Acta Biomed 2021; Vol. 92, Supplement 7: e2021530 DOI: 10.23750/abm.v92iS7.12401

Review

Non-invasive biomarkers of eosinophilic esophagitis

Martina Votto[1,2], Maria De Filippo[1,2], Riccardo Castagnoli[1,2], Francesco Delle Cave[1,2], Francesca Giffoni[1,2], Viola Santi[1,2], Marta Vergani[1,2], Carlo Caffarelli[3], Mara De Amici[4], Gian Luigi Marseglia[1,2], Amelia Licari[1,2]

[1] Pediatric Unit, Department of Clinical, Surgical, Diagnostic, and Pediatric Sciences, University of Pavia, Pavia, Italy; [2] Pediatric Clinic, Fondazione IRCCS Policlinico San Matteo, University of Pavia, Pavia, Italy; [3] Pediatric Clinic, Department of Medicine and Surgery, University of Parma, Parma, Italy; [4] Immuno-Allergology Laboratory, Clinical Chemistry Unit, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy

Abstract. Eosinophilic esophagitis (EoE) is an emerging allergen-mediated disease characterized by symptoms of esophageal dysfunction and eosinophilic inflammation. EoE diagnosis requires 15 eosinophils per high power field (eos/HPF) in tissue biopsies endoscopically obtained. The need for several endoscopies to monitoring the disease and the absence of validated non-invasive biomarkers or tools are the main reasons for the significant burden on affected patients and the healthcare system. There is a critical need for non-invasive or minimally invasive biomarkers. In the last years, several efforts have been made to identify potential biomarkers for diagnosing and monitoring the disease that we summarized in this review. The future of EoE is exciting from both a diagnostic and therapeutic standpoint. Further research is required to confirm phenotypes and histological or serological biomarkers to provide a novel endotype classification based on different cytokine or genetic signatures relevant to precision medicine. (www.actabiomedica.it)

Keywords: eosinophilic esophagitis, biomarkers, cytokines, genes, atopy.

Introduction

Eosinophilic gastrointestinal disorders (EGIDs) are emerging inflammatory diseases which may involve any part of the gastrointestinal (GI) tract and lead to the eosinophilic mucosal infiltration in the absence of secondary causes of intestinal eosinophilia (1, 2). Based on the site of the eosinophil inflammations, EGIDs are classified into eosinophilic esophagitis (EoE) and nonesophageal EGIDs, distinct in eosinophilic gastritis (EoG), gastroenteritis (EoGE), and colitis (EoC) (1). While nonesophageal EGIDs still represent a clinical enigma for clinicians, EoE is considered the prototype of EGIDs with standardized guidelines (1, 3). EoE is a chronic/remittent, allergen-mediated disease characterized by esophageal dysfunction and eosinophilic infiltration, affecting both children and adults, with a male-female ratio of 3:1 (4). The prevalence of EoE is significantly increased in the last decade. It is currently considered one of the most common causes of upper gastrointestinal morbidity, detected in 12% - 23% of patients undergoing endoscopy for dysphagia and about 50% of subjects with food impaction (4, 5). EoE diagnosis requires 15 eosinophils per high power field (eos/HPF) in tissue biopsies endoscopically obtained, without concomitant eosinophilic infiltration in other GI tracts (3). The need for several endoscopies to monitoring the disease and the absence of validated non-invasive biomarkers or tools are the main reasons for a significant burden on affected patients and the healthcare system (6). In the last years, several efforts have been made to identify

Table 1. Biomarker classification and definition.

Biomarker classification	Definition
Diagnostic Biomarker (DB)	A DB detects or confirms the presence of a disease or identifies an individual with a disease subtype.
Monitoring Biomarker (MB)	An MB assesses the status of a disease or detects the clinical (efficacy and safety) and pharmacodynamic effects of treatment (i.e., biological therapy).
Predictive Biomarkers (PreB)	A PreB assesses if the exposure to therapy or environmental agent induces favorable or unfavorable effects in a patient or group of individuals.
Prognostic Biomarkers (ProB)	A ProB can identify the likelihood of a clinical event, disease recurrence, or progression in affected patients.
Risk Biomarker (RB)	An RB indicates the potential for developing a disease in a healthy individual.

potential non-invasive biomarkers for diagnosing and monitoring the disease. Biomarkers may provide new insight into the understanding of EoE pathogenesis and defining potential endotypes with relevant impact on precision medicine.

