The Optimal Number of Biopsy Fragments to Establish a Morphologic Diagnosis of Eosinophilic Esophagitis

Jennifer A. Nielsen, BA[1], Donna J. Lager, MD[2], Matthew Lewin, MD[2], Gabriel Rendon, MD[3] and Cory A. Roberts, MD[2]

OBJECTIVES: **Eosinophilic esophagitis (EoE) is characterized clinically by dysphagia, chest pain, and food impaction, and morphologically by increased numbers of intraepithelial eosinophils and marked basal hyperplasia of the squamous mucosa. The consensus criteria for a diagnosis of EoE include the presence of ≥15 eosinophils/HPF in biopsies from both proximal and distal esophagus in the absence of other causes of esophageal eosinophilia, and the lack of clinical response to proton pump inhibitor therapy. Because of the variability in the distribution of intraepithelial eosinophils among biopsy fragments and the lack of standardized biopsy practices, we sought to determine the optimal number of esophageal biopsies from the mid and distal esophagus needed to reach the minimum morphologic criteria of ≥15 eosinophils/HPF.**

METHODS: **From 5 January 2009 to 26 September 2011, 771 patients were diagnosed with EoE at our institution. From that patient population, 102 sequential cases were chosen for further study, all of whom had biopsies taken from the mid and distal esophagus. Cases with only gastric mucosa present and biopsies taken from patients with a previous diagnosis of EoE were excluded. The original H&E-stained slides were reviewed, and the number of biopsy fragments containing squamous mucosa was recorded. By using a ×40 objective and ×10 oculars (field diameter = 0.52 mm, field area = 0.21 mm^2), the number of eosinophils per high power field (EOS/HPF) in up to three HPFs was counted in each biopsy fragment.**

RESULTS: **The EOS/HPF were counted in 1,342 biopsy fragments. The number of biopsy fragments obtained from the mid esophagus ranged from 1 to 20 (mean 7; median 7) and those obtained from the distal esophagus ranged from 1 to 18 (mean 6; median 5). There was no significant difference between the mean number of EOS/HPF from the mid (26) and lower (25) esophagus or between the mean peak number of EOS/HPF from the mid (69.1) and lower (60.4) esophagus. The probability of one, four, five, and six biopsy fragments containing >15 EOS/HPF was 0.63, 0.98, 0.99, and >0.99, respectively.**

CONCLUSIONS: **From these data, at least four biopsy fragments should be submitted from the mid and/or proximal esophagus to optimize the chances of a positive diagnosis of EoE in populations not known to have undergone previous proton pump inhibitor therapy. However, the yield is not increased beyond six biopsy fragments. In order to morphologically exclude a diagnosis of reflux esophagitis as the cause of intraepithelial eosinophilia, distal esophageal biopsies, if obtained, must be accompanied by more proximal biopsies (i.e., mid esophagus or higher).**

Am J Gastroenterol 2014; 109:515–520; doi:10.1038/ajg.2013.463; published online 21 January 2014

INTRODUCTION

Esophageal eosinophilia is associated with a number of diseases, most notably gastroesophageal reflux disease (GERD) and eosinophilic esophagitis (EoE) (1). EoE is a chronic immune-mediated disease characterized clinically by symptoms of esophageal dysfunction and esophageal eosinophilia on biopsy (1). Reported across all age groups, EoE is clinically characterized by eating difficulties and a failure to thrive, particularly in children, chest and/or abdominal pain, dysphagia, food impaction, and the lack of response to proton pump inhibitor (PPI) therapy (1,2).

[1]Research, ProPath, Dallas, Texas, USA; [2]Gastrointestinal Pathology Division, ProPath, Dallas, Texas, USA; [3]Texas Digestive Disease Consultants, Southlake, Texas, USA. **Correspondence:** Donna J. Lager, MD, Gastrointestinal Pathology Division, ProPath, 1355 River Bend Dr., Dallas, Texas 75247, USA. E-mail: Donna.lager@propath.com

Data from this study was presented in part at the annual meeting of the United States and Canadian Academy of Pathology in March, 2013 in Baltimore, MD.
Received 20 June 2013; accepted 29 November 2013

Commonly associated with allergic or atopic diseases, studies have shown that EoE is associated with immune-mediated hypersensitivity reactions to various food and environmental allergens (1,3,4). Endoscopic features of EoE include a diminished vascular pattern, linear furrowing, surface white spots or exudate, and proximal corrugated rings or trachealization (1,4). However, in a study by Liacouras *et al.* (5) of 381 pediatric patients with histologic evidence of EoE, 32% of patients had biopsies from endoscopically unremarkable mucosa. Other studies have also demonstrated endoscopically normal mucosa in patients with EoE (4–8).

