



Biomarkers for eosinophilic esophagitis: a review

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ABSTRACT

Background: The complex pathophysiology of eosinophilic esophagitis (EoE) provides several candidate biomarkers that could be used to establish diagnosis, assess response to therapy, and document disease recurrence.

Objective: To review the literature on various biomarkers of EoE, with respect to their correlation to disease activity and response to treatment.

Data Sources: A literature search was performed using PubMed and OVID with keyword combinations of EoE and various potential biomarkers.

Study Selections: Between 2006 and 2012, 26 studies that investigated the correlation of various tissue and serum biomarkers with EoE were identified.

Results: The markers investigated included eotaxins-1, -2, and -3, interleukin-5 (IL-5), interleukin-13 (IL-13), eosinophil-derived neurotoxin, mast cell markers, absolute eosinophil count, and micro-RNAs. Several studies have shown a positive correlation between eotaxin-3, IL-5, and IL-13 messenger RNA (mRNA) expression in esophageal tissue and disease activity. Eotaxin-3 mRNA staining was found to have 89% sensitivity for diagnosing EoE. Staining for mast cells and their products has also shown promise. More recently, a microRNA signature that can potentially distinguish EoE from non-EoE esophagitis has been identified.

Conclusion: The studies are quite heterogeneous with respect to their methodology and the biomarker(s) studied, but most have investigated tissue biomarkers. Eotaxin-3 and IL-13 have emerged as the most promising ones with respect to sensitivity and degree of positive correlation to disease process. Future research on biomarkers for EoE should include longitudinal studies, establishment of normal values, effects of concomitant atopic diseases, age and gender, and validation of methodology of the tests.

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Introduction

Eosinophilic esophagitis (EoE) is an emerging worldwide disease characterized by marked esophageal eosinophilic infiltration (≥ 15 eosinophils/high-power field) and variable response to acid suppression therapy.^{1,2} It affects both children and adults. The presenting symptoms, which vary by age, include difficulty eating, failure to thrive, chest or abdominal pain, dysphagia, and food impaction.³ Diagnostic guidelines published in 2007, and revised in 2011, require demonstration of esophageal eosinophilia in the absence of gastroesophageal reflux disease (GERD) as determined through a clinical trial with proton pump inhibitors or the use of pH-monitoring studies.^{1,4} EoE is associated with other atopic diseases, and many patients have evidence of food and aeroallergen sensitivities. T-helper 2 (Th2) cells and their associated cytokines are involved in the pathogenesis of EoE.³

Currently, esophagogastroduodenoscopy and histological examination of esophageal mucosal biopsy specimens are required to establish the diagnosis, objectively assess response to therapy, document disease remission, and evaluate symptom recurrence.^{1,5} Reliable, noninvasive biomarkers have not yet been identified. An ideal biomarker for EoE should correlate with disease activity, reflect changes with therapy, have high sensitivity and specificity, be reproducible and cost-effective, and be performed on noninvasively and easily obtained specimens.⁵ The complex pathogenesis of EoE and eosinophil physiology provide several candidate biomarkers. Most studies have focused on eosinophil-derived neurotoxin (EDN), eotaxin-1, eotaxin-2, eotaxin-3, interleukin-5 (IL-5), interleukin-13 (IL-13), eosinophil peroxidase, absolute eosinophil count (AEC), and mast cells (MC). We conducted a literature search on PubMed and OVID using keyword combinations of EoE and each of these biomarkers. Twenty-six studies that investigated the correlation between tissue and serum biomarkers and EoE disease activity between 2006 and 2012 were selected. All the included studies used tissue or serum samples from patients who were diagnosed with EoE based on the widely accepted criteria per consensus recommendations published in 2007 and revised in 2011.^{1,4} The studies are quite heterogeneous with respect to their

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Table 1
Summary of studies on potential biomarkers for eosinophilic esophagitis

Name of biomarker	Number of studies	Tissue/serum based	Correlation with disease activity	Response to steroid treatment (if studied)
Eotaxin-3	9	8/1	7: Positive correlation	3: Down regulation
Eotaxins-1 and -2	4	3/1	3: Positive correlation	1: Down regulation of CCL11 (eotaxin-1) gene expression
IL-5	8	4/4	6: Positive correlation	2: Down regulation
IL-13	3	All tissue based	2: Positive correlation	1: Decrease in mRNA levels
EDN	4	2/2	All: Positive correlation	1: Decrease in serum levels
MicroRNA	2	1 mixed/1 tissue	miR-21 and -223 upregulated, miR-375 downregulated in tissue. miR-146a, -146b and -223 increased in plasma	Tissue miR-675 induced by and miR-375 normalized with disease remission. Plasma miR-146a and -223 normalized with remission, miR-146b remained elevated
Mast cells	7	All tissue based	All: Positive correlation	2: Decreased tissue mast cell numbers with treatment

