A Gene Expression Panel is Accurate for Diagnosis and Monitoring Treatment of Eosinophilic Esophagitis in Adults

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OBJECTIVE: Eosinophilic esophagitis (EoE) can be difficult to diagnose. We aimed to evaluate whether a gene expression score could differentiate adult EoE cases from non-EoE controls and to determine whether scores normalized after treatment for EoE.
METHODS: We analyzed prospectively collected esophageal biopsies from EoE patients (diagnosed as per consensus guidelines and after a proton pump inhibitor trial) and non-EoE controls. Gene expression for a previously constructed 94 gene panel was quantified for a single RNA-later preserved biopsy. For diagnosis, a summary expression score and the area under the receiver operating characteristic curve (AUC) were calculated. For treatment response (defined as < 15 eosinophils per high-power field), pretreatment and posttreatment EoE samples were compared.
RESULTS: For 91 EoE cases and 174 controls, gene scores for EoE cases were lower than non-EoE controls (mean 198 vs. 420; $P < 0.001$), with an AUC of 0.927. A score ≤ 263 yielded a positive predictive value = 91%; a score ≥ 349 yielded a negative predictive value = 90%; only 12% of subjects had an indeterminate score (264–348) by this classification scheme. For the 89 EoE cases with paired pretreatment and posttreatment samples, overall gene scores improved after treatment from 199 to 343 ($P < 0.001$). This normalization was seen only in cases with histological response (202 vs. 425; $P < 0.001$); scores were unchanged in non-responders (189 vs. 226; $P = 0.25$).
CONCLUSIONS: A gene expression score has high diagnostic utility for distinguishing EoE patients from non-EoE controls in adults and can be used in clinical algorithms. Because it is highly responsive to treatment, the test could be used to monitor disease status.
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INTRODUCTION

The current paradigm for diagnosing eosinophilic esophagitis (EoE) requires the presence of symptoms of esophageal dysfunction, an esophageal biopsy with at least 15 eosinophils per high-power field (eos/hpf) after a course of a proton pump inhibitor (PPI), and exclusion of other potential causes of esophageal eosinophilia.[1,2] Although this definition has helped to provide more consistency in the field,[3,4] diagnosis of EoE remains challenging. Symptoms of esophageal dysfunction can be seen in multiple conditions, including gastroesophageal reflux disease and esophageal motility disorders, and esophageal eosinophilia, even at very high levels, is not specific for EoE.[5,6] Moreover, histological assessment of eosinophilia in clinical practice is fraught with problems related to tissue sampling, section thickness, field selection, degranulation, and even microscope used.[3,7] Finally, although endoscopic signs and biopsy findings can be highly suggestive, there are no pathognomonic signs of EoE and there is significant clinical overlap between EoE, gastroesophageal reflux disease, and other causes of dysphagia.[8]

Because of these issues, there has been significant research interest in optimizing the diagnosis of EoE, including clinical symptom scores,[9–12] immunohistochemical staining of esophageal biopsies,[13–17] endoscopic severity scores,[18–20] biomarkers,[21–24] and gene expression.[25–27] Recently, analysis of the EoE transcriptome identified a panel of 94 differentially expressed genes that held promise for diagnosis of EoE.[28] Genes were selected based on the degree to which they were upregulated or downregulated, their relation to EoE pathogenesis, and their involvement in pathways related to EoE inflammation, and the panel contains pro-inflammatory genes, epithelial/barrier function genes, and mast cell–related genes, among others.[28,29] The study by Wen et al.[28] showed that a gene expression summary score was highly sensitive and specific for distinguishing EoE cases from non-EoE controls, but the score was primarily derived and validated in a pediatric population, with relatively few adults included in the study. In addition, although the score appeared to normalize in EoE cases after successful treatment, assessment of treatment response was not a major focus of that study, and the

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utility of this test has not been validated in an independent external population.

The aim of this study was to evaluate whether a gene expression score could accurately differentiate adult EoE cases from non-EoE controls and to determine whether scores were affected by treatment for EoE.