EoE was initially reported in an adult patient with achalasia in 1978 (9) and was further characterized in 1993 in a report of 12 patients with numerous intraepithelial eosinophils on biopsy and normal acid exposure on 24-h pH monitoring (10). Originally considered quite rare (10,11), epidemiological reports confirm up to a fourfold increase in the diagnosis of EoE in the last decade, with an estimated prevalence of 3 or 4 cases per 10,000 people (11). EoE has been reported worldwide and throughout the human lifespan, with a strong male prevalence (8,11,12).

The endoscopic features of EoE are not pathognomonic (1,4,7), therefore esophageal biopsy is necessary to establish the presence of increased numbers of intraepithelial eosinophils and other features of EoE, which are supported by clinical symptoms and endoscopic features. However, due to the heterogeneous, patchy distribution of esophageal involvement, multiple biopsy samples are necessary to either detect or rule out EoE (7,11,13) (**Figure 1**). Furthermore, lack of a diagnostic standard impedes the ability to accurately compare studies on biopsy practices for the detection of EoE (7,13,14). Dellon *et al.* (8) examined the variability of diagnostic criteria for EoE in the literature and found at least 10 histologic threshold points of esophageal eosinophilia (ranging from 5 to 30 eosinophils/high-power field (EOS/HPF)), definitions of HPF that could render a variability of eosinophil density up to 23-fold, and disparity among esophageal biopsy protocols and methods of counting eosinophils. Current consensus on the diagnostic criteria for EoE includes the presence of ≥15 EOS/HPF in 2–4 biopsy samples taken from both the proximal and distal esophagus in the absence of other causes of esophageal eosinophilia, and lack of a clinical response to proton pump inhibitor (PPI) therapy (1,15). Consequently, unless a previous PPI therapy trial is conducted to rule out GERD, biopsies from the lower esophagus alone may not be sufficient to exclude reflux as the cause of increased eosinophils. Because of the variability in the distribution of intraepithelial eosinophils among biopsy fragments and the lack of standardized biopsy practices, we sought to determine the optimum number of esophageal biopsies from the mid and distal esophagus that were needed to support a morphologic diagnosis of EoE based on numbers of intraepithelial eosinophils.

METHODS

Patient/specimen selection

From 5 January 2009 to 26 September 2011, 771 adult patients were diagnosed with EoE at our institution according to the current consensus criteria for diagnosis (1,15). From that patient population, 610 were excluded because they did not have both a mid and lower biopsy, leaving 161 cases. A total of 39 more cases were excluded because the submitted vial contained biopsies from multiple sites, such that each biopsy's location in the esophagus could not be determined. Fourteen more were excluded because the patient had a previous diagnosis of EoE, and six final cases were excluded because the biopsy contained only glandular or gastric mucosa instead of esophageal squamous mucosa. Consequently, tissues from the remaining 102 cases with both mid and distal esophageal biopsies were chosen for further study. The Western Institutional Review board determined this study to be exempt under 45 CFR.101(b) (4).

Procedure for counting pieces

The original hematoxylin and eosin (H&E)-stained slides were reviewed, and the number of biopsy pieces containing viable squamous mucosa was recorded. The slides for each specimen were reviewed at low power (Nikon eclipse 50iPOL microscope, Nikon Instruments, Tokyo, Japan) to determine which of the three routine levels per specimen showed the most complete sectioning of