Abbreviations: EDN, Eosinophil-derived neurotoxin; IL-5, interleukin-5; IL-13, interleukin-13.

methodology and biomarker(s) studied. Most studies used the number of eosinophils in the esophageal mucosa rather than symptoms as a marker of disease activity. In the following review, we have attempted to summarize the available knowledge so far on potential biomarkers for EoE. A summary of the included studies is presented in Table 1.

Eotaxin-3

Eosinophil localization to the lamina propria in the gastrointestinal (GI) tract is regulated by eotaxins, chemokines constitutively expressed throughout the GI tract.⁶ The eotaxin receptor, CCR3, is primarily expressed on eosinophils.⁷ Among the 3 eotaxins (-1, -2, and -3), eotaxin-3 has been shown to have the strongest correlation with disease pathogenesis and therefore is presented first. Blanchard et al⁸ in 2006 analyzed esophageal tissue by a genome-wide microarray expression analysis. The gene encoding eotaxin-3 was found to be the most highly induced gene in EoE patients compared with healthy individuals. Esophageal eotaxin-3 messenger ribonucleic acid (mRNA) and protein levels strongly correlated with tissue eosinophilia and mastocytosis. A single-nucleotide polymorphism in the human eotaxin-3 gene was associated with disease susceptibility. Mice deficient in CCR3 were found to be protected from experimental EoE.⁸ Another study in 2007 showed that eotaxin-3 tissue expression positively correlated with esophageal eosinophil numbers.⁹ Interestingly, Gupta et al¹⁰ showed that the expression of eotaxin-3 in esophageal biopsy specimens was similar between controls and EoE patients. In the same year, Bhattacharya et al¹¹ showed that the mean mRNA expression levels of eotaxin-3 were markedly elevated in patients with EoE as compared with patients with GERD and healthy control groups. A study in 2008 evaluated the changes induced by topical steroid treatment to eotaxin-3 esophageal gene expression levels in EoE patients.¹² The levels were significantly increased in EoE. They were down-regulated on steroid treatment, but control expression levels were never reached.

Another study by Blanchard et al in 2007 was based on real-time polymerase chain reaction and microarray analysis performed on RNA extracted from esophageal biopsy specimens and primary esophageal epithelial cell cultures stimulated with IL-13. Esophageal epithelial cells were found to markedly produce eotaxin-3 after IL-13 stimulation.¹³ Bullock et al⁹ showed that eotaxin-3 tissue expression positively correlated with esophageal eosinophil numbers and peripheral blood CCR3 mean channel of fluorescence in pediatric EoE patients. A recent study in 2011 quantified cytokine mRNA in esophageal biopsies and blood samples.¹⁴ Analysis of esophageal biopsies revealed that eotaxin-3 mRNA level had 89% sensitivity for distinguishing patients with and without EoE. A marked overexpression of approximately 1% of the genome was

found in EoE patients compared with healthy individuals. A prospective cross-sectional analysis on 47 pediatric patients undergoing endoscopic evaluation of possible EoE was conducted in 2006. Blood levels of eotaxin-3 significantly correlated with esophageal eosinophil density and were increased in patients with active EoE versus controls.¹⁵ Based on these studies, a preponderance of evidence exists for the positive correlation of eotaxin-3 tissue expression and disease activity in terms of esophageal eosinophilia and response to corticosteroid therapy.