METHODS

Study design and patients. This was an analysis of esophageal biopsies collected during a prospective cohort study conducted at the University of North Carolina from 2009 through 2015, and details of this study design have been previously described.[12,20,21,30,31] We consecutively enrolled patients aged 18–80 years who were undergoing outpatient upper endoscopy for evaluation of symptoms of esophageal dysfunction such as dysphagia, food impaction, heartburn, reflux, or chest pain. Patients were enrolled prior to the endoscopy and before their final diagnosis was clinically determined. Exclusion criteria were: a known diagnosis of EoE or a different eosinophilic gastrointestinal disorder, gastrointestinal bleeding, active anticoagulation, esophageal cancer, prior esophageal surgery, esophageal varices, medical instability or multiple comorbidities precluding enrollment in the clinical opinion of the endoscopist, or inability to read or understand the consent form. In that parent study, of the 586 subjects screened, 280 were ineligible owing to these exclusion criteria.[12] Informed consent, including consent for future use of stored biopsy specimens, was obtained prior to the endoscopy. This study was approved by the UNC Institutional Review Board and registered on clinicaltrials.gov (NCT 01988285).

We used consensus guidelines as the gold standard to clinically diagnose EoE cases.[1,2] Cases were required to have at least one symptom of esophageal dysfunction, ≥ 15 eos/hpf on esophageal biopsy after an 8-week proton pump inhibitor trial (20–40 mg twice daily of any of the available agents, prescribed at the discretion of the clinician), and exclusion of other causes of esophageal eosinophilia. Controls were subjects who did not meet clinical or histological criteria for EoE after endoscopy and biopsy. Subjects with proton pump inhibitor–responsive esophageal eosinophilia were not included in this study based on prior data that gene expression profiling could not distinguish them from EoE cases.[32] In addition, we included a set of patients who were "clinically challenging" from a diagnostic standpoint: they had ≥ 15 eos/hpf on esophageal biopsy but could not be readily classified as a case or control based on initial clinical presentation alone.

Data, bio-sample collection, and follow-up. Patient demographics, symptoms, and endoscopic findings were recorded prospectively on standardized case report forms. During the endoscopy, esophageal biopsies were obtained per research protocol (two from the proximal, one from the middle, and two from the distal esophagus) for determination of tissue eosinophil counts and to maximize EoE diagnostic sensitivity.[33,34] We also collected research-protocol gastric and duodenal biopsies to exclude concomitant eosinophilic gastroenteritis. Esophageal eosinophil counts were determined based on our previously validated methodology.[35] The maximum eosinophil density (eos/mm^2) was quantified in five hpfs and then converted to an eosinophil count (eos/hpf) based on a microscopic field size of 0.24 mm^2, the most commonly reported size in the literature.[3] Slides were masked as to case/control status.

In addition to biopsies obtained for histological assessment, we also collected additional biopsy samples that were labeled with a de-identified study number, masked as to case/control status, and stored at $-80\,°C$ RNA-later (Life Technologies/Thermo-Fisher Scientific, Grand Island, NY) for future use.

Table 1 Baseline characteristics of cases and controls

	Non-EoE controls ($n=174$)	EoE cases ($n=91$)	P
Age (mean years ± s.d.)	52.1 ± 14.2	37.1 ± 12.7	<0.001
Male (*n*, %)	73 (42)	54 (59)	0.007
White (*n*, %)	143 (82)	86 (95)	0.005
Symptoms (n, %)			
Dysphagia	120 (69)	89 (98)	<0.001
Heartburn	97 (56)	12 (13)	<0.001
Abdominal pain	20 (11)	7 (8)	0.33
Nausea/vomiting	11 (6)	1 (1)	0.05
Any atopic disease (n, %)	99 (58)	68 (76)	0.006
Asthma	40 (24)	26 (29)	0.36
Rhinitis/sinusitis	83 (49)	60 (67)	0.006
Dermatitis	11 (7)	7 (8)	0.70
Food allergies	30 (18)	35 (39)	<0.001
Endoscopic findings (n, %)			
Rings	21 (12)	72 (79)	<0.001
Stricture	35 (20)	22 (24)	0.45
Narrowing	6 (3)	32 (35)	<0.001
Furrows	12 (7)	79 (87)	<0.001
White plaques/exudates	6 (3)	43 (47)	<0.001
Edema/decreased vascularity	5 (3)	52 (57)	<0.001
Hiatal hernia	84 (48)	10 (11)	<0.001
Dilation performed	55 (32)	29 (32)	0.97
Eosinophil count (max eos/hpf ± s.d.)	1.5 ± 3.3	135.9 ± 123.4	<0.001
Associated histological findings (n, %)			
Eosinophil degranulation	16 (9)	84 (92)	<0.001
Eosinophil microabscesses	0 (0)	57 (63)	<0.001
Basal layer hyperplasia	18 (10)	39 (73)	<0.001
Spongiosis	57 (33)	77 (85)	<0.001
Diagnosis (n, %)			
EoE	—	91 (100)	—
GERD	74 (43)	—	—
Peptic stricture	14 (8)	—	—
Schatzki's ring	10 (6)	—	—
Other stricture	12 (7)	—	—
Achalasia	5 (3)	—	—
Esophageal spasm	9 (5)	—	—
Ineffective esophageal motility	3 (2)	—	—
Non-specific dysmotility	9 (5)	—	—
Functional	34 (19)	—	—
Normal/no esophageal pathology	4 (2)	—	—