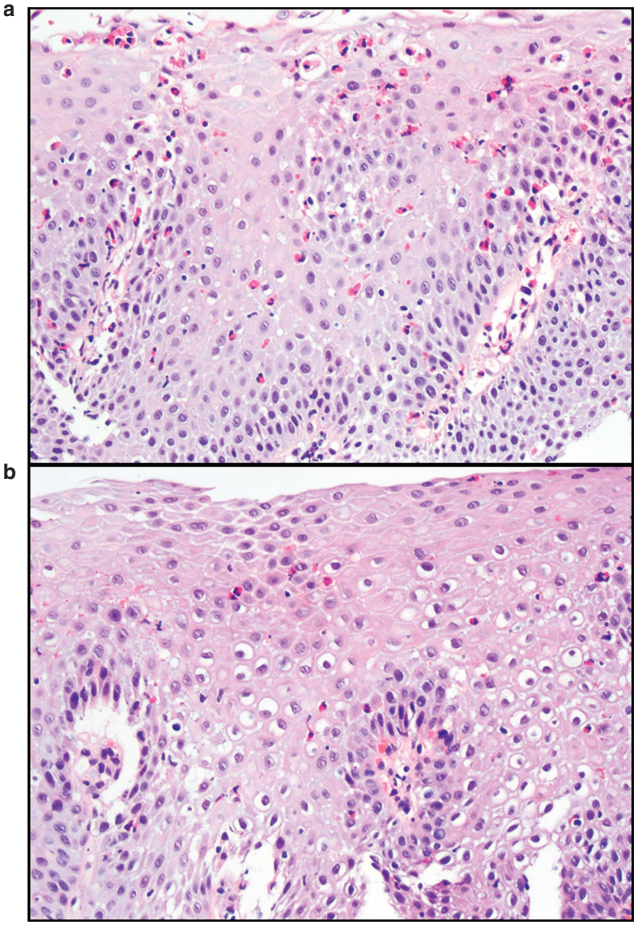

Figure 1. The heterogeneous distribution of eosinophilic esophagitis. Two fragments from the same biopsy with numerous (**a**) and fewer (**b**) intraepithelial eosinophils, demonstrating the patchiness of the disease.

all pieces on the slide. Routinely, three levels are cut such that the first sections are taken at ~25% of the full thickness, the second at 50%, and the third at 75%. All pieces and levels were observed and used in the pathologists' original diagnosis of EoE. Because all the pieces embedded in paraffin are different sizes, the first level the histotechnologist cuts may not completely sample every piece of tissue. Consequently, in our procedure for counting pieces the number of biopsy pieces was counted at the most complete level, which was usually the deepest. The tissue levels on each slide were compared, to assure that two adjacent pieces were not originally one piece that was broken during processing or that had not been completely sectioned. For example, two pieces with their surfaces opposed or two pieces with the surface of one adjacent to the deep edge of another were counted as two pieces, whereas two pieces aligned horizontally but separated by a small space were considered to be one piece for the study. All of the pieces from the most complete level were used in counting eosinophils in order to evaluate the largest amount of tissue possible.

Procedure for counting eosinophils

The original diagnosis of EoE was made by one of three pathologists (D.J.L., M.L., or C.A.R.) at the time of biopsy. The biopsy pieces on each slide were subsequently reviewed by J.A.N. at medium power to identify the areas of highest eosinophil density. The intraepithelial eosinophils/HPF (field diameter = 0.52 mm, field area = 0.21 mm^2) on each biopsy piece were counted by a trained investigator (J.A.N.) using a ×40 objective and ×10 oculars in three fields of highest eosinophil density. Occasionally small pieces did not span three HPF, and in these pieces, fewer than three HPF were counted and recorded. Degranulated eosinophils were counted by grouping cytoplasmic material around the closest nucleus. The number of eosinophils in densely packed areas such as microabscesses was estimated by counting nuclei. Eosinophils detached from the main tissue fragment and those in the submucosa were not counted. The number of biopsy pieces and eosinophil counts were reviewed and agreed upon by a second investigator (D.J.L.). Disagreements were resolved by a reassessment by both parties.

Calculations

The number of eosinophils in each of the three HPFs was recorded for each piece from biopsies of mid and distal esophagus. The average and peak numbers of eosinophils/HPF from the mid and distal esophageal biopsies were compared. Calculations were performed with several thresholds of EOS/HPF, including ≥15, ≥20, ≥25, and ≥30. Statistical correlation was made using online T-test calculator http://studentsttest.com/.

RESULTS

The number of biopsy pieces obtained from the mid esophagus ranged from 1 to 20 (mean 7; median 7) and from the distal esophagus from 1 to 18 (mean 6; median 5). The distribution of the number of biopsy pieces from mid and distal esophagus is shown in **Figure 2**.

Of the 1,342 total esophageal biopsy pieces that were submitted and counted, 841 pieces met the minimal established histological criteria for EoE (assuming a diagnostic threshold of ≥15 EOS/HPF). By using these criteria, one biopsy had a calculated sensitivity of 62.6%, which increased to 94.8, 98.1, and 99.3% after three, four, and five biopsies, respectively. Using higher thresholds of ≥20, ≥25, and ≥30 eosinophils/HPF, necessitated an increased number of biopsy pieces to reach the same diagnostic sensitivity.

Of the 742 total esophageal biopsy pieces submitted and counted from just the mid esophagus, 455 pieces met the minimal established histological criteria for EoE (assuming a diagnostic threshold of ≥15 EOS/HPF). By using these criteria, one biopsy had a calculated sensitivity of 61.3%, which increased to 94.2%, 97.8%, and 99.1% after three, four, and five biopsies, respectively. Using higher thresholds of ≥20, ≥25, and ≥30 eosinophils/HPF, increased the number of biopsy pieces that were necessary to reach the same diagnostic sensitivity (**Figure 3**).

