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Original article

Newly developed and validated eosinophilic esophagitis histology scoring system and evidence that it outperforms peak eosinophil count for disease diagnosis and monitoring

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SUMMARY. Eosinophilic esophagitis (EoE) is diagnosed by symptoms, and at least 15 intraepithelial eosinophils per high power field in an esophageal biopsy. Other pathologic features have not been emphasized. We developed a histology scoring system for esophageal biopsies that evaluates eight features: eosinophil density, basal zone hyperplasia, eosinophil abscesses, eosinophil surface layering, dilated intercellular spaces (DIS), surface epithelial alteration, dyskeratotic epithelial cells, and lamina propria fibrosis. Severity (grade) and extent (stage) of abnormalities were scored using a 4-point scale (0 normal; 3 maximum change). Reliability was demonstrated by strong to moderate agreement among three pathologists who scored biopsies independently ($P \leq 0.008$). Several features were often abnormal in 201 biopsies (101 distal, 100 proximal) from 104 subjects (34 untreated, 167 treated). Median grade and stage scores were significantly higher in untreated compared with treated subjects ($P \leq 0.0062$). Grade scores for features independent of eosinophil counts were significantly higher in biopsies from untreated compared with treated subjects (basal zone hyperplasia $P \leq 0.024$ and DIS $P \leq 0.005$), and were strongly correlated (R-square >0.67). Principal components analysis identified three principal components that explained 78.2% of the variation in the features. In logistic regression models, two principal components more closely associated with treatment status than log distal peak eosinophil count (PEC) (R-square 17, area under the curve (AUC) 77.8 vs. R-square 9, AUC 69.8). In summary, the EoE histology scoring system provides a method to objectively assess histologic changes in the esophagus beyond eosinophil number. Importantly, it discriminates treated from untreated patients, uses features commonly found in such biopsies, and is utilizable by pathologists after minimal training. These data provide rationales and a method to evaluate esophageal biopsies for features in addition to PEC.

KEY WORDS: eosinophilic esophagitis, pathology, pediatrics, scale.

INTRODUCTION

Eosinophilic esophagitis (EoE), a relapsing chronic disease, is diagnosed by clinical symptoms, lack of response to proton pump inhibitor therapy, and eosinophil-rich

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inflammation in esophageal biopsies.[1–3] Peak eosinophil count (PEC), ≥ 15 intraepithelial eosinophils in at least one high power field (HPF), in an esophageal biopsy is the gold standard for the pathologic portion of the diagnosis. Reduced PEC constitutes an endpoint in clinical trials of therapies for EoE, and is a common goal in clinical management.[1–8] However, rereview of slides with PECs 1–14/HPF yields counts at or above the diagnostic threshold value in >20% of such biopsies, a limitation of using PEC as the sole pathologic diagnostic feature.[9]

Pathology other than eosinophil-rich inflammation has been described in EoE: basal zone hyperplasia

(BZH), dilated intercellular spaces (DIS), and thickened lamina propria fibers (LPF) are commonly seen. Only quantifying eosinophils has been emphasized in the diagnosis and treatment of EoE, yet standard methods to evaluate and quantify other common pathologic features could increase reproducible results (multiple pathologists using uniform methods to count eosinophils in esophageal biopsies achieve nearly perfect interobserver agreement[10]) and diagnostic yield (a comprehensive esophageal biopsy scoring system that includes inflammation and other pathologic variables distinguishes patients who have gastroesophageal reflux disease from control subjects[11]).

The current focus of EoE pathology evaluation is the maximum severity of eosinophil inflammation (EI), i.e. PEC. Severity is an important metric, but the amount of diseased tissue may also be important. Indeed, for other inflammatory diseases affecting the bowel both severity (grade) and extent (stage) of inflammation provide important clinical information.[12–14]

Our objective is to develop and validate a histologic scoring system (HSS) to objectively evaluate grade and stage of multiple pathologic features in esophageal biopsies from patients with EoE. We show that pathologists can successfully use the HSS after minimal training. Importantly, the EoEHSS both provides information not imparted by PEC, and better discriminates esophageal biopsies by EoE therapy status compared with PEC.

