inflammatory response mediated by cytokines such as IL-5, which attracts the eosinophils to the injured epithelium and causes the release of eosinopoietic factor to stimulate bone marrow production of eosinophils, resulting in a rise of the peripheral blood eosinophil count.

Our study is limited by its retrospective design, lack of a control group composed of healthy individuals, and the inability to assess the effect of acid-suppression therapy in these patients. It would be interesting to measure the peripheral blood eosinophil count in patients with squa-

mous cell carcinoma of the esophagus, which is not associated with GERD. It would also be clinically pertinent to measure the peripheral blood eosinophil count in patients with GERD before and after medical therapy or antireflux surgery and in patients with esophageal adenocarcinoma before and after esophagectomy in a prospective fashion. In addition, we did not investigate the degree of intraepithelial eosinophils in patients with dysplasia and intramucosal carcinoma. Furthermore, it is known that the eosinophil count has a diurnal variation,[31] with the lowest count in the morning and the highest count in the evening. This time-related variation did not affect our results, as the blood samples were drawn during the day. We also did not collect information about food allergies, which made it difficult to control for this factor.

Despite these limitations, this study is the first to focus on the significance of peripheral blood eosinophil count in patients with GERD and shows that an independent increase in peripheral eosinophil count was associated with the progression in the sequence of mucosal injury from GERD to metaplasia to dysplasia to intramucosal carcinoma. Our study further indicates that GERD is a chronic disease with a progressive degree of inflammation reflected by an increasing peripheral blood eosinophil count with each epithelial change. The microscopic resolution of this inflammatory process should be an integral part of the treatment of patients with GERD, and effective therapy should prevent the progression of the mucosal changes associated with the inflammation.

Accepted for Publication: May 21, 2009.
Correspondence: Farzaneh Banki, MD, 11914 Astoria Blvd, Medical Plaza 1, Ste 260, Houston, TX 77089 (farzaneh.banki@uth.tmc.edu).
Author Contributions: *Study concept and design:* Banki and Lipham. *Acquisition of data:* Banki, Flanagan, Zehetner, Armstrong, Sinha, Oh, Oezcelik, Abate, and Lipham. *Analysis and interpretation of data:* Banki, Hagen, Joshi, S. DeMeester, Lipham, Chandrasoma, Crookes, and T. DeMeester. *Drafting of the manuscript:* Banki, Flanagan, Zehetner, Armstrong, Sinha, Oh, Oezcelik, Abate, and Chandrasoma. *Critical revision of the manuscript for important intellectual content:* Banki, Hagen, Joshi, S. DeMeester, Lipham, Crookes, and T. DeMeester. *Statistical analysis:* Joshi. *Obtained funding:* T. DeMeester. *Administrative, technical, and material support:* Joshi, Chandrasoma, and T. DeMeester. *Study supervision:* Banki, Hagen, and Lipham. *Institutional review board approval:* Zehetner.
Financial Disclosure: None reported.
Previous Presentation: This study was presented at the 80th Annual Meeting of the Pacific Coast Surgical Association; February 14, 2009; San Francisco, California.

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