Eotaxins-1 and -2

Eotaxins along with IL-5 are the only cytokines that selectively regulate eosinophil recruitment.¹⁶ In 2006, Gupta et al studied mRNA expression of eotaxins-1 and -2 in normal and inflamed esophageal mucosa of children. Eotaxin-1 mRNA expression was significantly up-regulated in EoE patients, whereas eotaxin-2 expression was up-regulated in controls.¹⁰ A retrospective study compared mRNA expression of eotaxins between groups of EoE patients, GERD patients, and healthy individuals. Mean expression levels of eotaxins-1 and -2 were significantly elevated in EoE patients, although at lower levels than eotaxin-3.¹¹ In 2008, Lucendo et al¹² evaluated the effect of topical steroid treatment on eotaxin-1 (CCL11) esophageal gene expression levels in EoE patients. Half of the patients showed a notably decreased expression of eotaxin-1 on steroid treatment. The other half only showed a moderate or no change in gene expression.¹²

A prospective cross-sectional analysis performed on 47 pediatric patients measured eotaxin-1 and eotaxin-2 blood levels in 2006. The levels did not correlate with esophageal eosinophil density and were not increased in active EoE vs controls or those with inactive EoE.¹⁵ The above studies do not establish a strong and consistent correlation of eotaxins-1 and -2 tissue and serum levels with EoE disease activity.

Interleukin-5 (IL-5)

Interleukin-5 plays a critical role in eosinophil trafficking into inflammatory sites along with other Th2 cytokines, IL-4 and IL-13. IL-5 increases the pool of eotaxin-responsive eosinophils and primes eosinophils to respond to CCR3 ligands.⁷ Several studies have evaluated IL-5 mRNA expression in esophageal biopsy specimens of EoE patients in comparison with controls. Bhattacharya et al¹¹ in 2007 conducted a retrospective study using esophageal biopsy specimens from 33 EoE patients, 20 GERD patients, and 17 healthy controls. Tissue IL-5 mRNA expression levels were significantly elevated in patients with EoE compared with GERD and controls, although at lower levels than eotaxin-3.¹¹ Similar results were reported in a study in 2006 by Gupta et al.¹⁰ Lucendo et al in 2008 evaluated the effect of topical steroid treatment on IL-5

esophageal gene expression levels in patients with EoE. Esophageal biopsies were taken from 8 adult patients at the time of diagnosis and after 3-month treatment with fluticasone propionate. Interleukin-5 was not detected in control samples, and expression levels were variably down-regulated after treatment in 6 of the patients.¹² Another study in 2011 showed a 4-fold induction of esophageal transcripts of IL-5 receptor alpha in individuals with EoE. The presence of allergy was associated with significantly increased esophageal expression of IL-5 mRNA in EoE patients.¹⁴

Serum levels of IL-5 in EoE patients have also been studied. In 2006, Konikoff et al¹⁵ showed that blood levels of IL-5 did not correlate with esophageal eosinophil density and were not increased in active EoE vs controls or those with inactive EoE. In 2007, Bullock et al⁹ used flowcytometry to measure eosinophil expression of IL-5 receptor-alpha and intracellular expression of IL-5. EoE patients with active disease had increased percentage of CD4+ T cells expressing IL-5 compared with those in disease remission.⁹ In another study in 2010, Huang et al¹⁷ used a cytometric bead array to demonstrate significantly increased levels of plasma IL-5 in EoE vs GERD.¹⁷ A longitudinal study in children with EoE showed that serum IL-5 levels in patients with EoE were not statistically different from those of the controls.¹⁸ Tissue expression of IL-5 mRNA could potentially be used for diagnosis as well as monitoring response to therapy in patients with EoE, but serum levels have not been shown to correlate consistently in all studies.

Interleukin-13 (IL-13)

Interleukin-13 is a Th2 cell-derived cytokine produced at increased levels in allergic tissues and thought to be one of the central regulators of eosinophilic diseases. It is one of the key cytokines involved in eosinophil trafficking to inflammatory sites.⁷ Gupta et al¹⁰ in 2006 studied the mRNA expression of IL-13 in normal and inflamed esophageal mucosa of children. The expression of IL-13 was similar between controls and patients with EoE. Blanchard et al in 2007 performed real-time polymerase chain reaction and microarray analysis on RNA extracted from esophageal biopsy specimens and primary esophageal epithelial cell cultures stimulated with IL-13. The IL-13 mRNA levels were markedly increased in esophageal biopsy specimens from EoE patients compared with those from healthy individuals. IL-13 mRNA levels were largely reversible with glucocorticoid treatment. They identified an *in vivo* IL-13-induced transcriptome that may be potentially useful to assess efficacy of anti-IL-13 therapy. Interleukin-13 stimulation of esophageal epithelial cells was shown to induce eotaxin-3 production.¹³ Another study in 2011 identified IL-13 as one of the 8 cytokines whose blood levels retrospectively distinguished EoE patients from healthy controls with 100% specificity and sensitivity. It proposed the development of a cytokine panel scoring system for predicting the diagnosis of EoE.¹⁴ The above studies establish that tissue and serum expression levels of IL-13 correlate with tissue eosinophilia in EoE, and it is 1 of the most promising potential biomarkers for the disease.

