HHS Public Access

Author manuscript

Ann Allergy Asthma Immunol. Author manuscript; available in PMC 2019 August 01.

Published in final edited form as:

Ann Allergy Asthma Immunol. 2018 August ; 121(2): 218–228. doi:10.1016/j.anai.2018.05.005.

Minimally-Invasive Biomarker Studies in Eosinophilic Esophagitis: A Systematic Review

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Abstract

Background: Eosinophilic esophagitis (EoE) is a chronic, inflammatory disease of the esophagus which currently requires repeated endoscopic biopsies for diagnosis and monitoring as no reliable non-invasive markers have been identified.

Objective: To identify promising minimally-invasive EoE biomarkers and remaining gaps in biomarker validation.

Methods: We performed a systematic review of EMBASE, Ovid Medline, PubMed, and Web of Science from inception to June 6, 2017. Studies were included if subjects met the 2007 consensus criteria for EoE diagnosis, a minimally-invasive biomarker was assessed, and the study included at least 1 control for comparison.

Results: The search identified 2094 studies, with 234 reviewed at full text level, and 49 included in the analysis (20 adult, 19 pediatric, 7 pediatric and adult, and 3 not stated). The majority (26 of 49) were published after 2014. Thirty-five studies included normal controls, 9 analyzed atopic

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Conflicts of interest: none

controls, and 29 compared samples from subjects with active and inactive EoE. Minimally-invasive biomarkers were obtained from peripheral blood (n=41 studies), sponge/string samples (3), oral/throat swab secretions (2), breath condensate (2), stool (2), and urine (2). The most commonly reported biomarkers were peripheral blood eosinophils (16), blood and string eosinophil granule proteins (14), and eosinophil surface or intracellular markers (12). EoE biomarkers distinguished active EoE from normal controls in 23 studies, atopic controls in 2 studies, and inactive EoE controls in 20 studies.

Conclusion: Several promising minimally-invasive biomarkers for EoE have emerged; however, few are able to differentiate EoE from other atopic diseases.

Keywords

Eosinophilic esophagitis; biomarker; diagnosis; monitor; string test; esophageal brushings; noninvasive; minimally invasive

INTRODUCTION

Eosinophilic esophagitis (EoE) is a clinicopathologic diagnosis characterized by symptoms of esophageal dysfunction and eosinophilia of the esophageal epithelium. For many years, esophageal eosinophilia was considered a manifestation of gastroesophageal reflux disease (GERD)[1]; however, a retrospective case series by Attwood et al[2] in 1993 described 12 adults with dysphagia, dense intraepithelial esophageal eosinophils in the absence of reflux. One year later, Straumann et al described 10 patients with acute recurrent dysphagia with discrete endoscopic findings and high concentrations of intraepithelial eosinophils, responsive to treatment with systemic steroid and antihistamine; further defining a new disease entity termed *Idiopathic Eosinophilic Esophagitis*.[3] Finally, in 1995 Kelly et al[4] demonstrated disease remission with institution of an elemental diet, suggesting that EoE is a food-driven disorder. Despite these descriptions, formalized diagnostic criteria for IEE were lacking until 2007, when the first consensus guidelines were developed for evaluation and management of the condition now termed eosinophilic esophagitis (EoE).[5]

The clinical presentation of EoE varies by age. Children suffer mainly from feeding difficulty and failure to thrive, with symptoms including vomiting and abdominal pain. In older children, complaints may include chest pain and dysphagia. This is in contrast to adolescents and adults, who present with symptoms of dysphagia, chest pain, and food impaction.[6] The 2007 consensus definition of EoE requires at least one symptom of esophageal dysfunction along with at least 15 eosinophils per high-power field on esophageal biopsy. Other causes of esophageal eosinophilia – in particular GERD – must be excluded before the diagnosis can be established.[5] Newer guidelines[7] have been published further refining the diagnosis of EoE in which GERD and EoE may coexist and interact. We have chosen to use the 2007 consensus definition of EoE to ensure that all pertinent papers prior to the publication of these updated guidelines would be included in our review. Current treatment modalities are elimination diets (empiric, skin test-directed, or elemental), swallowed topical corticosteroids, and proton pump inhibitors. For patients who develop esophageal narrowing, esophageal dilation is used as treatment to alleviate symptoms. Controversy remains regarding the diagnostic and therapeutic long-term management given

no evidence this is a pre-malignant condition, and few studies have investigated long-term outcomes associated with diet or topical corticosteroid therapy after symptom and histologic remission is achieved. Nevertheless, in the vast majority of patients, EoE is a chronic disease process, and if therapy is discontinued, inflammation recurs which can affect quality of life and result in complications (e.g. stricture formation).[6,8–10] Current expert consensus recommends maintenance therapy for patients with evidence of chronic esophageal remodeling, a history of food impactions or severe symptoms, or rapid recurrence of symptoms while not on therapy.[6]

One of the challenges with EoE is discordance between symptoms and histopathologic features, making diagnosis and monitoring response to therapy challenging. For example, some patients with minimal symptoms may have significantly elevated eos/hpf on esophageal biopsy indicating ongoing inflammation and active disease. The current recommendations for initial diagnosis and disease monitoring involve serial endoscopic evaluations with biopsies. This invasive approach poses risk to patients especially in children younger than 3 years. In April 2017, the U.S. Food and Drug Administration (FDA) issued a new warning of possible negative effects on brain development in children below 3 years undergoing recurrent or lengthy procedure requiring sedation or general anesthesia.[11] The negative effects on brain development associated with short duration anesthesia required for one upper endoscopy is unknown; however, patients with EoE typically undergo multiple procedures. In addition to the risks posed to patients, there are also significant health care costs associated with these procedures.[12–14] Identifying a reliable, non-invasive or minimally invasive biomarker for diagnosing and monitoring could help reduce the need for risky, invasive procedures, potentially increasing safety and reducing health care expenditures.[13]

Several non-invasive biomarkers have been studied in patients with EoE but none have yet been incorporated into treatment guidelines or routine clinical practice. Recent efforts to identify EoE biomarkers have rapidly expanded;[13,15] and while there are some published reviews on this subject, these publications do not employ systematic review methods in order to ensure all relevant studies are identified. Therefore, we undertook a systematic review to: (1) identify study design strengths and weaknesses that inform design of future EoE biomarker studies and (2) identify the most promising biomarkers so that attempts at reproduction and validation in other populations may propel the field forward.