Of the 600 total esophageal biopsy pieces submitted and counted from just the distal esophagus, 386 pieces met the minimal established histological criteria for EoE (assuming a diagnostic threshold of ≥15 EOS/HPF). By using these criteria, one biopsy had a calculated sensitivity of 64.3%, which increased to 95.5%, 98.4%, and 99.4% after three, four, and five biopsies, respectively. Using higher thresholds of ≥20, ≥25, and ≥30 eosinophils/HPF increased the number of biopsy pieces necessary to reach the same diagnostic sensitivity.

There were greater peak counts of EOS/HPF in the mid esophageal biopsies (range, 0–272; mean, 69) compared with those from the distal esophagus (range, 1–272; mean, 60). This difference was not statistically significant; however, a greater percentage of biopsies from the distal esophagus met the diagnostic criteria of ≥15 EOS/HPF.

DISCUSSION

Assessing the optimal number and location of gastrointestinal mucosal biopsies has become important in the clinical management of several inflammatory, metaplastic, and dysplastic

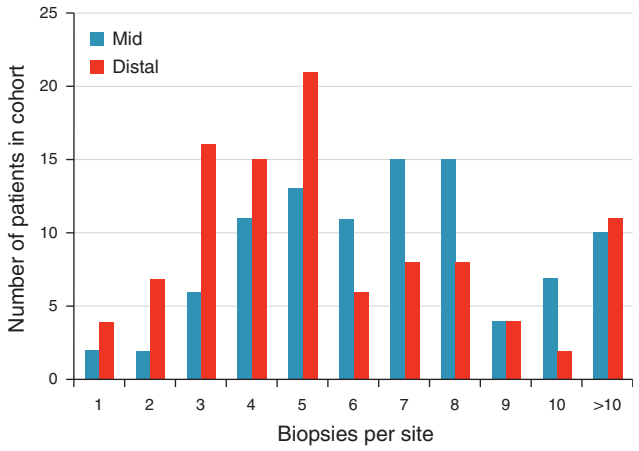

Figure 2. The number of patients in eosinophilic esophagitis cohort and their respective number of biopsies per site (mid and distal).

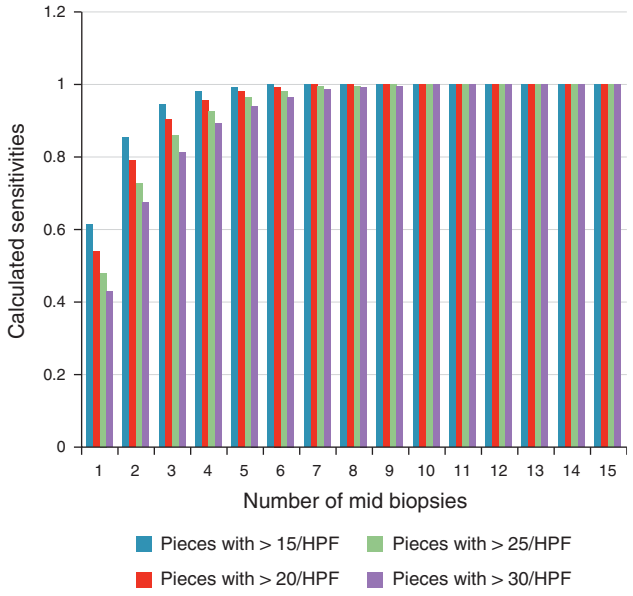

Figure 3. The calculated sensitivities of the number of biopsies taken from the mid esophagus at diagnostic thresholds of >15, >20, >25, and >30 number of eosinophils per high power field (EOS/HPF).

disorders of the gastrointestinal tract. Yantiss *et al.* (7) summarized the current recommendations for several of these disorders, such as EoE (13–15), Barrett's esophagus (16–18), chronic gastritis (19,20), celiac disease (21–23), microscopic colitis (24,25), and inflammatory bowel disease (26,27). Two studies similar to ours correlated diagnostic sensitivity with the number of biopsy pieces in adult and pediatric patients, respectively, with EoE (13,14).

Gonsalves *et al.* (13) reviewed charts from 66 adult patients diagnosed with EoE (based on ≥15 EOS/HPF) and subsequently examined the corresponding 341 esophageal biopsy specimens. The median EOS/HPF was 107 (range, 0–557 EOS/HPF), calculated from an average of five HPFs of 0.44 mm diameter each. The likelihood of detecting EoE with one biopsy was 55%, which reached 100% after five biopsies. However, because biopsy location was known in only 40 of these patients, it is difficult to compare this sensitivity calculation with the results from other studies. Subgroup analysis was conducted in only 20 patients to assess the variability of esophageal eosinophilia between distal specimens (taken within 5 cm from the squamocolumnar junction) and proximal specimens (taken 10 cm proximal to the distal site). Although the density of esophageal eosinophilia was found to be greater in distal biopsies (mean, 82 EOS/HPF; range, 23–557 EOS/HPF) than it was in the proximal biopsies (mean, 68 EOS/HPF; range, 0–456 EOS/HPF), the difference was not great enough to be statistically significant. This study recommends that at least five biopsies be taken from a variety of esophageal locations to optimize detection (13). A subsequent study by the same group concluded that this original study was "limited in that patients with GERD were not systematically excluded by means of a trial of PPI therapy or pH testing" (14).