EoE, eosinophilic esophagitis; eos/hpf, eosinophils per high-power field; GERD, gastroesophageal reflux disease.

Table 2 Baseline and follow-up gene scores

	Non-EoE controls (*n* = 171)	EoE cases (*n* = 90)	*P*[a]
Gene score (mean ± s.d.)			
Baseline	419.7 ± 66.1	197.8 ± 138.4	<0.001
Posttreatment[b]		342.5 ± 138.1	
P[a]		<0.001	
Gene score (mean ± s.d.) in histological responders (< 15 eos/hpf) (n = 51)			
Baseline		201.5 ± 137.0	
Posttreatment		425.3 ± 50.1	
P[a]		<0.001	
Gene score (mean ± s.d.) in histological non-responders (< 15 eos/hpf) (n = 36)			
Baseline		189.1 ± 142.8	
Posttreatment		226.4 ± 142.1	
P[a]		0.25	

EoE, eosinophilic esophagitis; eos/hpf, eosinophils per high-power field.
[a]Cases and controls compared with a two-sample *t*-test; cases before and after treatment compared with a paired *t*-test.
[b]*n* = 89 EoE cases with posttreatment samples.

This study utilized a single RNA-later-preserved biopsy from the mid-esophagus (10 cm above the gastroesophageal junction) for gene expression determination. The decision to use a single mid-esophageal biopsy was based on our prior work showing that gene expression in EoE and controls was similar throughout the esophagus.[31]

Patients diagnosed with EoE were treated as clinically indicated by their gastroenterologist. They could receive treatment with topical corticosteroids (either oval viscous budesonide 1 mg twice daily or fluticasone from a multi-dose inhaler, 880 mcg twice daily) for 8 weeks[36–38] or dietary therapy with the six-food elimination diet for 6 weeks[39,40] based on personal preference. When the initial course of therapy was completed, patients had a repeat upper endoscopy with biopsy during which time a repeat set of esophageal biopsies was obtained using identical protocols as the baseline endoscopy.

Gene expression determination. After patient enrollment was complete, the mid-esophageal biopsies in RNA-later were removed from − 80 °C storage and sent on dry ice for processing at Miraca Life Sciences (Phoenix, AZ) for gene expression testing. The supernatant was removed after thawing and the tissue was homogenized. RNA extraction was performed on the homogenized specimens using the RNeasy Mini Extraction Kit (Qiagen, Valencia, CA) per the manufacturer's instructions, and the concentration was measured using spectrophotometry (NanoDrop, Wilmington, DE). A concentration of 16.5 ng/μl of RNA for a total of 500 ng was considered acceptable. cDNA synthesis was carried out using the iScript cDNA Synthesis Kit (Bio-Rad, Hercules, CA) with PCR performed on ABI 9700 (Applied Biosystems, Foster City, CA). The cDNA and TaqMan Universal Master Mix II, no UNG (Life Technologies) were loaded onto custom Taqman TLDA cards containing preloaded Taqman gene expression assays in a 384-well format. This consisted of the 94 gene panel that was previously developed for EoE[28] and 2 housekeeping genes (GAPDH and 18S) (EoGenius, Miraca Life Sciences, Irving, TX). PCR was performed on Quant Studio 7 (Life Technologies) to determine the gene expression levels measured as threshold cycles (Ct). Samples with a GAPDH value of <30 Ct were considered acceptable for analysis.

Using this expression data, a summary score was calculated using a previously established algorithm.[28] Specifically, the Ct value of the housekeeping gene was subtracted from the Ct value of each gene of interest to acquire the ΔCT, and then the absolute values of the normalized gene Ct values were summed for each gene in the gene expression panel.