METHODS

Eligibility criteria and literature search

This systematic review contains the elements of the 27-item checklist put forth in the Preferred Reporting Items for Systematic Reviews and Meta-analysis statement (PRISMA) statement.[16] Articles were included that diagnosed EoE based on 2007 consensus definition which requires at least one symptom of esophageal dysfunction and at least 15 eosinophils/hpf on esophageal biopsy.[5] The 2007 consensus definition was chosen so that articles prior to 2011 would not be excluded if they did not fulfill all of the 2011 EoE diagnosis criteria. In addition, articles were also required to study a non-invasive or minimally invasive biomarker. A non-invasive or minimally invasive test was defined as one

that can be collected without an endoscope. Human case reports, case series, cross-sectional and cohort studies, and clinical trials were included. All non-human studies and studies not containing new clinical information were excluded. We also excluded studies investigating allergy testing (serum-specific IgE, prick skin testing, atopy patch testing), and radiologic modalities based on consensus of the authors.

We performed a systematic review of English-language and non-English-language articles using MEDLINE, PubMed, Web of Science and Embase (inception to June 6, 2017) with the assistance of an experienced medical librarian (LAM). The following search terms were used: eosinophil*, hypereosinophil*, serologic marker, peripheral blood, marker, biomarker, Cytosponge, string test, non-invasive, minimally invasive, semi-invasive, brush, and assay. The search strategy used for MEDLINE is detailed in Table 1. A similar search strategy was adapted by an experienced medical librarian for the other electronic databases. To identify additional relevant articles, bibliographies of included articles were searched. Published proceedings from 2013–2016 American College of Gastroenterology (ACG), Digestive Diseases Week (DDW), American Academy of Allergy, Asthma and Immunology (AAAAI), and United European Gastroenterology (UEG) annual meetings were searched online using the term eosinophilic esophagitis in portable document format (PDF). When a PDF was not available, meeting programs were searched. Once all studies had been reviewed and appropriate articles included, the bibliographies from all included articles were compared to our original search and duplicates removed. Content experts among the authors (BLW, MG, AS, ESD) were also queried regarding their knowledge of unpublished data or studies omitted from the list of eligible studies.

Study selection

Two abstract reviewers worked independently to consider whether each of the abstracts identified would meet eligibility criteria. The reviewers were not blinded to the author, institution or journal of publication. If it was unclear whether the article met inclusion criteria based on the abstract or if the reviewers disagreed on whether to include or exclude the study, a full text review was performed. In order to include all possible relevant studies, we did not specifically exclude other causes of esophageal eosinophilia such as GERD when reviewing at the abstract level. Disagreement at full text inclusion levels was resolved by consensus.

Consensus was obtained upon discussion and agreement among 3 authors. Once all duplicates had been removed from the bibliography search of the included articles, the titles were reviewed by a single reviewer.

Data Collection

One author (MAR) independently extracted relevant data into a spreadsheet and these data were rechecked by a second reviewer (BTH). Any discrepancies were resolved through author consensus. Extracted data from each study included: author, year, age of participants, number of participants, information on study controls (normal, atopic, disease activity), the non-invasive collection method (e.g. blood, urine, sponge), a complete list of all biomarkers studied, and a list of all biomarkers where statistical significance was found when compared

to normal controls, atopic controls, and disease responsiveness to treatment measures. Authors were contacted if data were missing. The method of author contacts were: (1) e-mail briefly explaining the study and asking specifically for data relevant to the review and (2) a 2nd email 1 week later if no response with a scaled-back request to share the most important missing data.

Assessment of methodological quality

To assess risk of bias, 2 independent reviewers followed instructions from the Cochrane Handbook for Systematic Reviews of Diagnostic Test Accuracy.[17] For each study, we used the recommended quality items derived from the QUADAS-2 tool by determining the spectrum of patients represented, the likelihood the reference standard was to identify the target condition, time between the index and reference tests, the allocation of reference standard on the study population with regard to whole group and independence of index test, whether interpretation of tests was independent, clinical data available when tests were interpreted and whether this would be available in practice.[17,18]

RESULTS:

Our initial search of electronic databases yielded 1454 articles after removal of duplicates. By searching bibliographies, reviewing meeting programs, and contacting content experts an additional 640 studies were identified after removal of duplicates. Of these 2094 articles, 234 met criteria for full-text review. Forty-nine studies[19–67] were included after full-text review. The bibliographies of these 49 studies were searched and there were 614 non-duplicate references derived which were reviewed by a single reviewer at title level. Seven articles were identified for review at the abstract level. Four articles did not include minimally invasive biomarkers, 1 article was a duplicate that had already been included, 1 article was an abstract for an article we had already included, and 1 article was an abstract only that described a minimally invasive biomarker. We were unable to identify an associated full-length manuscript for these abstract data, and therefore elected to contact the authors to see if they could share their data (author did not respond). Figure 1 describes the flow of information through the different phases of the systematic review using the PRIMSA flow-sheet template.

Table 2 details the study characteristics and biomarkers studied for each of the included articles. The search identified 20 adult, 19 pediatric, and 7 combined age studies, in addition to 3 studies which did not state the age of the participants. The majority of the publications were published after 2014 (26 of 49). Biomarkers were assessed from peripheral blood (41 studies), Cytosponge/esophageal string test (3), breath sampling (2), oropharyngeal swabs (2), stool (2), and urine (2). With regards to study controls, 35 studies included a normal control group, 9 included atopic controls, and 29 studies compared active to a “treated EoE” (trEoE) control group. There were 23 studies that noted significant differences in a variety of markers in subjects with EoE versus normal controls. Only increased urinary 3-bromotyrosine (3-BT)[26] and decreased total IgE[61] (EoE vs allergic rhinitis) demonstrated significant difference when compared to atopic controls.