Subsequently, Shah *et al.* (14) evaluated 221 esophageal biopsy specimens from 30 pediatric patients diagnosed with EoE on the basis of persistent esophageal eosinophilia of ≥15 EOS/HPF after 8 weeks of PPI therapy. All patients had biopsies taken from the mid and distal esophagus (8–10 cm and 2–3 cm proximal to the gastroesophageal junction, respectively). Of the 22 patients with a negative pH probe study, the median EOS/HPF was 37 (range: 0–288 EOS/HPF), calculated from an average of five HPFs of 0.4 mm^2 each. One biopsy detected EoE with a sensitivity of 73%, which reached 100% after six biopsies. Histologic analysis of the mid and distal biopsies demonstrated more eosinophils in the distal esophagus than it did in the mid esophagus, but this difference was not statistically significant. Interestingly, six patients would not have met the diagnostic criteria (using 15 EOS/HPF) if in five (17%) of them only mid esophageal and in one patient (3%) only distal easophageal biopsies had been taken. In this study, a diagnosis of EoE was made on a biopsy from the distal esophagus alone because these patients previously underwent PPI trial therapy, excluding GERD as a differential diagnosis. As a result of these findings, it was recommended that three biopsy pieces be taken from the distal esophagus and three additional pieces from the mid esophagus.

To our knowledge, our study is the largest to date that seeks to determine the optimal number and location of esophageal biopsies that are necessary to meet morphologic criteria for a diagnosis of EoE. Many factors can influence this calculated sensitivity, including the methods for determining a cohort population, the biopsy protocol of the gastroenterologist, the diagnostic threshold of EOS/HPF, and the counting style of the pathologist (8).

Adult patients with EoE usually present with chief complaints related to esophageal dysfunction, the most common of which is dysphagia (1). The subtlety of gross endoscopic findings is considered one of the reasons EoE has been under-recognized in the past (4). A correlation between symptom severity, endoscopic findings, and the density of esophageal eosinophilia is still controversial (1,10,13,14,28,29). In a population of 222 patients presenting with dysphagia, who had subsequent endoscopy and esophageal biopsy, only 15% showed histologic features of EoE. Furthermore, only 8 of 21 (38%) patients with endoscopic features of EoE actually met histologic criteria for the diagnosis (30). Not only can pediatric and adult populations present differently (8), but up to 32% of patients with EoE have completely normal-appearing esophageal mucosa endoscopically (5,7,30). Therefore, patient demographics and the method of selecting the patient cohort can affect the results of the study (14). Considering the myriad of non-pathognomonic clinical symptoms (dysphagia, food impaction, reflux, abdominal pain, nausea, vomiting) and uncorrelated endoscopic findings (rings, strictures, corrugated surfaces, crepe-paper mucosa, linear furrowing, white exudes) (1,4), a diagnosis of EoE requires histological examination. However, the diagnostic parameters have unfortunately been applied differently among investigators (8).

The biopsy protocol among gastroenterologists can vary by location in the esophagus, the number of biopsies taken, and how the samples are submitted to the laboratory. It has been suggested that targeting esophageal biopsies to areas with the most involved-appearing mucosa (such as white plaques or exudates) may increase the diagnostic sensitivity of detecting EoE, compared with obtaining biopsies at predetermined distances regardless of the mucosal

condition (4,13,14); however, this has yet to be confirmed. It is also unclear whether or not there is a uniform distribution of eosinophilia in the esophagus in EoE (13), or if there is a higher density in the distal esophagus (5,31). Of studies that describe biopsy protocols, one review found that 35% gastroenterologists biopsy the distal esophagus alone, whereas 65% biopsy multiple levels (8). Our data and that of other studies demonstrate that although there is an increased number of eosinophils in the distal esophagus of patients with EoE compared with other regions of the esophagus, the difference is not statistically significant (13,14). In a study to assess the utility of proximal esophageal biopsies in establishing a diagnosis of EoE, Lee *et al.* (32) found that the diagnosis would have been missed in 4 out of 23 (17%) cases, if proximal biopsies had not been taken and a diagnosis had consequently been made on distal biopsies alone. Our study supports this concept that taking biopsies from places other than the distal esophagus, will increase the diagnostic yield and is especially necessary in patients not known to have previously undergone PPI therapy (1,7,33).