Biomarkers are measures of biological status. According to the Food and Drug Administration (FDA) - National Institutes of Health (NIH) definition, a biomarker is a "defined characteristic measured as an indicator of normal biological processes, pathogenic processes or responses to an exposure or intervention" (7). This definition is broad and encompasses therapeutic interventions and molecular, histologic, radiographic, or physiologic characteristics. According to their putative applications, several categories of biomarkers have been identified, and often, they may overlap each other (Table 1) (8). Notably, an ideal biomarker should present different features, such as reasonable costs and a significant impact on clinical management (Table 2). This review aimed to summarize current evidence on non-invasive biomarkers for EoE diagnosis and monitoring, highlighting promising tools and future potential candidates. We performed a non-systematic review of articles via the online database PubMed, combining the terms "eosinophilic esophagitis" AND "biomarkers." The literature review was performed in May 2021. All studies that met the following criteria were included: 1) case series, cross-sectional and cohort studies, published in English in peer-reviewed journals in the last ten years, 2) participants were children and adult patients diagnosed with EoE, according to current guidelines (3). Articles were also required to assess non-invasive biomarkers. Potentially eligible publications were manually screened and reviewed, and non-relevant publications were excluded (Figure 1).

Serological and biochemical markers

Blood eosinophils, eosinophil granule, and cell-surface proteins

Considering the allergic pathogenesis, most studies have focused on the rationale that EoE patients

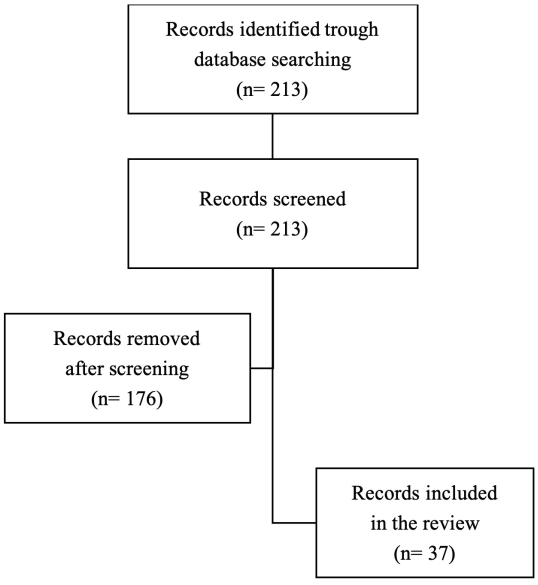

Figure 1. Methods and search strategy.

Table 2. Features of an ideal biomarker for the diagnosis and monitoring of EoE.

Features of an ideal biomarker
Correlate with the EoE state
Connect with EoE severity
Non-invasive and easy to collect or perform
Standardized
Have high sensitivity
Carry high specificity
Cost-effective
Low biological variation

may have elevated peripheral eosinophils compared to healthy controls or subjects with gastroesophageal reflux disease (GERD) (Table 3) (9-11). Many of these studies showed that peripheral eosinophil levels might increase during active disease, but whether this marker alone reflected mucosal inflammation is still unclear. Recently, Wechsler et al. have demonstrated that absolute eosinophil count (AEC), together with a panel of plasma biomarkers, such as galectin-10 (GAL-10), eosinophil cationic protein (ECP), eosinophil-derived neurotoxin (EDN), eotaxin-3 (EOT3), and major basic protein 1 (MBP-1) were useful to identify EoE subjects and predicted esophageal eosinophilia (10). Another study showed that AEC, ECP, EDN, and interleukin-(IL)-5 had statistically significant correlations with esophageal eosinophilia (11). Less recently, Rodriguez-Sanchez et al. assessed the potential usefulness of eosinophil activity markers (peripheral eosinophils, total serum IgE, ECP) as a predictor of diet response. Authors demonstrated that peripheral blood eosinophils decreased significantly in responders but not in non-responders patients (9).

Other studies have evaluated blood eosinophil progenitors (EoP) and eosinophil-surface markers with promising results (12-14). Johansson et al. recently reported that platelet activation and platelet-eosinophil association pathways might be involved in EoE pathogenesis, showing that CD41 (aIIb-integrin subunit) expressed on eosinophils surface was a potential non-invasive biomarker for esophageal eosinophilic inflammation (14). Another study examined whether phenotypic analysis of eosinophil surface markers could

Table 3. Serum biomarkers of EoE.