The most common EoE biomarker assessed by 16 studies was the peripheral blood absolute eosinophil count (AEC). Among these studies, 7 reported a significant difference in AEC between subjects with active versus trEoE (Table 3). Only 5 studies had an atopic control group for comparison (no significant differences noted). Four studies were noted that observed a change in AEC compared to normal controls.

Table 4 summarizes the findings for 14 studies reporting granule proteins as biomarkers, including eosinophilic cationic protein (ECP) (9 studies), eosinophil-derived neurotoxin (EDN) (7 studies) eosinophil peroxidase (EPX) (2 studies), and major basic protein (MBP) (2 studies). Only two of these studies (one assessing EDN[39] and another assessing ECP[60]) had an atopic control group for comparison, and neither study noted a significant difference between groups. Compared to normal controls, EDN was significantly increased in four studies, ECP in 2, and MBP in 1. Four studies identified significantly different ECP levels in samples from patients with active vs treated EoE. One additional study was a case report (n=1) which noted normalization of the ECP level following EoE treatment.[31] There were 12 studies which analyzed a variety of eosinophil surface/intracellular markers. Four of these studies compared EoE to an atopic control group, but none identified any significant difference between groups (Table 5).

We identified 29 studies which assessed for potential biomarkers to monitor response to treatment. Only 3 of these studies were randomized clinical trials,[25,60,63] which noted significant changes in AEC, ECP, chemokine ligand-26 (CCL-26), chemokine ligand-17 (CCL-17), and mast cell tryptase (MCT) in patients with active versus treated EoE (Table 6). Finally, we identified 3 studies that assess RNA (Benitez, Nguyen and Sawant) and note that these are distinct from all of the other studies which measured proteins (Table 2).

Quality Assessment:

In 22 of the 49 included studies, there was clear declaration that patient samples were obtained either by sampling consecutive patients or randomly selected, and therefore, at low risk of multiple biases. However, almost all of the included studies were derived from samples obtained from patients seen at specialty referral centers, resulting in possible selection biases and issues related to uncertain generalizability to other patient populations. Four studies were determined to have a high risk of bias based on the increased time frame between collection of the esophageal biopsies and measurement of the non-invasive biomarker while 16 studies did not clearly state what time frame separated the collection of biopsies and the measurement of the minimally invasive biomarker. Details of the quality assessments are displayed in Table 7.

DISCUSSION:

This study is the first to utilize a systematic approach to identify relevant articles, in contrast to other literature reviews for minimally invasive EoE biomarkers. Using a systematic approach, we identified 2094 potential articles, of which 49 met our inclusion criteria. Twenty-six of these articles have been published since 2014, a testament to the rapid pace at which the EoE biomarker field is moving; however, only 3 of these studies were randomized-controlled studies.

A key objective of this review was to identify methodological strengths and weaknesses of the identified studies. Many weaknesses were identified including specimen timing, retrospective design of many studies, absence of an atopic control group and selection of patients which would represent the general population--suggesting there is opportunity for improvement in the design of future studies. First, we strongly recommend that study protocols specify that biomarkers be measured at the same time as the reference standard (i.e. peak number of eosinophils in esophageal histology). This recommendation favors prospectively designed studies which can pre-specify sample collection timing. For example, blood, saliva, or urine biomarkers should ideally be collected immediately prior to esophageal biopsy to account for the possibility that the biopsy itself could impact the biomarker measurement. In addition, randomized-controlled studies (representing only 3 of 49 included studies) allow for comparison in both placebo and intervention groups and most importantly, reduce the risks posed by confounding factors. Second, EoE biomarker studies should incorporate a prospective design using random or consecutive selection to prevent selection bias in determining which patients are tested; a phenomenon that is difficult to avoid in retrospective studies. Third, the generalizability of samples derived primarily from highly specialized tertiary referral centers should be considered. Attempts to include community-based samples or design studies that utilize a population-based sampling frame may improve applicability of study findings. Finally, we identified only 9 studies that included atopic controls--only 2 of which identified biomarkers (increased urinary 3-BT and decreased total IgE) that distinguish EoE from other allergic disease states. Inclusion of atopic controls is critical given the fact that a large proportion of patients with EoE have allergic comorbidities associated with eosinophilia (i.e. asthma, allergic rhinitis). Consequently, biomarker comparison in EoE subjects and other atopic diseases enhances the ability to control for a more robust range of possible confounders.

The other objective of this study was to identify the most promising EoE biomarkers. Future plans include a meta-analysis of biomarkers which were investigated across multiple studies to determine whether pooled data can enhance power and provide more robust point estimate compared to estimates derived from smaller, individual studies. AEC, ECP, eotaxin, and chemokine receptor type 3 (CCR3) are of interest for meta-analysis based on the number of studies which measured these biomarkers along with selecting biomarkers representing different general categories of biomarkers (granule protein, chemokine, eosinophil cell surface protein). We note that almost all of the studies performed to date were investigating blood-based biomarkers (AEC more often than any other), which identifies a need to develop alternative sampling techniques. Given the risk of potential confounding due to other eosinophilic/atopic disorders, minimally-invasive sampling of the esophagus or a contiguous site may prove critical. Early findings from esophageal string test and Cytosponge are encouraging but represent only a small fraction of EoE biomarker studies. Additional controlled studies are also needed to validate mass spectroscopy assessment of brominated urinary tyrosine in EoE subjects. While the degree of 3-BT elevation may distinguish EoE subjects, this has also been used as a marker of pediatric asthma.[68] Another potential approach might be to combine a biomarker (e.g. AEC) with symptom assessment in order to confer site specificity. This too has certain pitfalls particularly because subjects with EoE /or asthma may have subclinical or comorbid disease. Further efforts to build the

evidence base around non-blood-based EoE biomarkers is an important focus of ongoing research efforts.

In summary, we identified 49 studies that examined minimally invasive EoE biomarkers, the majority of which were identified over the past 3 years. Blood-based biomarkers are the most frequently investigated however early findings from other non-invasive methods (esophageal string test and Cytosponge) seem promising. We identified timing of specimen collection, patient selection, and inclusion of an atopic control group as important study design considerations for future EoE biomarker studies. The absence of meta-analysis is the main limitation of this study; however, this is being actively pursued. Despite the increased interest in this area and the clear clinical need for minimally-invasive biomarkers, there is still not a minimally-invasive biomarker that has been incorporated into guideline recommendations or routine clinical practice. Fortunately, several promising biomarkers are under study which may reduce the need for repeated endoscopic biopsies.