MATERIALS AND METHODS

Three EoE cohorts were evaluated: #1 patients ($N = 41$; 79 biopsies, 41 proximal, 38 distal) prospectively recruited for a study correlating PEC with symptoms,[15] some of whom participated in a prospective placebo-controlled study of swallowed fluticasone propionate to treat EoE[4]; #2 well-characterized EoE patients ($N = 40$; 79 biopsies, 40 proximal, 39 distal) prospectively recruited to develop a patient reported outcome scale[16]; #3 randomly selected patients ($N = 23$; 43 biopsies, 20 proximal, 23 distal) undergoing endoscopy as part of ongoing clinical care (July 16-August 21, 2012; see Supporting Information for additional information). Study subjects and/or their legal guardians from cohorts #1 and #2 signed informed consent forms. Cohort #3 was designed to capture a random set of patients; therefore, not all had consented for research. A Health Insurance Portability and Accountability Act (HIPAA) waiver of consent was obtained from the Institutional Review Board (IRB) to assess data of individuals who did not sign consent forms. This study was performed with the approval of the Cincinnati Children's Hospital Medical Center (CCHMC) IRB.

Eight features of esophageal biopsies were defined and evaluated. EI was graded using PEC obtained by
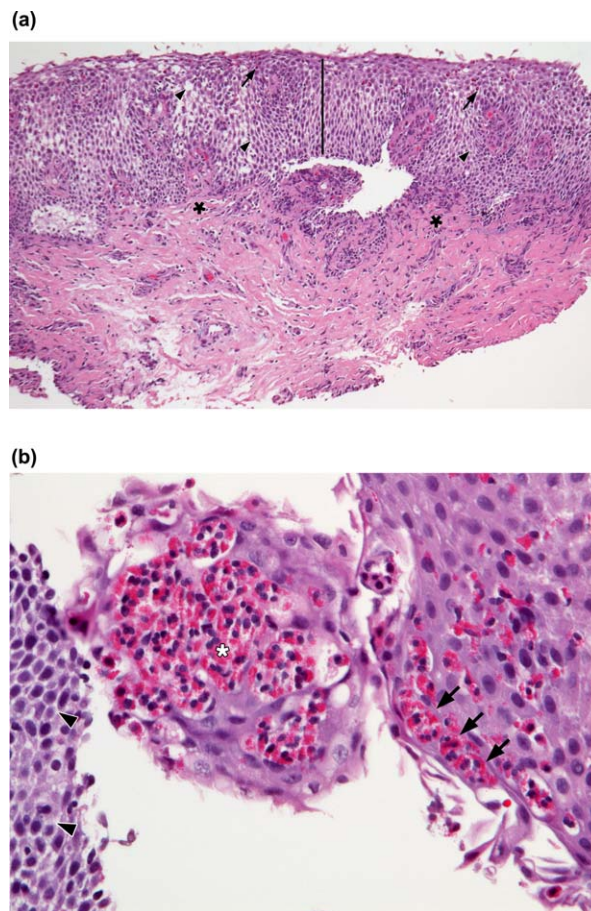

Fig. 1   EoE biopsies. (A) EI is present (arrows). BZH (bar) occupies >66% of total epithelial height (grade 3). Intercellular bridges in DIS (arrowheads) are easily identified at 100X (grade 3). LPF fibers exceed the diameter of basal cell nuclei (asterisks) (grade 3). (B) EA (white asterisk) contains more than 20 eosinophils (grade 3). SL (arrows) consists of 5–10 eosinophils in a row (grade 2). DIS are present (arrowheads).