Author, year	Population	Study	Biomarkers	Outcome
Rodriguez-Sanchez et al, 2013 (9)	30 Adults	Cross-sectional	ECP, total IgE, peripheral blood eosinophils, and the maximum peak of eosinophils/hpf	Serum total IgE and ECP do not act as markers for EoE activity
Wechsler et al, 2021(10)	71 Children and adolescents	Prospective case-control study	Blood AEC. Plasma EDN, ECP, MBP-1, GAL-10, EOT2, EOT3. Urine OPN and MMP-9	Plasma (GAL-10, ECP, EDN, Eotaxin-3, MBP-1), and urine (OPN) biomarkers were increased in EoE compared to control. Therefore, GAL-10 is a potential biomarker for EoE screening
Min et al, 2017 (11)	115 Children and adults	Prospective case-control study	Serum analysis of AEC, EOT3, EDN, ECP, and IL-5	AEC, ECP, and EDN were higher in EoE subjects compared to controls and correlated with the degree of esophageal eosinophilia
Nguyen et al, 2011 (12)	77 Children and adolescents	Case-control study	CD66b, phospho-STAT1, and phospho-STAT6	Measurements of CD66b and phospho-STAT levels in peripheral eosinophils may be beneficial for identifying EoE
Morris et al, 2017 (13)	31 Children and adolescents	Case-control study	Peripheral blood EoP.	EoP levels were increased in patients with active EoE and significantly correlated with esophageal eosinophilia
Johansson et al, 2020 (14)	25 Adults	Prospective study	IIb-integrin (CD41)	CD41 associated with circulating eosinophils is a potential non-invasive biomarker for esophageal eosinophilic inflammation
Schwartz et al, 2019 (15)	31 Children and adolescents	Retrospective study	Peripheral blood EoP	Blood EoP correlates with tissue pathology during active EoE

Table 3. Serum biomarkers of EoE.

Author, year	Population	Study	Biomarkers	Outcome
Henderson et al, 2020 (16)	34 Children and adolescents	Prospective study	Circulating eosinophil progenitors	Blood EoP levels may be used as a biomarker to detect active EoE disease
Subbarao et al, 2011 (17)	80 Children and adolescents	Case-control study	Serum IL-5 and EDN	Serum EDN levels were significantly higher in subjects with EoE than controls
Schlag et al, 2013 (18)	15 Adults	Prospective observational study	ECP and TRP	ECP but not TRP could be a promising non-invasive biomarker to assess response to topical corticosteroid therapy
Doménech Witek et al, 2017 (18)	19 Adults	Retrospective study	Serum ECP	The serial determination of ECP was proper to monitor patients with EoE
Cengiz, 2019 (20)	29 Adults	Case-control study	Serum ECP	Serum ECP level was significantly higher in patients with EoE than in controls. In addition, ECP is strongly correlated with EREFS and the symptom of food impaction
Wright et al, 2018 (21)	39 Adults	Prospective case-control study	Serum EPX	EoE subjects had significantly lower median EPX levels
Lu et al, 2018 (23)	31 Children and adolescents	Case-control study	Serum 15-HETE	15(S)-HETE may aid in the diagnosis of EoE
Dellon et al, 2016 (24) Dellon et al, 2015 (25)	61 Adults	Case-control study	Serum periostin. Serum IL-4, IL-5, IL-6, IL-9, IL-13, TGF-α, TGF-β, TNF-α, EOT-1, -2, and -3, TSLP, MBP, and EDN	Serum periostin and cytokines levels were similar in cases and controls, and there were no changes post-treatment
Dellon et al, 2017 (27)	48 Adults	Case-control study	Autoantibodies (IgG1 and IgG4) to DSG1, DSG3, and to collagen XVII (NC16A)	Anti-NC16A and anti-DSG3 IgG4 autoantibodies were strongly associated with EoE. Anti-NC16A levels decreased significantly in EoE cases with a histologic response after topical corticosteroid treatment

AEC, absolute eosinophil count; CD, cluster of differentiation; DSG, desmoglein; ECP, eosinophil cationic protein; EDN, eosinophil-derived neurotoxin; EoPs, eosinophil progenitors; EOT, eotaxin; EPX, eosinophil peroxidase; GAL-10, galectin-10; HETE, hydroxyeicosatetraenoic acid; Ig, immunoglobulin; IL, interleukin; MBP-1, major basic protein-1; MMP, matrix metalloproteinase; OPN, osteopontin; STAT, signal transducer and activator of transcription; TGF, transforming growth factor; TLSP, thymic stromal lymphopoietin; TNF, tumor necrosis factor; TRP, tryptase.

distinguish treated from untreated disease. In 2011, Nguyen et al. found elevated surface CD66 intracellular phospho-STAT1 and phospho-STAT6, which differentiated children with active EoE from treated and healthy controls (12, 15, 16). Three studies recently assessed the levels of blood EoP as potential biomarkers of active EoE, esophageal inflammation, and response to treatments both in children both adults (13, 15, 16).

