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Original contribution

Determination of esophageal eosinophil counts and other histologic features of eosinophilic esophagitis by pathology trainees is highly accurate☆,☆☆

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Summary Many studies of eosinophilic esophagitis (EoE) use expert pathology review, but it is unknown whether less experienced pathologists can reliably assess EoE histology. We aimed to determine whether trainee pathologists can accurately quantify esophageal eosinophil counts and identify associated histologic features of EoE, as compared with expert pathologists. We used a set of 40 digitized slides from patients with varying degrees of esophageal eosinophilia. Each of 6 trainee pathologists underwent a teaching session and used our validated protocol to determine eosinophil counts and associated EoE findings. The same slides had previously been evaluated by expert pathologists, and these results comprised the criterion standard. Eosinophil counts were correlated, and agreement was calculated for the diagnostic threshold of 15 eosinophils per high-power field as well as for associated EoE findings. Peak eosinophil counts were highly correlated between the trainees and the criterion standard (ρ ranged from 0.87 to 0.92; $P < .001$ for all). Peak counts were also highly correlated between trainees (0.75-0.91; $P < .001$), and results were similar for mean counts. Agreement was excellent for determining if a count exceeded the diagnostic threshold (κ ranged from 0.83 to 0.89; $P < .001$). Agreement was very good for eosinophil degranulation ($\kappa = 0.54$-0.83; $P < .01$) and spongiosis ($\kappa = 0.44$-0.87; $P < .01$) but was lower for eosinophil microabscesses ($\kappa = 0.37$-0.64; $P < .01$). In conclusion, using a teaching session, digitized slide set, and validated protocol, the agreement between pathology trainees and expert pathologists for determining eosinophil counts was excellent. Agreement was very good for eosinophil degranulation and spongiosis but less so for microabscesses.

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1. Introduction

Eosinophilic esophagitis (EoE) is an allergy/immune-mediated condition defined clinically by symptoms of esophageal dysfunction and histologically by eosinophilic infiltration of the esophageal mucosa [1,2]. The clinical presentation consists of a spectrum of symptoms depending on age, with children having feeding difficulties, failure to thrive, vomiting, heartburn, and abdominal pain, and with adolescents and adults complaining of dysphagia and food impaction [3]. A subsequent esophagogastroduodenoscopy is required to obtain esophageal biopsy samples. The hallmark pathologic feature of EoE is a peak eosinophil count of at least 15 eosinophils per high-power field (eos/HPF), which persists after high-dose proton pump inhibitor and after other potential causes of secondary esophageal and systemic eosinophilia have been excluded [1,2,4,5]. In addition to the presence of eosinophils, there are a number of other associated histologic findings. Although these are not specific for EoE, they include epithelial spongiosis, basal layer hyperplasia, lamina propria fibrosis, eosinophilic microabscesses, eosinophilic degranulation, and superficial infiltration of the epithelium [4,5]. However, all findings may not be noted in a single biopsy [6-9].

The histopathologic findings of EoE are well described, and recently, a new summary score has been developed and validated for use in EoE that may be more accurate than the eosinophil count alone [10]. However, much of the research on EoE has been performed at referral centers in conjunction with expert pathology review [11-20]. There are few studies of the reproducibility of determining eosinophil counts and assessing the other associated findings outside this setting [21-23]. For example, we have previously validated a protocol for determining eosinophil counts with excellent interobserver and intraobserver reliability, but these results were limited to expert gastrointestinal pathologists [21]. It is currently unknown how well this protocol would perform outside an expert setting. However, it is important to understand how diagnosis could translate to other settings.

The aim of this study was to determine whether nonexpert trainee pathologists could accurately quantify esophageal eosinophil counts and identify associated histologic features of EoE, as compared with expert pathologists.

2. Materials and methods

2.1. Patients and pathology samples

This study used a previously constructed set of esophageal biopsy samples from 40 patients in the University of North Carolina EoE clinicopathological database. These were selected specifically to represent a wide spectrum of eosinophilia (ranging from 0 to 400 eos/HPF), and the methodology for selecting these patients has been described previously [21,24-27]. Archived slides were deidentified (and there was no linked reference to clinical or endoscopic findings) and scanned using the Aperio ScanScope CS slide scanner (Aperio Technologies, Vista, CA). The digitized slides were evaluated by 6 second- and third-year pathology residents after a training session as detailed below. The images were viewed using the Aperio ImageScope software (12.1.0.5029) (Aperio Technologies). This study was approved by the University of North Carolina institutional review board.

2.2. Training curriculum

Before analysis of the 40-patient slide set, each trainee pathologist reviewed our previously published protocol for determination of eosinophilic counts and associated histologic findings of EoE [21]. In addition, each pathologist participated in a teaching session to acclimate to the Aperio software (Aperio Technologies), quantify eosinophil counts, characterize the eosinophil infiltrate, and identify microabscesses, eosinophil degranulation, spongiosis, and lamina propria fibrosis. Questions could be addressed to the senior study pathologist (J. T. W.) or the senior author (E. S. D.) during this time but not during the evaluation of the study slide set. The pathology residents were either in their second or in their early third year of pathology training at the time of participation.

2.3. Histologic analysis

Our previously validated protocol, which also showed excellent agreement between scanned and glass slides, was used for analysis [8,21]. In brief, after reviewing the entire biopsy sample, 5 HPFs on the digitized slides were evaluated for maximum eosinophil density (eos/mm^2). Trainees were instructed to quantify eosinophils in the most highly inflamed field, in the second most highly inflamed field, and in 3 additional fields that were representative of the biopsy overall. The field was selected on the computer screen using the ImageScope software (Aperio Technologies), and this provided an area (mm^2). The eosinophil density (eos/mm^2) was then converted to an eosinophil count (eos/HPF) for an assumed HPF size of 0.24 mm^2, which is the most commonly reported HPF size in the literature [28].

Next, each HPF was evaluated for the other histologic features of EoE. These included the presence of eosinophilic microabscesses (defined as clusters of ≥4 eos), eosinophilic degranulation (defined as release of eosinophilic granules from eosinophils into the surrounding epithelium), basal layer hyperplasia (expansion of the basal zone by >25% of epithelial height; evaluated in properly oriented specimens only), spongiosis (also termed *dilated intercellular spaces*), and lamina propria fibrosis (increased deposition of collagen in the lamina propria, if sufficient subepithelial stroma was present for evaluation).

2.4. Statistical analysis

We calculated the peak eosinophil count (highest count of all of the 5 HPFs examined) as well as the mean count of the