Statistical analysis. We summarized clinical features of the study populations with descriptive statistics. Baseline data for cases and controls, including the mean gene scores, were compared using two-sample *t*-tests for continuous variables and chi-square for categorical variables. We assessed the gene score cut point (<333 vs. ≥333) that was previously found to maximize diagnostic accuracy (a score <333 was shown to be characteristic of EoE)[28] evaluated the correlation between the maximum eosinophil counts and the gene scores and performed additional receiver operator characteristic curve analyses to calculate the area under the curve (AUC) and operating characteristics for our data set. With these data, we explored gene score ranges, rather than a single cut point, that would optimize both positive and negative predictive values (PPV and NPV, respectively) while minimizing indeterminate results. Next we analyzed the baseline and posttreatment gene scores for EoE cases using paired *t*-tests. The posttreatment gene scores were also stratified by histological response status, defined as 15 eos/hpf.[41] Finally, we examined the gene scores for clinically challenging patients (≥15 eos/hpf on esophageal biopsy but with an indeterminate initial clinical presentation). For these cases, the gold standard was the final clinical diagnosis based on all available testing, treatment, and follow-up data in the medical record. Analyses were performed with Stata 9.2 (StataCorp, College Station, TX).

RESULTS

Patient characteristics. A total of 174 non-EoE controls and 91 incident EoE cases had samples analyzed in this study. Compared with controls, EoE cases were younger (37 vs. 52
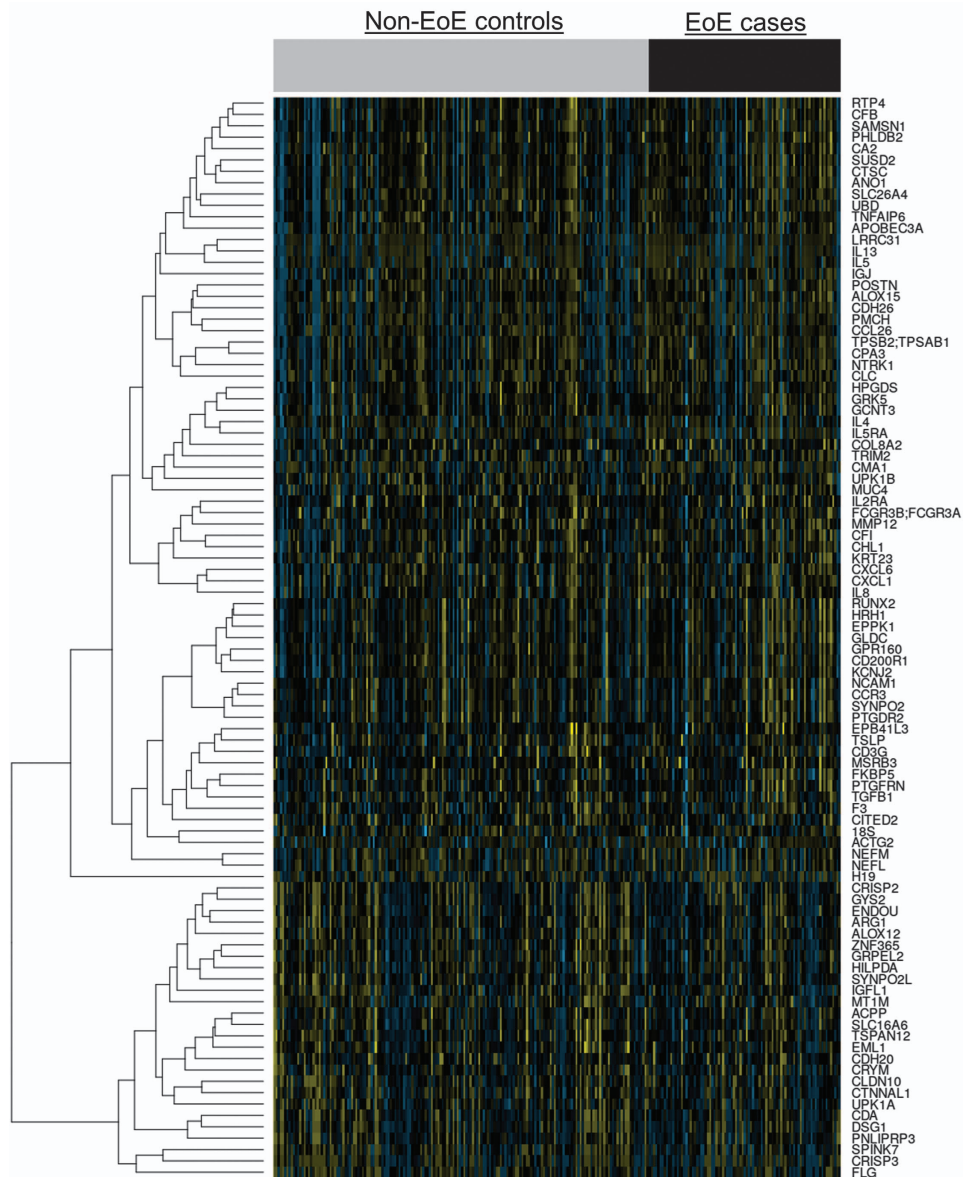
Figure 1 Gene expression heat map for controls (gray bar) and eosinophilic esophagitis (EoE) cases (black back). Yellow indicates more highly expressed genes and blue indicates less highly expressed genes.