Eosinophil granule proteins have been investigated as other potential markers of disease, showing inconsistent and conflicting results (17-21). Subbarao et al. determined that EDN levels provided a sustained decrease following treatment in 66 children with EoE (17). More recently, a small prospective study of 15 adults showed that serum ECP, but not tryptase (TRP), significantly correlated with tissue eosinophils

after swallowed steroid therapy (18). Moreover, ECP was high in adults with EoE, and its serial determination was also helpful in monitoring the disease (19-20).

Recent evidence suggested a pathogenetic role for arachidonate 15-lipooxygenase (ALOX15) in EoE. ALOX15 is upregulated and overexpressed in mucosal biopsies of EoE patients (22). 15(S)-hydroxyeicosatetraenoic acid (15(S)-HETE), a metabolite of ALOX15, detectable in peripheral blood, was found elevated in the EoE compared to the non-EoE group, suggesting its potential role as a disease indicator (23).

Type 2 (T2) cytokines

With an advanced understanding of EoE pathogenesis, several studies sought to assess whether T2 cytokines, including interleukin (IL)-4, IL-5, IL-6, IL-9, IL-13, TGF-α, transforming growth factor (TGF)-β, tumor necrosis factor (TNF)-α, EOT-1, -2, -3, thymic stromal lymphopoietin (TSLP) and periostin were increased in the peripheral circulation of affected patients (24, 25). Therefore, peripheral cytokine measurements did not consistently characterize the esophageal inflammation or disease activity. In addition, the results of these studies are limited by the confounding influence of other concomitant allergic diseases.

Autoantibodies

EoE has been associated with a range of autoimmune conditions, such as inflammatory bowel diseases, coeliac disease, vasculitis, or type 1 diabetes mellitus (26). Moreover, esophageal epithelial barrier dysfunction is essential in EoE pathogenesis. Antibodies against epithelial adhesion molecules are founded in several autoimmune skin conditions. Therefore, EoE may even be associated with these specific autoantibodies. Dellon et al. recently demonstrated that anti-collagen XVII (NC16A) and anti-desmoglein 3 (DSG3) IgG4 autoantibodies were strongly associated with EoE. Moreover, anti-NC16A levels decreased significantly in EoE patients after topical corticosteroid treatment (27).

Histopathological biomarkers

Immunohistochemical markers

Diagnosis of EoE requires more than 15 eos/HPF in the esophageal tracts. Therefore, other diagnostic histological findings, including a thickened mucosa with basal layer hyperplasia and papillary lengthening, eosinophil surface layering, and eosinophilic microabscesses, have been proposed (28). Several studies assessing histological biomarkers have been reported. Extracellular deposition of eosinophil granule proteins, such as eosinophil peroxidase (EPX), is present in the esophagus of patients with EoE and positively correlates with the peak of tissue eosinophils (Table 4) (29, 30). Moreover, EPX levels decreased in treatment responders (29). On the contrary, Schroeder et al. demonstrated that the less invasive assessment of pharyngeal EPX did not correlate with the esophageal eosinophil count in children with EoE compared to healthy controls (31).

Other eosinophil granule proteins, such as MBP-1, TRP, EDN, and EOT-3, have been evaluated as potential histological biomarkers of EoE and response to therapy, with conflicting results. (32-36). Notably, EDN in brushing samples obtained with the nasogastric endoscopy was significantly higher in children and young adults with active EoE than patients in remission, healthy controls, and GERD. (37).

Other tissue markers

ALOX15 plays an essential role in the metabolism of fatty acids and the production of various cytokines and chemokines. ALOX15 is expressed in blood eosinophils and respiratory epithelium. ALOX15 is also upregulated in the esophageal epithelium from patients with active EoE in contrast to esophageal fragments from patients in remission, subjects with GERD, or healthy controls (38). Thus, ALOX15 immunohistochemistry may be helpful in the diagnosis of cases with clinical features of EoE but that do not meet the histological criteria (39).

IgG4

The role of immunoglobulin G4 (IgG4) in EoE pathogenesis has not been precisely defined, and

Table 4. Immunohistochemical biomarkers.