Acknowledgments

Funding Source: NIH Grant R01 DK101856

Abbreviations:

3-BT	3-bromotyrosine
AAAAI	American Academy of Allergy, Asthma and Immunology
ACG	American College of Gastroenterology
AEC	absolute eosinophil count
CCL-26	chemokine ligand-26
CCL-17	chemokine ligand-17
CCR3	chemokine receptor type 3
DDW	Digestive Diseases Week
ECP	eosinophilic cationic protein
EDN	eosinophil-derived neurotoxin
EoE	eosinophilic esophagitis
EPX	eosinophil peroxidase
FDA	Food and Drug Administration
GERD	Gastroesophageal reflux disease
MBP	major basic protein
MCT	mast cell tryptase

PDF	portable document format
PRIMSA	Preferred Reporting Items for Systematic Reviews and Meta-analysis
trEoE	treated EoE
UEG	United European Gastroenterology

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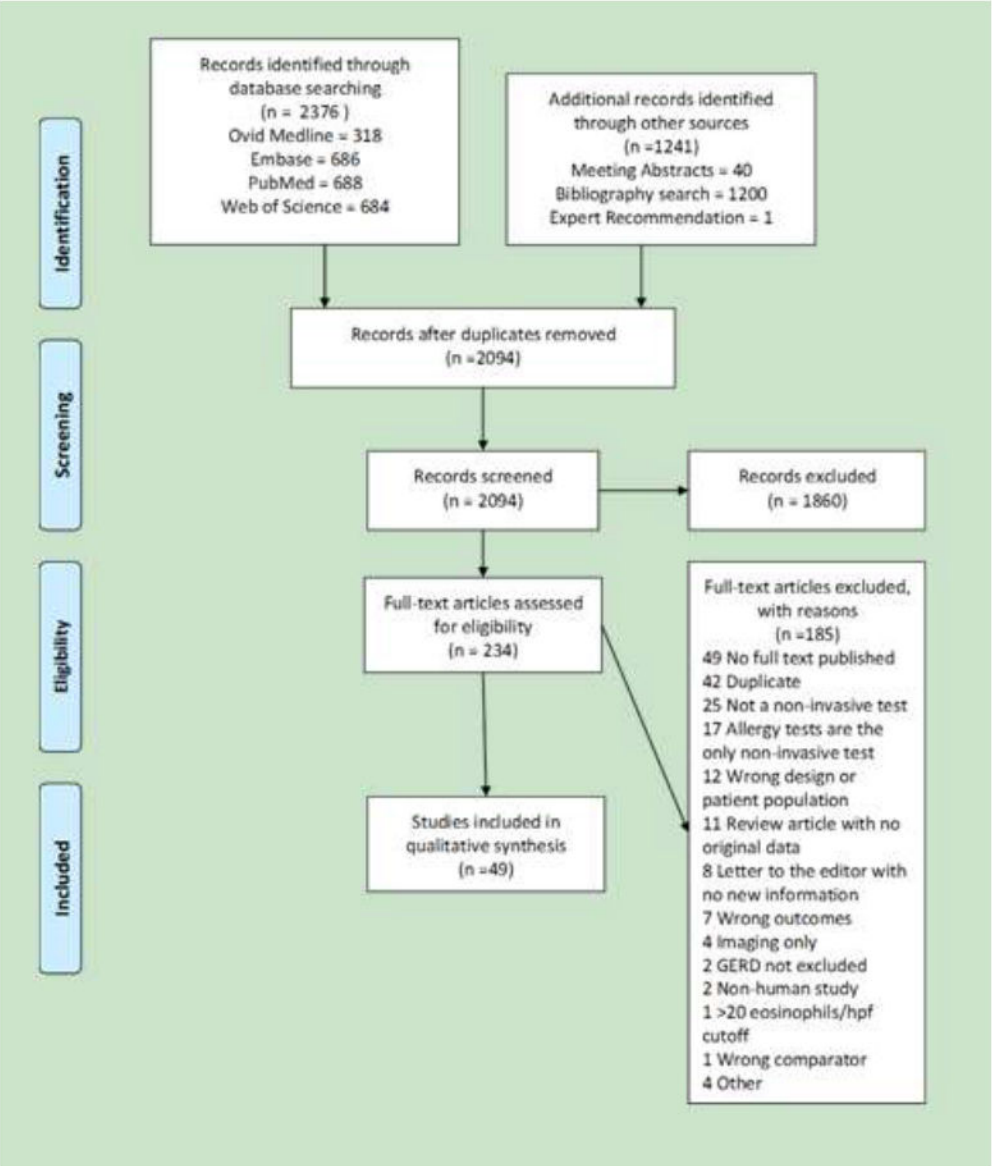

Figure 1.
Legend: The PRISMA diagram details the search and selection process[14]

Table 1:

Search Strategy

	Search Terms
1	(eosinophil*or hypereosinophil*) or exp eosinophil/or exp eosinophilia/
2	1 and (exp esophagitis/ or (esophag* or oesophag*)
3	serologic *marker*
4	peripheral blood
5	marker*
6	3 or 4 or 5
7	exp biomarkers/or (biomarker* or bio-marker* or cytosponge* or enterotest* or brush*or assay or (((sponge or string)adj2 (techn*1 or capsule*or sampl*)) or (((gel or gelatin)adj2 capsule*)))
8	6 or 7
9	(noninvasive* or non-invasive* or non-endoscop* or nonendoscop* or ((minim* or less)adj3 invasive*))
10	(semi-invasive or semi invasive) [title, abstract, original title, name of substance word, subject heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier]
11	9 or 10
12	2 and (8 or 11)
13	remove duplicates from 12

Table 2:

Characteristics of All Included Studies

Author	Year	Age group	Number of EoE patients	Number of Controls	Normal Control	Atopic Control	Disease Activity Control	Biomarker(s) Studied	Biomarker source
Benitez	2015	Pediatric	33	35	Yes	No	Yes	16S RNA bacterial sequencing	Oral swab
Blanchard	2011	Not stated	226	14 GERD, 14 Normal	Yes	No	Yes	Cytokine array	Blood
Botan	2017	Both	31	10	Yes	No	No	“activated” appearance of eosinophils	Blood
Bullock	2007	Pediatric	12	8 Normal, 5 Atopic	Yes	Yes	Yes	AEC, CCR3 on eosinophils, CD4 expression of IL-5	Blood
Clayton	2014	Adults (Except for 3 subjects age 15–17)	15	41 Normal	Yes	No	No	IgG4 (total and food-specific)	Blood
Colson	2014	Pediatric	59	0	No	No	Yes	AEC	Blood
Conus	2009	Not stated	11 (5mepolizumab, 6placebo)	0	No	No	Yes	AEC, IL-5Rα, CCR3	Blood
Cunnion	2016	Pediatric	27	24 Normal, 24 Atopic	Yes	Yes	No	3-BT	Urine
Dellon	2015	Adult	61	87	Yes	No	Yes	IL-4, IL-5, IL-6, IL-9, IL-13, TGF-α, TGF-β, TNF-α, Eotaxin 1/2/3, TSLP, MBP, EDN	Blood
Dellon	2016	Adult	61	87	Yes	No	Yes	Periostin	Blood
Domenech Witek	2017	Adult	19	0	No	No	Yes	tIgE, AEC, ECP	Blood
Fuentebella	2010	Pediatric	33	7 GERD, 8 Normal	Yes	No	No	Treg: CD4+CD25hi CD127lo	Blood
Fujiwara	2002	Adult	1	0	No	No	Yes	AEC, Eotaxin (total and free), ECP	Blood
Furuta	2013	Pediatric	14 Active,8 Disease remission	4 GERD, 15 Normal	Yes	No	Yes	MBP, EDN, ECP, EPX, CLC/Gal-10	Esophageal String
Huang	2010	Pediatric	35 Newly diagnosed 9 Treated	8 GERD, 5 Ulcerative colitis, 5 Crohn’s disease, 8 Normal	Yes	No	Yes	35 chemokine/cytokines including: bFGF or FGF-2; eotaxin 1/2/3; IL-1α; IL-1β; IL-1RA; IL-2; IL-4; IL-5; IL-6; IL-7; IL-8; IL-10; IL-12-p40; IL-12-p70; IL-13; IL-15; IL-17; IL-17F; ENA78; GCSF; GM-CSF, GRO-α; IFN-γ; IP10; leptin; MCP-3; MIG; MIP-1α; MIP-1β;NGF;PDGF-BB; RANTES; TGF-β;TNF-α; TNF-β, and VEGF	Blood
Johnsson	2011	Adult	12	8 Ulcerative colitis, 10 Airway allergy 10 Normal	Yes	Yes	No	AEC, CD23, CD54, CRTH2, CD11c, CCR3, CD44, CD11b, CD18, CD58, CCL5(RANTES), CCL11 (eotaxin-1), CCL26, IL-2, IL-3, IL-4, IL-5, GM-CSF	Blood
Jyonouchi	2013	Pediatric	10 Active 10 Disease Remission	16 Normal	Yes	No	Yes	iNKTs	Blood
Katzka	2015	Adult	13 Active 7 Disease Remission	0	No	No	Yes	Eos/hpf, EDN	Cytosponge
Kinoshita	2012	Adult	18	18 EGID 30 Normal	Yes	No	No	IL-5, IL-13, IL-15, Eotaxin-3, TSLP	Blood
Knipping	2014	Pediatric	91	45	Yes	No	No	TSLP, TARC, KFLC, L-FLC	Blood
Konikoff	2006	Pediatric	16 Active 16 Disease remission 1 Intermediate	9 Normal5 EGID	Yes	Yes	Yes	AEC, IL-5, Eotaxins 1/2/3 EDN (blood/stool)	Blood, Stool

Author	Year	Age group	Number of EoE patients	Number of Controls	Normal Control	Atopic Control	Disease Activity Control	Biomarker(s) Studied	Biomarker source
Krupp	2016	Pediatric	33	37	Yes	No	No	IL-5, IL-9, Eotaxin, EGF, FGF-2	Blood
Lanz	2012	Pediatric	18	23 Gastritis 14 Normal	Yes	No	No	eNO	Breath
Leung	2012	Both	14	0	No	No	Yes	eNO	Breath
Lexmond	2013	Pediatric	30	20 Reflux, 20 Normal	Yes	No	No	Urine LTE4, Serum LTC4	Urine, Blood
Lingblom	2014	Adult	21	15	Yes	No	Yes	CD18, CD44, CD40, CCR3, CD23, CD54, FPR, CRTH2	Blood
Lingblom	2017	Both	53	51	Yes	No	No	CD23, CD44, CD54, CRTH2, FoxP3, Galectin-10	Blood
Lucendo	2013	Adult	17	0	No	No	Yes	AEC, tIgE, ECP	Blood
Min	2016	Both	46	53	Yes	No	Yes	AEC, Eotaxin-3, EDN, ECP, IL-5	Blood
Morris	2016	Pediatric	17 Active, 14 Disease Remission	10 Atopic	No	Yes	Yes	AEC, Eosinophil progenitor	Blood
Nguyen	2011	Pediatric	35 Newly Diagnosed EoE off therapy 7 Known EoE on therapy	35	Yes	Yes	Yes	PBMC transcript analysis of STAT1, STAT6, and CD66b, Surface CD66b on peripheral eosinophils	Blood
Patel	2010	Pediatric	10	11 GERD,10 Normal	Yes	No	No	HLA-DR	Blood
Paterson	2016	Adult	6	166 esophagitis 10 Candida 638 Normal	Yes	No	No	Eos/hpf	Cytosponge
Paz Zafra	2012	Both	25	17	Yes	No	No	AEC, tIgE, C5a, CD40 ligand, GCSF, GM-CSF, CXCL1, CCL1, CD54, IFN-c, IL-1a, IL-1b, IL-1ra, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12 p70, IL-13, IL-16, IL-17, IL-17E, IL-23, IL-27, IL-32a, CXCL10, CXCL11, CCL2, MIF, CCL3, CCL4, Ser-pin E1, RANTES,CXCL12, and TNFa	Blood
Philpott	2015	Adult	85	193	Yes	No	No	AEC	Blood
Rawson	2016	Pediatric	27	11	Yes	No	Yes	TGF-β, PAI-1, PF4	Blood
Rayapudi	2014	Not stated	7	6	Yes	No	No	iNKTs	Blood
Rodriguez-Sanchez	2013	Both	22 Responders, 8 Nonresponders	0	No	No	Yes	AEC, ECP, tIgE	Blood
Saffari	2017	Adult	8	21	Yes	No	Yes	EPO activity	Throat swab
Sawant	2015	Pediatric	7	8 Asthma8 Normal	Yes	Yes (asthma)	No	miR-21	Blood
Schlag	2014	Adult	15	0	No	No	Yes	ECP, MC tryptase	Blood
Schlag	2015	Adult	69	39 Atopic controls (with EoE)	No	Yes	Yes	AEC, ECP, CCL-17, CCL-18, CCL-26, MC tryptase	Blood
Soylu	2016	Adult	7 (also with allergic rhinitis)	60 Allergic rhinitis	No	Yes	No	tIgE	Blood
Straumann	2005	Adult	8	4 Dyspepsia, 6 Normal	Yes	No	No	AEC; CD25, IL-4, IL-5, IL-13, IL-10 expression on eosinophils	Blood
Straumann	2010	Adult	11 (5mepolizumab, 6placebo)	0	No	No	Yes	ECP, EDN, Eotaxin, and TNF-α, IL-5Rα on eosinophils	Blood
Subbarao	2011	Pediatric	60	20	Yes	No	Yes	IL-5 (Blood); EDN (Blood/stool)	Blood, stool