years; $P<0.001$), more frequently male (59% vs. 42%; $P=0.007$) and white (95% vs. 82%; $P=0.005$), and had more atopic diseases (76% vs. 58%; $P=0.006$) (Table 1). Cases also more commonly had endoscopic findings of esophageal rings, narrowing, linear furrows, white plaques/exudates, and edema/decreased vascularity. The baseline maximum eosinophil counts were 135.9 ± 123.4 in the EoE cases and 1.5 ± 3.3 in the controls ($P<0.001$) (Table 1).

Baseline gene expression scores. Sufficient RNA was available for 171 controls and 90 cases. At baseline, the mean gene expression score was significantly lower in EoE cases compared with controls (197.8 ± 138.4 vs. 419.7 ± 66.1; $P<0.001$) (Table 2). The heat map showing gene expression for the gene panel used for all subjects is presented in Figure 1. The maximum eosinophil count

inversely correlated with the gene score (Pearson's $R=-0.65$; $P<0.001$). On receiver operator characteristic analysis, the AUC was 0.927 for diagnosis of EoE (Figure 2). The gene score cut point of 333 correctly classified 72 of the cases (79%) and 158 of the controls (91%), corresponding to a κ of 0.71. After optimizing potential gene score ranges, we found that a score ≤263 yielded a PPV$=91\%$, a score ≥349 yielded an NPV$=90\%$, and only 12% of subjects had an indeterminate score (range: 264–348) by this classification scheme (Table 3).

Posttreatment gene expression scores. There were 89 EoE cases with paired pretreatment and posttreatment samples. A total of 81 (91%) were treated with topical steroids, 75 of whom (93%) were prescribed a budesonide slurry at 2 mg/day, and 6 of whom were prescribed
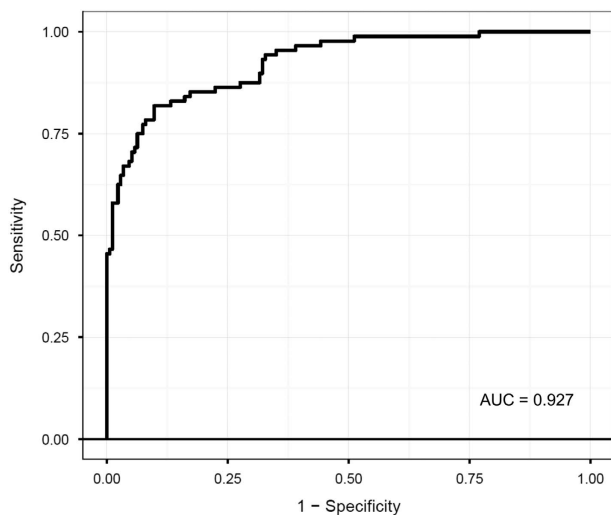
Figure 2 Receiver operator characteristic curve showing the utility of the gene expression panel for diagnosis of eosinophilic esophagitis. AUC, area under the receiver operating characteristic curve.

fluticasone in a multi-dose inhaler at 1760 mcg/day; the remaining patients were treated with dietary elimination. The overall gene scores after treatment increased to 342.5 ± 138.1 ($P < 0.001$) (Table 2). The heat map showing pretreatment and posttreatment gene expression for EoE cases is presented in Figure 3. Of note, this increase was seen only in those cases with histological response (201.5 ± 137.0 vs. 425.3 ± 50.1; $P < 0.001$); scores were unchanged in histological non-responders (189.1 ± 142.8 vs. 226.4 ± 142.1; $P = 0.25$; Figure 4).