counting eosinophils in the most densely inflamed HPF (Fig. 1, Supporting Information Fig. S1). Additional features were BZH: >15% of the total epithelial thickness (Fig. 1A); eosinophil abscess (EA): solid mass of intraepithelial eosinophils (Fig. 1B); eosinophil surface layering (SL): linear alignment of eosinophils parallel to the epithelial surface (Fig. 1B); DIS: spaces around squamous epithelial cells that exhibit intercellular bridges (Fig. 1A, Supporting Information Fig. S1); surface epithelial alteration (SEA): surface epithelial cells that exhibit altered tinctorial properties, manifest as dark red staining, with or without intraepithelial eosinophils (Supporting Information Fig. S1A); dyskeratotic epithelial cells (DEC): individual cells with deeply eosinophilic cytoplasm and hyperchromatic nuclei (Supporting Information Fig. S1B); LPF: thickened connective tissue fibers in the lamina propria (Fig. 1A). Each feature was scored separately for grade (severity) or stage (extent) of abnormality using a 4-point scale (0 = normal; 3 = most severe or extensive). Expanded definitions and detailed scoring methods are provided in Supporting Information.
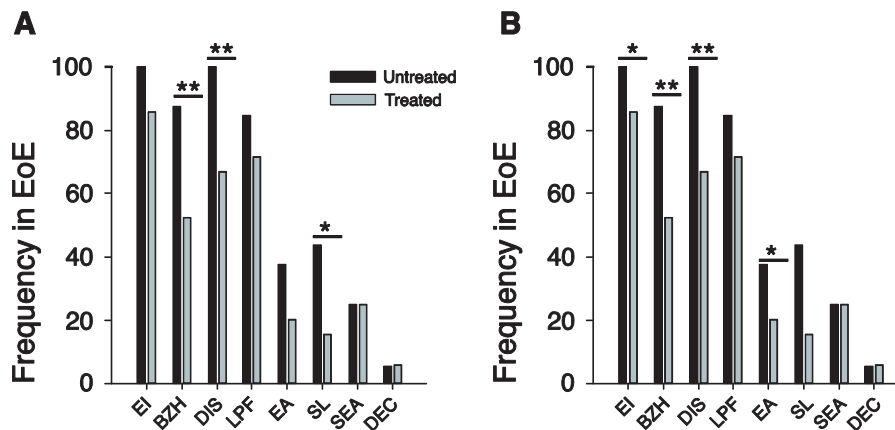
Fig. 2 EoEHSS feature frequency. Frequency of histologic abnormalities in untreated (black bars) and treated (gray bars) in distal (A) and proximal (EoE) biopsies (B). *$P \leq 0.05$, **$P \leq 0.0063$ (Bonferroni multiple testing threshold).

MHC used extensive experience to design the HSS, and scored all biopsies. To determine if others could similarly use the HSS, MHC invited additional CCHMC pathologists (JTB, RS) to apply it to biopsies encountered in their daily practice. MHC verbally described the features to JTB and RS, who stated they comprehended the definitions and the scoring methods, and that the features were present in EoE biopsies and were worthwhile to evaluate, thus providing face and content validity of the HSS. Visual aids, glass slides, or preselected images were not reviewed as part of training. JTB subsequently identified esophageal biopsies during his diagnostic biopsy evaluations that showed a spectrum of changes from few or no eosinophils to intense eosinophilic inflammation (cohort #3). These biopsies were independently scored by all three pathologists who were blinded to patient treatment status, or therapy, at the time that the biopsies were obtained. All features were evaluated and scored in the section on the slides showing the most marked pathology.

Data were analyzed using JMPGenomics6.1 (SAS Institute, Cary, NC, USA). The cohorts were compared using ANOVAs and contingency tables. Interobserver agreement was evaluated using Kendall's coefficient of concordance in the *R* package irr. To determine agreement across distal and proximal features, iota was estimated for grade and stage.

To compare untreated and treated patients, Wilcoxon nonparametric test was used for scores and contingency tables or Fisher's exact test for abnormalities. Features different at the Bonferroni correction threshold ($P \leq 0.05/8 = 0.00625$) are considered statistically significant. Features different at the nominal *P*-value threshold ($P \leq 0.05$) should be interpreted with caution.

Spearman nonparametric correlations between features within grade distal, grade proximal, stage distal, stage proximal were calculated. For each grouping, the multiple testing significance threshold is 0.0018 (0.05/28).

To determine if EoEHSS provides information beyond PEC, non-nested logistic regression models were compared using *R*-square and the area under the curve (AUC). Distal PEC counts were used because distal esophageal biopsies are virtually always obtained. Given the high degree of correlation among features, principal components analysis (PCA) with varimax rotation was used to develop a composite score. Due to a substantial degree of missing data for LPF (lamina propria was not present in many biopsies), this variable was excluded from the PCA. DEC and stage scores for SEA were also removed because they did not contribute substantially to the first three principal components (PC). The final PCA was based on the distal and proximal EoEHSS grade and stage scores for EI, BZH, DIS, EA, SL, and grade of SEA from 194 biopsies (97 subjects with both proximal and distal biopsies). Using the factor loadings, three composite measures were created: PC1, PC2, and PC3. Logistic regression models with PC1, PC1—PC2, and PC1—PC3 were compared with the model with distal PEC.