Eosinophil-derived neurotoxin

Eosinophil-derived neurotoxin is an eosinophil granule-derived secretory protein with ribonuclease and antiviral activity.¹⁹ Yang et al²⁰ in 2008 demonstrated that EDN enhances antigen-specific Th2-biased immune responses. Extracellular EDN has been used as an indicator of eosinophil activation and degranulation *in vitro*. Increased levels of EDN in body fluids have been observed in patients with various eosinophil-associated diseases.²¹

A prospective cross-sectional analysis of 47 pediatric patients undergoing endoscopic evaluation of possible EoE showed that plasma EDN levels significantly correlated with esophageal eosinophil density and were increased in patients with active EoE vs controls.¹⁵ More recently, Subbarao et al¹⁸ examined the correla-

tion between serum EDN levels and disease phenotype and activity in a longitudinal study involving children with EoE. Serum EDN levels were measured periodically in children with EoE for 24 weeks, and at baseline alone in controls. The levels were found to be significantly elevated in EoE patients compared with controls. Tissue EDN expression in EoE patients has also been examined. Alexander et al²² in 2008 published a study on immunofluorescence staining of esophageal biopsy specimens for EDN in 4 groups of patients: normals, low-level eosinophilia GERD patients, EoE patients with dysphagia with response to topical steroid therapy, and classical EoE patients with 25 to 100 eosinophils /high-power field. EDN scores were found to be higher in the last 2 categories of patients. Their study suggested that extracellular EDN immunofluorescence staining is a better marker for EoE than maximum eosinophil count.²² Another study evaluated the magnitude and distribution of EDN in esophageal specimens of EoE patients.²¹ Esophageal specimens were stained by immunofluorescence using polyclonal rabbit antibody to EDN. EDN staining was clearly observed in specimens from all EoE patients. In some EoE patients, marked extracellular EDN deposition was observed despite relatively small numbers of intact eosinophils, suggesting that tissue eosinophils may underestimate how extensively eosinophils are involved, particularly in individuals with marked eosinophil degranulation.²¹ These studies do establish a positive correlation of tissue and serum levels of EDN with EoE disease activity. Further studies are needed to define its place in the diagnostic algorithm of EoE.

Micro ribonucleic acids (miRNAs)

MicroRNAs (miRNAs) are single-stranded RNA molecules, 19 to 25 nucleotides long, that regulate posttranscriptional gene silencing of target genes.¹⁶ Multiple studies have demonstrated that EoE is associated with marked changes in tissue-specific gene expression, referred to as the EoE transcriptome.^{8,13} Lu et al²³ in 2012 investigated esophageal miRNA expression profile in patients with active EoE and those with glucocorticoid-induced remission. The most up-regulated miRNAs in EoE patients were: miRNA-21 and miRNA-223, and miR-375 was the most down-regulated. This miRNA signature correlated with the degree of tissue eosinophilia and was distinct from patients with chronic noneosinophilic esophagitis. The miRNA signature was largely reversible with glucocorticoid therapy. They, moreover, identified miRNA-675 as the only disease remission-induced miRNA.²³

Expression levels of a selected set of EoE-associated miRNAs in plasma samples from patients with EoE were also measured; miR-146a, miR-146b, and miR-223 were up-regulated in plasma of EoE patients compared with healthy controls.²³ In another study by the same author, miRNA array analysis was used to determine the differentially expressed miRNAs after IL-13 stimulation in esophageal squamous epithelial cells.²⁴ There was down-regulation of miR-375 expression levels in EoE patients compared with controls. The levels normalized with disease remission and inversely correlated with the degree of allergic inflammation.²⁴ These studies have opened up a whole new exciting family of potential biomarkers for EoE diagnosis and monitoring of treatment. Further studies will help refine their clinical utility.