Utility of gene expression scores in clinically challenging patients. There were 15 patients (mean age 53 years; 80% male) who had elevated eosinophil counts and clinically indeterminate features on their baseline endoscopy and biopsy (Supplementary Table S1). The mean peak eosinophil count in this group was 55 eos/hpf, and the mean baseline gene score was 313, in the indeterminate range by the classification scheme above. After all clinical features and subsequent testing were considered, only one patient was felt clinically to have overlapping EoE and gastroesophageal reflux disease; the gene score for this patient was 231, in the range suggestive of EoE. Ten patients (67%) had gene scores ≥ 264, putting them in either the indeterminate or non-EoE range, despite their elevated esophageal eosinophil count. None of these patients were clinically diagnosed with EoE.

DISCUSSION

Because the clinical, endoscopic, and histological features of EoE are non-specific, the diagnosis of EoE remains challenging. The description of a characteristic gene expression pattern in EoE patients, the EoE transcriptome,[25,26] was the foundation for the eventual development of a molecular strategy for diagnosis of EoE. A summary score based on a panel of 94 genes with differential expression in EoE compared with non-EoE controls showed great promise for diagnosis of EoE.[28] Our study focused on the clinical utility of

this gene expression summary score in adults and several results were notable. First, the score discriminated EoE cases from non-EoE controls with a high degree of accuracy, though not perfectly. Second, we were able to adapt a scoring system to provide clinically relevant probabilistic score ranges, rather than a single dichotomous threshold for diagnosis. Third, we showed that a positive gene score reliably normalizes after histological treatment response, suggesting that it may be used to assess treatment response in place of a set of biopsies obtained for histological analysis. Finally, there appeared to be some utility in applying the gene score to cases that were clinically indeterminate.

There have been several studies that have examined gene expression in EoE, showing differences in individual genes or microRNAs between EoE cases and controls and that these individual markers could normalize with treatment.[25–28,42–45] However, the landmark paper by Wen *et al.*[28] was the first to develop an overall gene score and then to go further by validating its use for EoE diagnosis. Although the score was primarily evaluated in a pediatric population with relatively few adults included, and while treatment response was not the main focus, the performance of this summary score was impressive. A cutoff of 333 perfectly distinguished EoE cases from non-EoE controls in the initial study phase (AUC = 1.00) and was nearly perfect in the follow-up validation phase (AUC = 0.97). Our results also showed an outstanding AUC (0.93). The main difference was that the threshold of 333, though good, was not perfect ($\kappa = 0.71$) and some patients were misclassified. This discrepancy may be explained by methodology. Because this is the first large-scale external validation of the gene expression panel, it is not surprising that a cut point developed in a different population does not have identical performance characteristics. In addition, the prior study processed samples individually in a research laboratory, while the present study utilized a more automated process that could be used in the clinical setting. To address this, we defined ranges, based on the clinical probability of EoE, that maximized PPV and NPV and minimized indeterminate results. In addition, the test had promise in cases that had high levels of eosinophilia on biopsy but had a clinically indeterminate presentation, something that no study to date has examined.