Protocols determining the sites within the esophagus and the number of biopsies to be obtained per site vary significantly among gastroenterologists. In one review of 116 original articles on the biopsy practices of gastroenterologists in diagnosing EoE, only 12 studies (10%) documented the exact number and location of the biopsy specimens such that the study design could be repeated (8). We determined the optimal number of biopsies to obtain in order to maximize the diagnostic yield in patients with EoE and calculated a sensitivity of around 60%, which is similar to previous reports (1,13,14). When three, four, and five biopsies were taken, this detection rate increased to 0.95, 0.98, and 0.99, such that taking more than six biopsies does not increase diagnostic yield. Because there was no significant difference between the EOS/HPF in the mid vs. the distal biopsies and because the patients in our cohort had not undergone a previous PPI trial therapy, we recommend that biopsies from sites other than the distal esophagus be taken in order to exclude reflux esophagitis as the cause for increased esophageal eosinophils.

There is also variation of the style of specimen submission among gastroenterologists. For instance, biopsies from the proximal and distal esophagus may be submitted to the pathology laboratory in the same specimen container, or the biopsy site may not be indicated. In the current study, 39 cases were not evaluated because the mid and distal esophageal biopsies were submitted in the same vial such that their respective sites could not be determined, and 97 additional specimens were submitted as "esophagus—not specified." Standardization of biopsy submission and labeling practices in cases of suspected EoE are also important in differentiating EoE from GERD morphologically with pathology specimens.

Perhaps the largest source of variability in diagnostic criteria for EoE is the method of counting and evaluating intraepithelial eosinophils. More than 10 different histological thresholds including >5, >7, >10, ≥15, 15-20, ≥20, 20-24, ≥24, ≥25, and ≥30 EOS/HPF have been used to diagnose EoE, the most common of which were ≥15 and ≥20 EOS/HPF (8). A magnification of ×400 (×40 objective × ×10 oculars) is fairly typical across studies, however because microscopes have different viewing areas, the exact area of an HPF is not standardized and is reported to range from 0.12 to 0.44 mm^2. This translates into a range of intraepithelial eosinophil densities from 34 to 125 EOS/mm^2, which is a 3.7-fold variation. Therefore, considering the span of published thresholds mentioned above, there is potential for a 23-fold difference of EOS/mm^2 between studies (8). After diagnostic guidelines for EoE were published in 2007 (15), the variability of eosinophil density used in studies decreased significantly, conforming more closely to the criteria suggested by consensus (34). In our study, we used the current consensus criteria of ≥15 EOS/HPF and defined one HPF as 0.21 mm^2 arriving at a density of 71 EOS/mm^2, which is within the range of other reported studies (8). In addition, the number of HPFs counted ranges in the literature from 1 to 10, some using a mean count and others the densest HPF (8). In a study by Lai *et al.* (35), the use of the peak count per HPF was more sensitive but less specific than the highest average count in identifying EoE.

A few studies have assessed the inter- and intraobserver reliability in determining eosinophil counts in the GI tract, with very high correlations up to 0.96 (36–38). Some investigators suggest that specific immunohistochemical stains (such as Luna eosinophil granule (LEG) (35) or eosinophil peroxidase-specific antibody directed against eosinophil secondary granule protein (39)) could be more sensitive in detecting EoE than traditional H&E slides, which potentially underestimate the number of eosinophils (40). In a study using digital images, Dellon *et al.* (36) not only demonstrated "substantial" to "near-perfect" correlation between the eosinophil counts in EoE populations among three pathologists but also established an excellent correlation between glass slides and digital images ($P < 0.001$ for all correlations).

CONCLUSIONS

The results of this study demonstrate that four or five biopsy fragments should be submitted to optimize the chances of achieving the morphologic criteria for a diagnosis of EoE, and that the yield is not increased beyond six biopsy fragments. Although there was no statistically significant difference in EOS/HPF between the mid and distal esophagus, biopsy of sites other than the distal esophagus is necessary to exclude the possibility that the increase of eosinophils is due to reflux esophagitis because our patient cohort did not undergo previous PPI trial therapy. In conclusion, unless GERD has already been ruled out, gastroenterologists should submit four or five biopsies from the mid or proximal esophagus in separate containers from each site to the pathology laboratory for evaluation to establish a morphologic diagnosis of EoE. Biopsies from the distal esophagus may also be submitted to evaluate for reflux esophagitis, but distal biopsies alone are not sufficient to establish a morphologic diagnosis of EoE.

CONFLICT OF INTEREST

Guarantor of the article: Donna J. Lager, MD.