RESULTS

Demographics

There were 104 study subjects (90 males, 14 females, mean age 8.66 years) (Supporting Information Table S1). Most subjects (82%) were on EoE therapy (diet therapy, swallowed fluticasone propionate, both, or systemic prednisone therapy).

Applying EoEHSS: reliability and ease of use

There was strong-to-moderate agreement among pathologists (Supporting Information Table S2, $P \leq 0.008$). Using PEC as the gold standard (Kendall's coefficient 0.93–0.96), we found equivalent-to-slight reductions for all features (0.80–0.94) except SEA and SL which exhibited modest agreement (0.61–0.72 and 0.73–0.88,

Table 1 EoEHSS grade scores[a] and PEC

	Distal			Proximal		
	Untreated	Treated	P	Untreated	Treated	P
EI	3 (2–3)	2 (1–3)	0.0035	3 (2–3)	1 (0–2)	0.0002
BZH	2 (1–3)	1 (0–2)	0.024	2 (1–3)	0 (0–1)	<0.0001
DIS	3 (3–3)	2 (0–3)	0.0051	3 (3–3)	2 (0–3)	0.0002
LPF	2 (2–3)	2 (0–2.5)	0.20	2 (0.75–2.25)	1 (0–2)	0.26
EA	0 (0–1)	0 (0–0)	0.14	0 (0–1)	0 (0–0)	0.041
SL	0 (0–2)	0 (0–0)	0.012	0 (0–1)	0 (0–0)	0.34
SEA	0 (0–0.75)	0 (0–0.75)	0.90	0 (0–0.25)	0 (0–0)	0.45
DEC	0 (0–0)	0 (0–0)	0.19	0 (0–0)	0 (0–0)	0.93
Non-PEC feature mean	0.47 (0.28–0.57)	0.29 (0.08–0.47)	0.0062	0.44 (0.28–0.51)	0.14 (0.05–0.38)	0.0003
PEC	131.5 (24.3–175)	26 (3–93)	0.008	69 (30.3–113.8)	3 (0–44)	0.008

[a]Median (IQR). Groups were compared using Wilcoxon rank sum analyses.

respectively). The multivariate measure of agreement exhibited moderate-to-strong agreement. Scoring was accomplished in less than 1 minute by all pathologists.

Frequencies of abnormalities

A total of 201 biopsies were reviewed: 101 (18 untreated, 83 treated) from proximal, 100 (16 untreated, 84 treated) from distal esophagus. Frequencies of abnormalities (grade score ≥ 1) for each feature were similar between distal and proximal biopsies (Fig. 2). Features commonly abnormal were EI, BZH, DIS, and LPF. These data support the importance of systematic survey of pathologic features other than eosinophil counts in inflamed and uninflamed esophageal biopsies from patients with EoE.

PEC differs by treatment status

PEC was greater in both proximal and distal biopsies from untreated compared with treated subjects ($P = 0.008$) (Table 1). Maximum PEC (greater of proximal and distal counts) was also greater ($P = 0.007$) in untreated (median 124; interquartile range (IQR) 69–172) compared with treated (median 33; IQR 3.5–126.4) subjects.

EoEHSS grade and stage scores differ by treatment status

We found marked differences in the grade and stage scores by treatment status (Tables 1 and 2) for proximal and distal biopsies ($P \leq 0.0062$) demonstrating EoEHSS responsiveness. After multiple-testing correction ($P \leq 0.00625$), several grade scores remained associated with treatment status: DIS ($P = 0.0051$) and EI ($P = 0.0035$) in distal biopsies; DIS ($P = 0.0002$), EI ($P = 0.0002$), and BZH ($P = 0.0001$) in proximal biopsies. Several stage scores remained significant after multiple-testing correction: EI ($P = 0.0049$) and DIS ($P = 0.0002$) in distal; EI ($P < 0.0001$), BZH ($P < 0.0001$), and DIS ($P = 0.0009$) in proximal biopsies. Importantly, grade scores of biopsies from 5/14 (36%)

individuals with 0 eosinophils/HPF exhibited LPF and/or DIS documenting that alterations may be present even if eosinophils are absent.