In addition to the test's diagnostic utility, we were able to show that it might have an even stronger role in monitoring of therapy. Currently, there are few studies that systematically assess or define the "best" histologicalal threshold in EoE,[41] clinical trials have substantial heterogeneity in the end points that have been used,[5,46] and guidelines do not recommend a specific cut point to monitor response.[2] In practice, multiple biopsies are obtained from multiple locations throughout the esophagus, and the posttreatment peak eosinophil count is determined from review of these samples. Because eosinophilia is patchy,[34,47] there could be sampling error during this evaluation. In contrast, it appears that gene expression may be more consistent throughout the esophagus,[31] and our results demonstrate that a single biopsy from a patient who had histological response also had normalization of a gene expression. It is therefore intriguing to speculate whether gene expression normalization might provide a more efficient and accurate way to define tissue response to treatment in
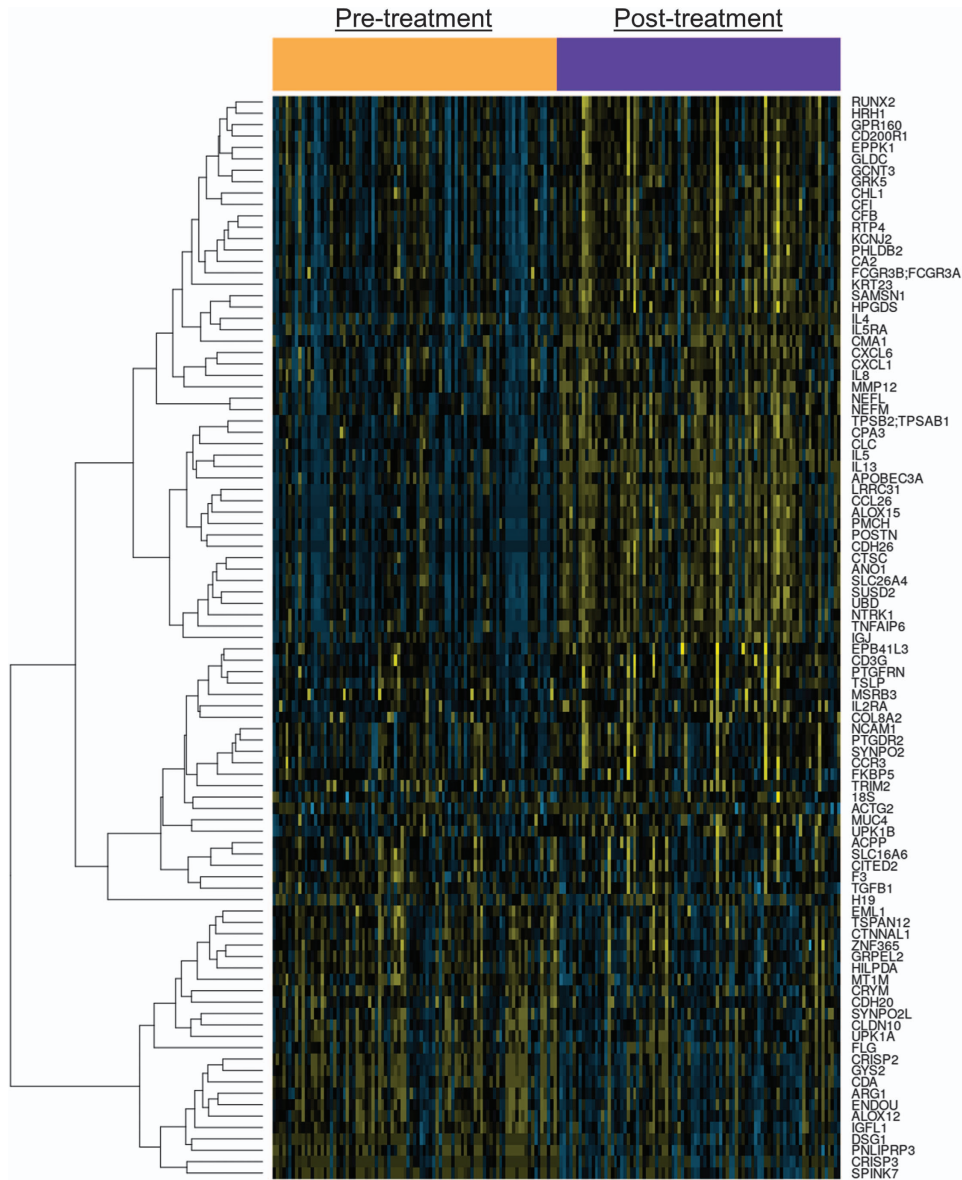
Pre-treatment   Post-treatment

Figure 3 Gene expression heat map for cases before (orange bar) and after (purple bar) treatment. Yellow indicates more highly expressed genes and blue indicates less highly expressed genes.

Table 3 Optimization of the gene score for clinical applicability

Predicted probability of EoE	Clinical test interpretation	Gene score range	Notes
0.70–1.0	Likely	≤263	PPV 91%
0.30–0.69	Indeterminate	264–348	12% of samples in this range
0–0.29	Unlikely	≥349	NPV 90%

EoE, eosinophilic esophagitis; NPV, negative predictive value; PPV, positive predictive value.

EoE. However, future studies will need to assess other measures of response (symptoms, endoscopic findings, histological findings besides eosinophil count alone) to fully explore the use of this gene panel as a treatment outcome measure. Additionally, even with the data presented here, the role and availability of this test in clinical diagnostic and treatment monitoring algorithms must still be defined. As of now, there may not be a need for use in clear-cut cases, but because diagnostic features of EoE are not specific, the test may have the most value in settings of clinical uncertainty or when a baseline value is needed for subsequent treatment monitoring.