Specific author contributions: Jennifer A. Nielsen: acquisition and tabulation of data, administrative support, drafting of the manuscript, final approval of the article; Donna J. Lager: conception and design, analysis and interpretation of histology, critical revision of

the manuscript for important intellectual content, final approval of the article; Matthew Lewin: conception and design, final approval of the article; Gabriel Rendon: analysis and interpretation of endoscopy, final approval of the article; Cory A. Roberts: conception and design, final approval of the article.

Financial support: This study was funded by ProPath Services, LLP.

Potential competing interests: None.

Study Highlights

WHAT IS CURRENT KNOWLEDGE

- ✓ Eosinophilic esophagitis (EoE) is characterized clinically by dysphagia, chest pain, food impaction, and no remarkable response to PPI therapy and morphologically by increased numbers of intraepithelial eosinophils and marked basal hyperplasia.
- ✓ EoE is patchy and shares many features of gastroesophageal reflux disease.
- ✓ According to consensus, a minimum of ≥15 EOS/HPF is necessary to diagnose EoE histologically.

WHAT IS NEW HERE

- ✓ We recommend that between 4 and 6 biopsies be taken from the middle or proximal esophagus to rule out EoE in cohorts with unknown PPI trial therapy status.
- ✓ This study supports the finding that additional biopsies from the lower esophagus do not increase the sensitivity of detecting EoE, unless GERD has already been ruled out by PPI therapy.