Correlations among EoEHSS features

The relationships among the different features (Fig. 3) were similar in proximal and distal biopsies. Based on clinical experience, EA and SL were anticipated to relate, and strong correlations for both grade and stage were found ($r^2 > 0.8$). For both grade and stage, EI correlated strongly with BZH ($r^2 \geq 0.76$), and with DIS ($r^2 \geq 0.68$); BZH and DIS were also strongly correlated ($r^2 \geq 0.67$) consistent with clinical experience. However, correlations between EI and EA or SL grade and stage were more attenuated ($r^2 \geq 0.56$) than anticipated. DEC had low correlations, possibly due to the low occurrence of this feature (Fig. 2).

EoEHSS composite score better explains treatment status than distal PEC

To determine if the EoEHSS is a better predictor of treatment status than PEC, we performed PCA to generate a composite score of the EoEHSS features. The first three PC explained a cumulative 78.2% of the variation (57.3, 11.1, and 9.9% for the first, second and third PC) of EoEHSS features. We then performed four logistic regression models with treatment status as the outcome variable and log distal PEC: PC1, PC1-PC2, and PC1-PC3. The model with log PEC had the lowest r^2 and AUC compared with the models using PCs (Table 3). PC1 and PC2 were associated with treatment status ($P = 0.0009$ and 0.02, respectively). PC1 was a size axis with similar factor loadings (weightings) suggesting that elevations across all features were associated with being untreated. PC2 was a contrast axis with DIS positively associated with being untreated and SL and EA negatively associated with being untreated.

Table 2   EoEHSS stage scores[a]

	Distal			Proximal		
	Untreated	Treated	P	Untreated	Treated	P
EI	2.5 (1–3)	1 (0–2)	0.0049	2 (1–3)	0 (0–2)	<0.0001
BZH	2.5 (2–3)	1 (0–3)	0.0070	3 (1–3)	0 (0–2)	<0.0001
DIS	3 (3–3)	1.5 (0–3)	0.0002	2.5 (2–3)	1 (0–3)	0.0009
LPF	3 (2.5–3)	3 (0–3)	0.12	3 (0.75–3)	2.5 (0–3)	0.48
EA	0 (0–1)	0 (0–0)	0.18	0 (0–1)	0 (0–0)	0.042
SL	0 (0–1)	0 (0–0)	0.011	0 (0–1)	0 (0–0)	0.28
SEA	0 (0–0.75)	0 (0–0.75)	0.98	0 (0–0.25)	0 (0–0)	0.40
DEC	0 (0–0)	0 (0–0)	0.19	0 (0–0)	0 (0–0)	0.94
Non-PEC feature mean	0.5 (0.31–0.54)	0.21 (0.04–0.43)	0.0024	0.46 (0.24–0.52)	0.13 (0–0.38)	0.0003

[a]Median (IQR). Groups were compared using Wilcoxon rank sum analyses.

DISCUSSION

We developed an EoEHSS that evaluates both PEC and additional pathologic features in EoE biopsies. Pathologists can apply the EoEHSS after minimal training and without substantially increasing time required to complete reporting. Many of the assessed features occur commonly and associate with treatment status. The EoEHSS composite score better discriminates treated from untreated patients than PEC, the current diagnostic gold standard, and also detects pathology in biopsies devoid of eosinophils, which may explain post-therapy disease activity. These results provide rationales and a method to evaluate esophageal biopsies for pathology in addition to PEC.