Author	Year	Age group	Number of EoE patients	Number of Controls	Normal Control	Atopic Control	Disease Activity Control	Biomarker(s) Studied	Biomarker source
Upparahal li Venkatesh aiah	2016	Both	2	0	No	No	Yes	CD274	Blood
von Arnim	2011	Adult	23	20 GERD	Yes	No	No	AEC, elevated or normal tIgE	Blood
Wright	2016	Adult	20	10	Yes	No	Yes	sIgG4 (total and food-specific)	Blood

Abbreviations Table 2:3-bromotyrosine (3-BT), absolute eosinophil count (AEC), chemokine receptor type 3 (CCR3), chemokine ligand 1 (CCL1), chemokine ligand 3 (CCL2), chemokine ligand 3 (CCL3), chemokine ligand 4 (CCL4), chemokine ligand 17 (CCL17), chemokine ligand 18 (CCL18), chemokine ligand 26 (CCL26), CXC chemokine ligand 1 (CXCL1), CXC chemokine ligand 10 (CXCL10), CXC chemokine ligand 11 (CXCL11), CXC chemokine ligand 12 (CXCL12), fibroblast growth factor basic (bFGF or FGF-2), IL-1 receptor antagonist (IL-1RA), IL-5 receptor alpha (IL-5Rα), eosinophil cationic protein (ECP),eosinophil derived neurotoxin (EDN), eosinophil peroxidase (EPX or EPO), eosinophil per high power field (eos/hpf), epidermal growth factor (EGF), epithelial cell-derived neutrophil-activating protein-78 (ENA78), exhaled nitric oxide (FeNO), formyl peptide receptor (FPR), granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), growth-related oncogene-alpha (GRO- α), interferon-gamma (IFN-g), invariant natural killer T cells (iNKTs), kappa free light chain (k-FLC), lambda free light chain (L-FLC), leukotriene E4 (LTE4), leukotriene C4 (LTC4), macrophage inflammatory protein 1 alpha (MIP-1a); macrophage inflammatory protein 1 beta (MIP-1b), major basic protein (MBP), MC (mast cell), nerve growth factor (NGF), peripheral blood mononuclear cell (PBMC), plasminogen activator inhibitor 1 (PAI-1), platelet derived growth factor-BB (PDGFBB), platelet factor 4 (PF4), regulated upon activation normal T cell expressed and secreted (RANTES), ribonucleic acid (RNA), thymic stromal lymphopoietin (TSLP), thymus-and activation-regulated chemokine (TARC), transforming growth factor-α (TGF-α), transforming growth factor- β (TGF-β), tumor necrosis factor-α , vascular endothelial growth factor (VEGF)

Table 3:

Studies Assessing Absolute Eosinophil Count (AEC)

Author	Year	Age group	Normal Control	Atopic Control	Disease Activity Control	Significant vs normal control	Significant vs atopic controls	Significant for responsiveness
Bullock	2007	Pediatric	Yes	Yes	Yes	Yes	No difference	Yes
Colson	2014	Pediatric	No	No	Yes	Not done	Not done	Yes
Conus	2009	Not stated	No	No	Yes	Not done	Not done	Yes
Domenech Witek	2017	Adult	No	No	Yes	Not done	Not done	No difference
Fujiwara	2002	Adult	No	No	Yes	Not done	Not done	Yes (n=1, case report)
Johnsson	2011	Adult	Yes	Yes	No	No difference	Possible difference but compared across 4 groups	Not done
Konikoff	2006	Pediatric	Yes	Yes	Yes	Yes	No difference	Yes
Lucendo	2013	Adult	No	No	Yes	Not done	Not done	No difference
Min	2016	Both	Yes	No	Yes	Yes	Not done	Yes
Morris	2016	Pediatric	No	Yes	Yes	Not done	No difference	No difference
Paz Zafra	2012	Both	Yes	No	No	Yes	Not done	Not done
Philpott	2015	Adults	Yes	No	No	No	Not done	Not done
Rodriguez-Sanchez	2013	Both	No	No	Yes	Not done	Not done	No difference
Schlag	2015	Adult	No	Yes	Yes	Not done	No difference	Yes
Straumann	2005	Adult	Yes	No	No	Not done	Not done	Not done
von Arnim	2011	Adult	Yes	No	No	No actual values (AEC elevated (y/n))	Not done	Not done