REFERENCES

1. Liacouras CA, Furuta GT, Hirano I *et al.* Eosinophilic esophagitis: updated consensus recommendations for children and adults. J Allergy Clin Immunol 2011;128:3–20 e26; quiz 21, 22.
2. Abonia JP, Rothenberg ME. Eosinophilic esophagitis: rapidly advancing insights. Annu Rev Med 2012;63:421–34.
3. Spergel JM, Andrews T, Brown-Whitehorn TF *et al.* Treatment of eosinophilic esophagitis with specific food elimination diet directed by a combination of skin prick and patch tests. Ann Allergy Asthma Immunol 2005;95:336–43.
4. Fox VL. Eosinophilic esophagitis: endoscopic findings. Gastrointest Endosc Clin N Am 2008;18:45–57; viii.
5. Liacouras CA, Spergel JM, Ruchelli E *et al.* Eosinophilic esophagitis: a 10-year experience in 381 children. Clin Gastroenterol Hepatol 2005;3:1198–206.
6. Chang F, Anderson S. Clinical and pathological features of eosinophilic oesophagitis: a review. Pathology 2008;40:3–8.
7. Yantiss RK, Odze RD. Optimal approach to obtaining mucosal biopsies for assessment of inflammatory disorders of the gastrointestinal tract. Am J Gastroenterol 2009;104:774–83.
8. Dellon ES, Aderoju A, Woosley JT *et al.* Variability in diagnostic criteria for eosinophilic esophagitis: a systematic review. Am J Gastroenterol 2007;102:2300–13.
9. Landres RT, Kuster GG, Strum WB. Eosinophilic esophagitis in a patient with vigorous achalasia. Gastroenterology 1978;74:1298–301.
10. Attwood SE, Smyrk TC, Demeester TR *et al.* Esophageal eosinophilia with dysphagia. A distinct clinicopathologic syndrome. Dig Dis Sci 1993;38:109–16.
11. Gonsalves N. Eosinophilic esophagitis: history, nomenclature, and diagnostic guidelines. Gastrointest Endosc Clin N Am 2008;18:1–9; vii.
12. Straumann A, Simon HU. Eosinophilic esophagitis: escalating epidemiology? J Allergy Clin Immunol 2005;115:418–9.
13. Gonsalves N, Policarpio-Nicolas M, Zhang Q *et al.* Histopathologic variability and endoscopic correlates in adults with eosinophilic esophagitis. Gastrointest Endosc 2006;64:313–9.
14. Shah A, Kagalwalla AF, Gonsalves N *et al.* Histopathologic variability in children with eosinophilic esophagitis. Am J Gastroenterol 2009;104:716–21.
15. Furuta GT, Liacouras CA, Collins MH *et al.* Eosinophilic esophagitis in children and adults: a systematic review and consensus recommendations for diagnosis and treatment. Gastroenterology 2007;133:1342–63.
16. Harrison R, Perry I, Haddadin W *et al.* Detection of intestinal metaplasia in barrett's esophagus: an observational comparator study suggests the need for a minimum of eight biopsies. Am J Gastroenterol 2007;102:1154–61.
17. Chandrasoma PT, Der R, Dalton P *et al.* Distribution and significance of epithelial types in columnar-lined esophagus. Am J Surg Pathol 2001;25:1188–93.
18. Abela JE, Going JJ, Mackenzie JF *et al.* Systematic four-quadrant biopsy detects barrett's dysplasia in more patients than nonsystematic biopsy. Am J Gastroenterol 2008;103:850–5.
19. Dixon MF, Genta RM, Yardley JH *et al.* Classification and grading of gastritis. The updated sydney system. International workshop on the histopathology of gastritis, Houston 1994. Am J Surg Pathol 1996;20:1161–81.
20. Eriksson NK, Farkkila MA, Voutilainen ME *et al.* The clinical value of taking routine biopsies from the incisura angularis during gastroscopy. Endoscopy 2005;37:532–6.
21. Oxentenko AS, Grisolano SW, Murray JA *et al.* The insensitivity of endoscopic markers in celiac disease. Am J Gastroenterol 2002;97:933–8.
22. Ravelli A, Bolognini S, Gambarotti M *et al.* Variability of histologic lesions in relation to biopsy site in gluten-sensitive enteropathy. Am J Gastroenterol 2005;100:177–85.
23. Bonamico M, Mariani P, Thanasi E *et al.* Patchy villous atrophy of the duodenum in childhood celiac disease. J Pediatr Gastroenterol Nutr 2004;38:204–7.
24. Thijs WJ, van Baarlen J, Kleibeuker JH *et al.* Microscopic colitis: prevalence and distribution throughout the colon in patients with chronic diarrhoea. Neth J Med 2005;63:137–40.
25. Matteoni CA, Wang N, Goldblum JR *et al.* Flexible sigmoidoscopy for the detection of microscopic colitis. Am J Med 2000;108:416–8.
26. Rubin CE, Haggitt RC, Burmer GC *et al.* DNA aneuploidy in colonic biopsies predicts future development of dysplasia in ulcerative colitis. Gastroenterology 1992;103:1611–20.
27. Itzkowitz SH, Present DH. Consensus conference: Colorectal cancer screening and surveillance in inflammatory bowel disease. Inflamm Bowel Dis 2005;11:314–21.
28. Aceves SS, Ackerman SJ. Relationships between eosinophilic inflammation, tissue remodeling, and fibrosis in eosinophilic esophagitis. Immunol Allergy Clin North Am 2009;29:197–211 xiii-xiv.
29. Pentiuk S, Putnam PE, Collins MH *et al.* Dissociation between symptoms and histological severity in pediatric eosinophilic esophagitis. J Pediatr Gastroenterol Nutr 2009;48:152–60.
30. Prasad GA, Talley NJ, Romero Y *et al.* Prevalence and predictive factors of eosinophilic esophagitis in patients presenting with dysphagia: a prospective study. Am J Gastroenterol 2007;102:2627–32.
31. Noel RJ, Putnam PE, Collins MH *et al.* Clinical and immunopathologic effects of swallowed fluticasone for eosinophilic esophagitis. Clin Gastroenterol Hepatol 2004;2:568–75.
32. Lee S, de Boer WB, Naran A *et al.* More than just counting eosinophils: proximal oesophageal involvement and subepithelial sclerosis are major diagnostic criteria for eosinophilic oesophagitis. J Clin Pathol 2010;63:644–7.
33. Furuta GT. Eosinophilic esophagitis: update on clinicopathological manifestations and pathophysiology. Curr Opin Gastroenterol 2011;27:383–8.
34. Sperry SL, Shaheen NJ, Dellon ES. Toward uniformity in the diagnosis of eosinophilic esophagitis (eoe): The effect of guidelines on variability of diagnostic criteria for eoe. Am J Gastroenterol 2011;106:824–32; quiz 833.
35. Lai AL, Girgis S, Liang Y *et al.* Diagnostic criteria for eosinophilic esophagitis: a 5-year retrospective review in a pediatric population. J Pediatr Gastroenterol Nutr 2009;49:63–70.
36. Dellon ES, Fritchie KJ, Rubinas TC *et al.* Inter- and intraobserver reliability and validation of a new method for determination of eosinophil counts in patients with esophageal eosinophilia. Dig Dis Sci 2010;55:1940–9.
37. Kelly KJ, Lazenby AJ, Rowe PC *et al.* Eosinophilic esophagitis attributed to gastroesophageal reflux: improvement with an amino acid-based formula. Gastroenterology 1995;109:1503–12.
38. Lowichik A, Weinberg AG. A quantitative evaluation of mucosal eosinophils in the pediatric gastrointestinal tract. Mod Pathol 1996;9:110–4.
39. Protheroe C, Woodruff SA, de Petris G *et al.* A novel histologic scoring system to evaluate mucosal biopsies from patients with eosinophilic esophagitis. Clin Gastroenterol Hepatol 2009;7:749–55 e711.
40. Muller S, Puhl S, Vieth M *et al.* Analysis of symptoms and endoscopic findings in 117 patients with histological diagnoses of eosinophilic esophagitis. Endoscopy 2007;39:339–44.