EoEHSS measures esophageal pathology reliably and efficiently

Strong to moderate agreement among pathologists who independently scored biopsies demonstrates EoEHSS reliability. Successful application after minimal training demonstrates EoEHSS efficiency. Several factors may facilitate this ease of implementation. Grading and staging are skills surgical pathologists use evaluating tumors. Pathologists frequently examine multiple levels of small biopsies, a habit that aids scoring. Most EoEHSS features are common in EoE biopsies and familiar to surgical pathologists. The EoEHSS evaluates PEC up to, but not over, 60 eosinophils/HPF. Reducing the time to generate peak counts may both diminish fatigue from prolonged counting and
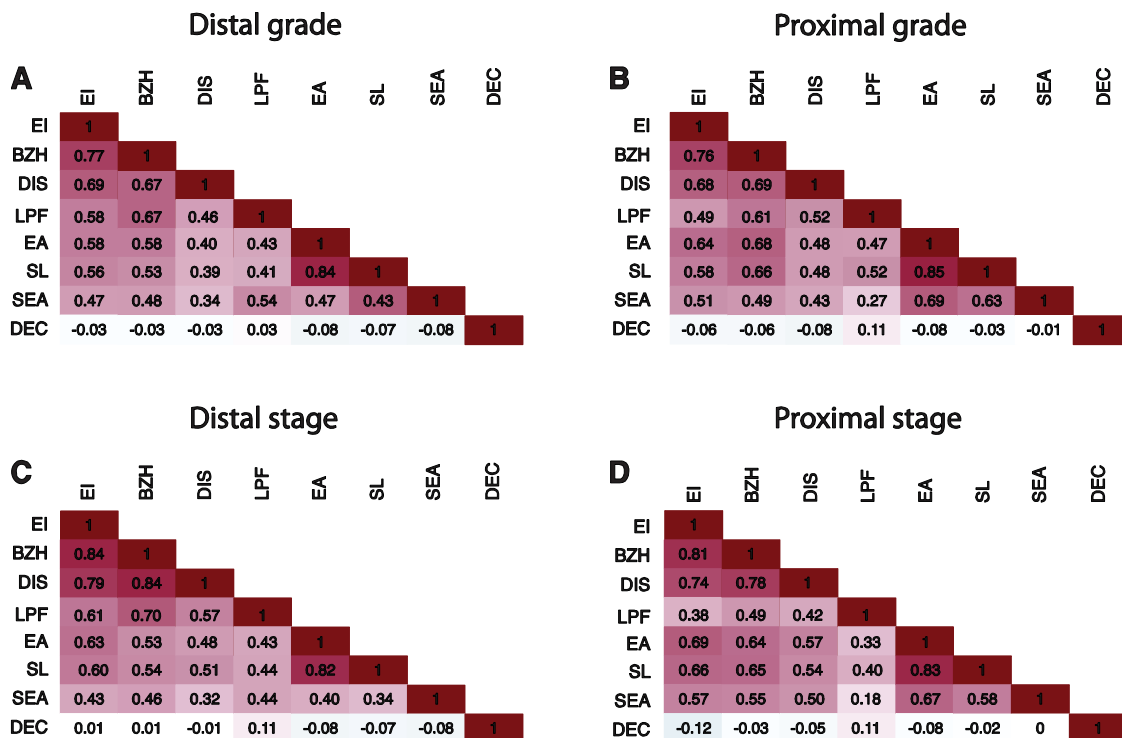

Fig. 3   Correlations between features. Panels A–D exhibit heatmaps of the Spearman correlations; A distal grade, B proximal grade, C distal stage, D proximal stage. Darker colors indicate higher correlation coefficients.

Table 3   Logistic modeling[a]

	Parameter	P	R-square (%)	AUC
Log distal PEC	PEC	0.006	9.0	69.8
PC1	PC1	0.003	10.6	77.6
PC1-PC2	PC1	0.0009	17.0	77.8
	PC2	0.02		
PC1-PC3	PC1	0.0006	17.8	78.3
	PC2	0.016		
	PC3	0.42		

[a]A composite measure of the EoEHSS scores was generated using PCA. Model fit was compared using R-square and AUC. PC1 included EI, BZH, DIS, EA, and SEA proximal and distal grade and stage scores weighted similarly. PC2 included negative weights for DIS and positive weights for SL and EA. PC3 included negative weights for SEA and SL and positive weights for EI.

increase consistency: correlations are generally better between initial and second reviews for PEC <60/HPF.[9] Thus the EoEHSS is a reliable, efficient method to evaluate multiple abnormalities in EoE biopsies.

Most EoEHSS abnormalities associate with treatment status

EI, BZH, DIS, EA, and SL exhibited significant reductions in treated compared with untreated biopsies from proximal and/or distal biopsies. EoE is defined pathologically by eosinophil-rich inflammation in esophageal biopsies and, therefore, pathology scoring systems must evaluate eosinophils. We, however, identified reductions in features that do not evaluate eosinophils, specifically BZH and DIS. The finding of noneosinophilic features associating with treatment status supports evaluating biopsies for more than eosinophils.