This paper has some limitations to acknowledge. This study was conducted in adults at a single tertiary care referral center, so it is possible that results are not generalizable. However, the study design enrolled all patients undergoing endoscopy for symptoms of esophageal dysfunction, a population that is likely to be similar to that in many other endoscopic suites. In addition, the biopsy samples that we used were preserved in RNA-later. Were a gene panel to be employed in clinical practice, formalin-fixed paraffin-embedded (FFPE) samples would most likely be used. The same gene expression
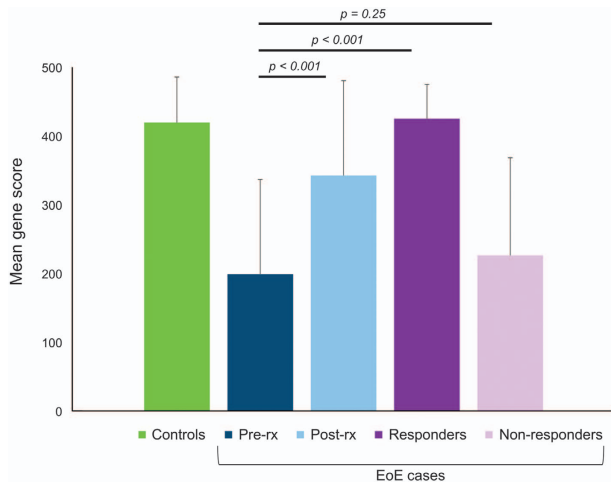
Figure 4 Mean gene expression scores (± s.d.) in controls (green bar) and eosinophilic esophagitis (EoE) cases (dark blue bar) at baseline, in all EoE cases after treatment, and in EoE cases after treatment stratified by histological response status (responders defined as < 15 eosinophils per high-power field).

panel has been shown to have good discriminative ability in FFPE samples, but we did not perform external validation, and this additional validation as well as confirmation of the proposed score ranges from this paper must still be explored for RNA derived from FFPE. Moreover, external validation of the cut points proposed in this study would also be required at different centers and in different populations. Although we included a patient population with high eosinophil counts and clinically indeterminate presentations, the clinical care and follow-up testing of these patients were not standardized, so those results should be interpreted with caution. The indeterminate patients also represented a relatively small sample, and in a number of cases a careful clinical evaluation could yield a correct diagnosis without using a gene expression test. In addition, our study cannot address the issue of whether gene expression can replace standard histological analysis in EoE diagnostic algorithms, and future research will need to elucidate the best way to utilize a test that could "rule in EoE", in contrast to an eosinophil count that is non-specific. The strengths of the study include the rigorous prospective design, meticulous sample handling that was identical for all subjects, and obtaining samples before case/control status was assigned. Moreover, this large case/control population was independent from the population used to develop and initially validate the test, the laboratory was masked to case/control status when gene scores were determined, and samples were processed and run with the same set of equipment that would be used in a clinical setting.

In conclusion, this prospective cohort study showed that a gene expression profile run on a single esophageal biopsy had an excellent ability to discriminate EoE cases from non-EoE controls, and we were able to generate probabilistic score ranges with high PPV and NPVs while minimizing samples with intermediate scores. In addition, patients with marked esophageal eosinophilia but with an indeterminate initial clinical presentation were able to be categorized as unlikely to have EoE based on higher gene scores. Although this test could be used in clinical algorithms, its exact place in these algorithms and utility above standard histological

analysis for diagnosis is still exploratory and yet to be determined. Finally, because the gene scores were responsive to treatment and normalized in patients who also had histological response, it is possible that in the future a single esophageal biopsy posttreatment could be used to define tissue response in EoE.

CONFLICT OF INTEREST

Study Highlights

WHAT IS CURRENT KNOWLEDGE

✓ Diagnosis of eosinophilic esophagitis (EoE) remains challenging as the clinical signs and biopsy findings are not specific.

✓ A gene expression panel for diagnosis of EoE has recently been developed, but its utility in adults and role for monitoring treatment response have not been extensively evaluated.

WHAT IS NEW HERE

✓ This large cohort study of adults with EoE and non-EoE controls analyzed a gene expression panel in prospectively collected esophageal biopsies and summary scores were calculated.

✓ Gene scores were markedly lower in newly diagnosed EoE cases compared with controls, with a very high diagnostic utility (area under the receiver operating characteristic curve was 0.927).

✓ A probabilistic range of gene scores was generated to divide patients suspected of EoE into being highly likely to have it, unlikely to have it, and indeterminate.

✓ Gene scores were highly responsive to treatment, with normalization of gene expression in EoE patients who were histological responders but not in non-responders. This suggests that the test could be used to monitor disease status.

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Supplementary Information accompanies this paper on the Clinical and Translational Gastroenterology website (http://www.nature.com/ctg)