Mast cells

Mast cells are important in the pathogenesis of EoE because they produce an abundance of cytokines that activate eosinophils and molecules that directly promote tissue remodeling.^{25,26} Gupta et al showed increased MC staining using anti-mast cell tryptase antibodies in EoE patients compared with controls in esophageal biopsy specimens of children with EoE.¹⁰ Another study quantified intraepithelial MCs using anti-tryptase antibodies. MC activation status was evaluated by immunohistochemistry for immunoglob-

ulin E. Esophageal biopsy specimens from patients with EoE showed higher intraepithelial MC counts and immunoglobulin E-bearing cells than GERD and control groups.²⁷ A case-control study showed that maximal epithelial tryptase density was higher in EoE than GERD. The tryptase density and eosinophil count were only weakly correlated.²⁸ Abonia et al²⁹ generated transcriptome expression profiles related to MC number and degranulation in patients with EoE and healthy controls. The expression of transcripts of mast cell proteases carboxypeptidase A3 and tryptase correlated with MC levels and distinguished EoE patients from controls. Treatment of patients with fluticasone propionate normalized levels of MCs and MC transcriptome in responder patients. Aceves et al³⁰ showed significantly increased numbers of tryptase-positive MCs in esophageal smooth muscle of EoE patients, with significant reduction in numbers with topical corticosteroid. In fact, the tryptase-positive mast cells were shown to express TGF- β 1 which increased contractility of cultured human esophageal smooth muscle cells in vitro.³⁰ Another study examined the expression of several well-defined MC-associated genes in biopsy specimens from distal esophagus from adult patients with EoE. Compared with those from control subjects, biopsy specimens from patients with EoE without treatment had significant increases in expression of MC genes. MC-associated gene expression was significantly reduced by treatment with swallowed fluticasone. Gene expression was also significantly reduced by food elimination diet and remained decreased during food reintroduction when patients denied any symptoms. Expression of the genes remained increased in nonresponders and was increased in patients experiencing food reintroduction-induced relapse.³¹ These studies show that mast cell products and dysregulated transcriptomes in EoE patients could potentially be useful for disease diagnosis and monitoring.

Absolute eosinophil count

Numerous studies have documented absolute and relative peripheral blood eosinophil counts in patients with EoE. The reported incidence of peripheral blood eosinophilia (PBE) shows a wide range of 10 to 100% over different age groups.⁵ Baxi et al³² reported a 67% incidence of PBE in children and adolescents in 2006. A prospective cross-sectional analysis of pediatric patients undergoing endoscopic evaluation of possible EoE showed that AEC levels significantly correlated with esophageal eosinophil density.¹⁵ In general, the reported degree of PBE when present in patients with EoE is modest.⁵ Very limited data are available correlating PBE with response to therapy. More data are needed for the effect of EoE therapy on AEC in the context of eosinophil counts on esophageal mucosal biopsy specimens.

Other markers

Various other cytokines, chemokines, and cell products involved in the pathogenesis of EoE are under investigation as potential markers of disease activity and response to therapy. Protheroe et al³³ developed a monoclonal antibody specific for an eosinophil secondary granule protein eosinophil peroxidase and used it to specifically identify intact eosinophils and detect eosinophil degranulation in formalin-fixed specimens. Based on this immunohistochemical staining, they developed a histopathologic scoring that was able to identify patients whose clinical course was suggestive of a diagnosis of EoE but failed to reach the critical threshold of 15 or more eosinophils per high-power field. Huang et al studied plasma fibroblast growth factor basic levels by cytometric bead array to differentiate EoE from GERD.¹⁷ Another protein of interest is FK506-binding protein 51 (FKBP51). Caldwell et al³⁴ in 2010 demonstrated increased esophageal FKBP51 mRNA levels in topical glucocorticoid responders compared with those in control subjects and patients with untreated active EoE. They suggested that in-

creased FKBP51 transcript levels could be used to distinguish glucocorticoid responders from untreated patients with active EoE.

Conclusion

Our understanding of the pathogenesis of EoE has significantly improved over the last decade, providing us with promising candidates as biomarkers with respect to disease activity and response to therapy. Eotaxin-3 and IL-13 have emerged as the most promising ones with respect to sensitivity and degree of positive correlation to the disease process. However, their place in the diagnostic algorithm of EoE is far from defined at this time. Special attention should also be paid to mast cell activation factors and products, IL-5, and development of a biomarker panel. Future research on biomarkers for EoE should include longitudinal studies, establishment of normal values, effects of concomitant atopic diseases, age and gender, and validation of methodology of the tests.

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