Scoring systems

A unique aspect of the EoEHSS is separate grade and stage scores for each feature. Determining the relative contributions of overall grade and stage scores, as well as the scores for individual features, to clinical signs and symptoms could lead to therapy endpoints. The correlations between the EoEHSS and symptoms is important to explore because of the lack of correlation of PEC with some symptom domains in a validated pediatric EoE symptom questionnaire.[16] Indeed, the effect of specific therapies on the area occupied by abnormal tissue (stage) has not been well-studied and is an important metric to pursue in future larger studies. For example, DIS are correlated with esophageal impedance abnormalities in EoE[17]; the relative contributions of DIS grade and stage to abnormal impedance studies could be tested using the EoEHSS. The effect of concomitant BZH and EI, which correlate well with DIS, could also be explored.

A previously published scoring system evaluates both severity and extent of eosinophil granule protein deposition but requires immunohistochemistry and is, therefore, not suited to daily pathology sign-out.[18] The EoEHSS does not require use of special stains for fea-ture recognition or scoring. Unlike the EoEHSS, another scoring system includes dichotomous scores, is used for biopsies from untreated subjects only, and yields final scores averaged among esophageal sites.[19]

EoEHSS identifies treatment status better than the gold standard—PEC

An important step in scale development is to compare a candidate scale to the existing gold standard. An important characteristic of a diagnostic test is to identify patients with the target disease, and also to discriminate them from patients who have undergone therapy, in other words, to identify treatment status. The subjects in this study were treated using a variety of therapies and were categorized as being treated without regard to the type of therapy, only that EoE therapy had been administered. PEC is the gold standard for the pathology portion of the diagnosis of EoE; therefore, we compared the performance of the EoEHSS composite score and PEC. The EoEHSS composite score, which includes both grade and stage, was more strongly associated with treatment status than log distal PEC as evidenced by greater R-square and AUC. Importantly, both BZH and DIS were part of this model, providing additional evidence that features not defined by eosinophils are important.

An interesting aspect of one PCA model was a negative association of SL and EA with untreated status. Perhaps SL and EA indicate disease chronicity; longer duration of illness among patients already on therapy than those not yet treated is likely. Future studies relating these features to duration of illness may be revealing.

Not all EoEHSS features were included in the models. LPF was excluded because of a large amount of missing data. However, LPF will remain in the EoEHSS until a sufficient number of biopsies in which lamina propria can be evaluated have been assessed to make meaningful conclusions about LPF's utility. Similarly, DEC appears disconnected from most EoEHSS features; however, DEC may yet prove to be an important marker of a particular phenotype which can be identified only with additional HSS-generated data and experience. At this point, removing any of the EoEHSS features would be premature until further scenarios can be examined.

This study was based on clinical practice and did not include subjects receiving specific therapy according to a clinical trial protocol. The EoEHSS outperformed PEC even in this heterogeneous real-world study and its performance might improve in a rigorous clinical trial. Prospective studies, including therapeutic trials, comparing the relative performances of the EoEHSS and PEC as diagnostic tools should be performed.

Future studies

These data document that evaluating the degree and extent of multiple pathologic features in EoE biopsies improves the ability to identify treated from untreated biopsies compared with the current gold standard—PEC. Further, prospective evaluation of the EoEHSS with respect to patient symptomology and the EoEHSS's impact on clinical care is required prior to finalizing this scale. Biopsies from adults who have EoE do not appear to differ from those from affected children, but evaluating biopsies from adults is also required.

In summary, we developed an EoEHSS that quantifies the frequency, severity, and extent of multiple features, including features that do not evaluate eosinophils. Pathologists can apply the HSS reliably and efficiently with minimal training. Further, the EoEHSS is more strongly associated with treatment status than the current gold standard, PEC only. Therefore, these results provide rationales and a method to evaluate esophageal biopsies for EoE.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site and includes:

Figure S1 EoEHSS features in esophageal biopsies from patients with EoE. (A) SEA manifest as

increased red staining of the surface layer of epithelium (arrowheads) is associated with few eosinophils large arrows (SEA grade 2). Narrow arrows point to intercellular bridges in dilated intercellular spaces. (B) DEC (arrowhead) cytoplasm is pink, but not as red as eosinophils (arrows), and is homogeneous, unlike the granular eosinophils.

Supplementary Table S1 Subject demographics

Supplementary Table S2 EoEHSS interobserver agreement among three pathologists