Table 4:

Studies Assessing Eosinophil Granular Proteins

Author	Year	Age group	Normal Control	Atopic Control	Disease Activity Control	Granule Protein(s) studied	Marker(s) significant vs normal controls	Marker(s) significant vs atopic controls	Marker(s) significant for responsiveness
Dellon	2015	Adult	Yes	No	Yes	MBP, EDN	No difference	Not done	No difference
Domenech Witek	2017	Adult	No	No	Yes	ECP	Not done	Not done	ECP
Fujiwara	2002	Adult	No	No	Yes	ECP	Not done	Not Done	Yes (n=1, case report)
Furuta	2013	Pediatric	Yes	No	Yes	MBP, EDN, ECP, EPX (string)	MBP, EDN, ECP	Not done	MBP, EPX
Katzka	2015	Adult	No	No	Yes	EDN	Not done	Not done	No difference
Konikoff	2006	Pediatric	Yes	Yes	Yes	EDN (blood/ stool)	EDN (Blood)	No difference	No difference
Lucendo	2013	Adult	No	No	Yes	ECP	Not done	Not done	No difference
Min	2016	Both	Yes	No	Yes	EDN, ECP	EDN, ECP	Not done	No difference
Rodriguez-Sanchez	2013	Both	No	No	Yes	ECP	Not done	Not done	No difference
Saffari	2017	Adult	Yes	No	Yes	EPX activity	No difference	Not done	No difference
Schlag	2014	Adult	No	No	Yes	ECP	Not done	Not done	ECP
Schlag	2015	Adult	No	Yes	Yes	ECP	Not done	No difference	ECP
Straumann	2010	Adult	No	No	Yes	ECP, EDN	Not done	Not done	ECP, EDN
Subbarao	2011	Pediatric	Yes	No	Yes	EDN (blood/stool)	EDN (Blood)	Not done	EDN (Blood)

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Table 5:

Studies Assessing Eosinophil Surface or Intracellular Markers

Author	Year	Age Group	Normal Control	Atopic Control	Disease Activity Control	Cell Surface/Intracellul ar Marker Studied	Marker(s) significant vs normal controls	Marker(s) significan t vs atopic controls	Marker(s) significant for responsiveness
Bullock	2007	Pediatric	Yes	Yes	Yes	AEC, CCR3 on eosinophils, CD4 expression of IL-5	CCR3 on eosinophil s, CD4 expression IL-5	No difference	CCR3, CD4 expression IL-5
Conus	2009	Not stated	No	No	Yes	AEC, IL-5Ra,CCR3	Not done	Not done	Not significant for IL-5Ra
Furuta	2013	Pediatric	Yes	No	Yes	MBP, EDN, ECP, EPX, CLC/Gal-10	CLC/Gal-10	Not done	CLC/Gal-10
Johnsson	2011	Adult	Yes	Yes	No	CD23, CD54,CRTH2, CD11c, CCR3, CD44, CD11b, CD18, CD58	CD23, CD54,CRTH2 CD1c,CCR3,CD44	Possible differenc e but compared across 4 groups	Not done
Lingblom	2014	Adult	Yes	No	Yes	CD18, CD44, CD40, CCR3, CD23, CD54, FPR, CRTH2	CD44,CCR3,CD23,CD54	Not done	CD18
Lingblom	2017	Both	Yes	No	No	CD23, CD44,CD54, CRTH2, FoxP3, Galectin-10	CD44,CRTH2, FoxP3, Galectin-10	Not done	Not done
Morris	2016	Pediatric	No	Yes	Yes	AEC, Eosinophil progenitor	Not done	No difference	Eosinophil progenitor
Nguyen	2011	Pediatric	Yes	Yes	Yes	PBMC transcript analysis of STAT1, STAT6, and CD66b, Surface CD66b on peripheral eosinophils	CD66b, STAT 6, STAT 1	Not reported	STAT1 (eosinophils), STAT6 (eosinophils/lymphocyte[s])
Patel	2010	Pediatric	Yes	No	No	HLA-DR	No difference	Not done	Not done
Straumann	2005	Adult	Yes	No	No	AEC; CD25, IL-4, IL-5, IL-13, IL-10 expression on eosinophils	Eosinophil expression of IL-5 and IL-13	Not done	Not done
Straumann	2010	Adult	No	No	Yes	Eos expression IL5 alpha receptor	Not done	Not done	IL-5Ra
Upparahalli Venkateshaiah	2016	Both	No	No	Yes	CD274	Not done	Not done	CD274 (case report, n=2)

Table 6:

Studies Assessing Biomarker Response to Treatment

Author	Year	Age group	Biomarker source	Biomarker(s) Studied	Biomarkers with significant difference in response to treatment
Randomized					
Conus	2009	Not stated	Blood	AEC, IL-5Ra, CCR3	AEC
Schlag	2015	Adult	Blood	AEC, ECP, CCL-17, CCL-18, CCL-26, MC tryptase	AEC, ECP, CCL-26, CCL-17, Serum MCT
Straumann	2010	Adult	Blood	ECP, EDN, eotaxin, and TNF-a, IL-5Ra on eosinphils	ECP, EDN
Non-Randomized					
Benitez	2015	Pediatric	Oral swab	16S RNA bacterial sequencing	No Difference
Blanchard	2011	Not stated	Blood	Cytokine array	No Difference
Bullock	2007	Pediatric	Blood	AEC, CCR3 on eosinophils, CD4 expression of IL-5	AEC, CCR3, CD4 expression IL-5
Colson	2014	Pediatric	Blood	AEC	AEC
Dellon	2015	Adult	Blood	IL-4, IL-5, Il-6, IL-9, Il-13, TGF-α, TGF-β, TNF-α, Eotaxin 1/2/3, TSLP, MBP, EDN	No Difference
Dellon	2016	Adult	Blood	Periostin	No Difference
Domenech Witek	2017	Adult	Blood	tIgE, AEC, ECP	ECP
Fujiwara	2002	Adult	Blood	AEC, Eotaxin (total and free), ECP	AEC, Eotaxin (total), ECP (n=1, case report)
Furuta	2013	Pediatric	Esophageal String	MBP, EDN, ECP, EPX, CLC/Gal-10	MBP, EPX, CLC/Gal-10
Huang	2010	Pediatric	Blood	35 chemokine/cytokines including: bFGF or FGF-2; eotaxin 1/2/3; IL-1α; IL-1β; IL-1RA; IL-2; IL-4; IL-5; IL-6; IL-7; IL-8; IL-10; IL-12-p40; IL-12-p70; IL-13; IL-15; IL-17; IL-17F; ENA78; GCSF; GM-CSF, GRO-α; IFN-γ; IP10; leptin; MCP-3; MIG; MIP-1α; MIP-1β; NGF; PDGF-BB; RANTES; TGF-β;TNF-α; TNF-β, and VEGF	bFGF, IL-5
Jyonouchi	2013	Pediatric	Blood	iNKTs	iNKTs
Katzka	2015	Adult	Cytosponge	Eosinophils, EDN	Eosinophils
Konikoff	2006	Pediatric	Blood, stool	AEC, IL-5, Eotaxins 1/2/3, EDN (blood and stool)	AEC
Leung	2012	Both	Breath	eNO	No Difference
Lingblom	2014	Adult	Blood	CD18, CD44, CD40, CCR3, CD23, CD54, PFR, CRTH2	CD18
Lucendo	2013	Adult	Blood	AEC, tIgE, ECP	No Difference
Min	2016	Both	Blood	AEC, Eotaxin-3, EDN, ECP, IL-5	AEC
Morris	2016	Pediatric	Blood	AEC, Eosinophil progenitor	Eosinophil progenitor
Nguyen	2011	Pediatric	Blood	PBMC transcript analysis of STAT1, STAT6, and CD66b, Surface CD66b on peripheral eosinophils	STAT1 (eosinophils), STAT6 (eosinophils/ lymphocytes)

Rawson	2016	Pediatric	Blood	TGF-β, PAI-1	No Difference
Rodriguez-Sanchez	2013	Both	Blood	AEC, ECP, tIgE	No Difference
Saffari	2017	Adult	Throat swab	EPX activity	No Difference
Schlag	2014	Adult	Blood	ECP, tryptase	ECP, tryptase
Subbarao	2011	Pediatric	Blood, stool	IL-5 (Blood); EDN (Blood and stool)	EDN (Blood)
Upparahalli Venkateshaiah	2016	Both	Blood	CD274	CD274 but case report (n=2)
Wright	2016	Adult	Blood	sIgG4 (total and foodspecific)	sIgG4

Table 7:

QUADAS-2 for Eosinophilic Esophagitis Minimally Invasive Biomarker Studies

Study	RISK OF BIAS				APPLICABILITY CONCERNS		
	PATIENT SELECTION	INDEX TEST	REFERENCE STANDARD	FLOW AND TIMING	PATIENT SELECTION	INDEX TEST	REFERENCE STANDARD
Benitez	☺	☺	☺	☺	?	☺	☺
Blanchard	?	☺	☺	☺	?	☺	☺
Botan	?	?	☺	?	?	☺	☺
Bullock	?	☺	☺	?	?	☺	☺
Clayton	☺	☺	☺	☹	?	☺	☺
Colson	☺	☺	☺	?	?	☺	☺
Conus	?	☺	☺	?	?	☺	☺
Cunnion	?	☺	☺	☺	?	☺	☺
Dellon-1	☺	☺	☺	☺	?	☺	☺
Dellon-2	☺	☺	☺	☺	?	☺	☺
Domenech	?	☺	☺	?	?	☺	☺
Fuentebella	☺	☺	☺	?	?	☺	☺
Fujiwara	?	☺	☺	☹	?	☺	☺
Furuta	?	☺	☺	☺	?	☺	☺
Huang	?	☺	☺	☺	?	☺	☺
Johnsson	?	☺	☺	☺	?	☺	☺
Jyonouchi	?	☺	☺	?	?	☺	☺
Katzka	?	☺	☺	☺	?	☺	☺
Kinoshita	?	☺	☺	?	?	☺	☺
Knipping	☺	☺	☺	☹	?	☺	☺
Konikoff	?	☺	☺	☺	?	☺	☺
Krupp	?	☺	☺	?	?	☺	☺
Lanz	?	☺	☺	?	?	☺	☺
Leung	☺	☺	☺	?	?	☺	☺
Lexmond	☺	☺	☺	☺	?	☺	☺
Lingblom-1	☹	☺	☺	?	☹	☺	☺
Lingblom-2	?	☺	☺	?	?	☺	☺
Lucendo	☺	☺	☺	?	?	☺	☺
Min	☺	☺	☺	☺	?	☺	☺
Morris	?	☺	☺	☺	?	☺	☺
Nguyen	?	☺	☺	☺	?	☺	☺
Patel	☺	☺	☺	☺	?	☺	☺
Paterson	☺	☺	☺	?	☺	☺	☺
Paz Zafra	☺	☺	☺	☺	?	☺	☺
Philpott	?	☺	☺	☹	?	☺	☺
Rawson	?	☺	☺	☺	?	☺	☺
Rayapudi	?	☺	☺	?	?	☺	☺
Rodriguez	☺	☺	☺	☺	?	☺	☺
Saffari	?	☺	☺	☺	?	☺	☺
Sawant	?	☺	☺	☺	?	☺	☺
Schlag-1	?	☺	☺	☺	?	☺	☺
Schlag-2	☺	☺	☺	☺	?	☺	☺
Soylu	?	☺	☺	☺	?	☺	☺
Straumann-1	☺	☺	☺	?	?	☺	☺
Straumann-2	☺	☺	☺	☺	?	☺	☺
Subbarao	☺	☺	☺	☺	?	☺	☺
Upparahalli	☹	☺	☺	?	☹	☺	☺
von Arnim	?	☺	☺	?	?	☺	☺
Wright	☺	☺	☺	☺	?	☺	☺

☺ Low Risk ☹ High Risk ? Unclear Risk