Author, year	Population	Study	Biomarkers	Outcome
Wright et al, 2021(29)	87 Adults	Case-control study	EPX	EPX was strongly correlated with tissue eosinophils accurately identified subjects with EoE and decreases in treatment responders
Saffari et al, 2017 (30)	36 Adults	Case-control study	EPX	EPX levels from esophageal mucosal samples correlated with eosinophilic inflammation
Schroeder et al, 2017 (31)	21 Children and adolescents	Case-control study	Pharyngeal and nasal EPX	EPX levels from the throat swabs do not correlate with esophageal eosinophil counts
Peterson et al, 2019 (32)	34 Adults	Retrospective study	MBP1	MBP1 is increased in esophageal biopsy specimens from symptomatic patients with EoE and may be a marker of disease activity
Kim et al, 2019 (33)	72 Adults	Retrospective study	TRP, EDN, and EOT3	TRP, EDN, and EOT3 could be promising biomarkers for disease activity, symptoms, and endoscopic response
Dellon et al, 2020 (34)	110 Adults	Retrospective study	MBP, EOT3, and TRP	Pretreatment MBP, EOT3, and TRP levels were not strongly associated with response to topical steroids. In contrast, elevated TRP levels may be associated with nonresponse compared with complete response
Dellon et al, 2014 (35)	196 Adults	Case-control study	MBP, EOT3, and TRP	Esophageal tissues from patients with EoE have substantially higher MBP, EOT3, and tryptase than controls
Dellon et al, 2012 (36)	105 Children and adults	Case-control study	MBP and EOT3	Patients with EoE had substantially higher levels of MBP and EOT3 staining than GERD patients
Smadi et al, 2018 (37)	94 Children and adults	Prospective cross-sectional study	EDN	EDN in brushing samples is significantly higher in patients having active EoE compared to healthy controls, GERD, and EoE in remission
Hui et al, 2017 (39)	21 Children and adolescents	Retrospective case-control study	ALOX15	ALOX15 immunohistochemistry helped support the diagnosis of EoE in situations with strong clinical suspicion
Clayton et al, 2014 (40)	30 Adults	Retrospective case-control study	IgG4	The level of IgG4-positive plasma cells was increased in the lamina propria and granular extracellular IgG4 deposits
Zukerberg et al, 2016 (41)	46 Adults	Case-control study	IgG4 deposits	76% of EoE cases showed int extracellular IgG4 deposits, whereas all GERD cases were negative
Rosenberg et al, 2018 (42)	36 Children and adolescents	Case-control study	IgG4	Tissue IgG4 levels correlated with esophageal eosinophil counts, histologic grade, stage scores, IL-4, IL-10, IL-13 expression, and had strong associations with a subset of the EoE transcriptome

ALOX, arachidonate lipoxygenase; EDN, eosinophil-derived neurotoxin; EPX, eosinophil peroxidase; GERD, gastroesophageal reflux disease; Ig, immunoglobulin; IL, interleukin; MBP-1, major basic protein-1; TRP, tryptase.

available studies reported conflicting data. One of the first studies showed an increased level of IgG4-positive plasma cells (IgG4-PC) in the lamina propria and granular extracellular IgG4 deposits (40). Zuckeberg et al. reported IgG4 deposits between the squamous cells in biopsies from patients with EoE. Additionally, IgG4-PC in submucosa were identified in 58% of EoE patients, but without significant difference compared to patients with GERD (41). A more recent study has demonstrated a significant relationship between IgG4 and EoE in adults and the pediatric population (42). Rosenberg et al. detected

increased IgG4 levels in children with EoE compared to healthy controls.

Moreover, IgG4 in the esophagus showed a positive correlation with concurrent peak tissue eosinophilia, histological grade, and stage according to the EoE histology scoring system (EoEHSS) (42). However, the high amount of IgG4 in esophageal mucosa still represents a conundrum. Thus, current data do not conclusively determine if high tissue IgG4 titers could be good predictors of diet response in EoE patients.

Microribonucleic acids (miRNAs) and DNA methylation

MiRNAs are single-stranded RNA molecules of 19-25 nucleotides involved in the post-transcriptional gene silencing. Several studies reported that EoE patients had a marked change in tissue-specific gene expression (Table 5). Lu et al. investigated esophageal miRNA expression profile in patients with active disease and responsive to steroids, finding that the expression levels of the most upregulated miRNAs (miR-21 and miR-223) and the most downregulated miRNA (miR-375) strongly correlated with esophageal eosinophil levels (43). More recently, Bhardwaj et al. found that the expression of salivary miR-4668 is higher in EoE compared to non-EoE subjects, suggesting its potential role as a non-invasive biomarker (44).

Other epigenetic mechanisms, different from miRNA and involved in EoE pathogenesis or response to therapies, have been recently assessed. For example, pediatric patients with EoE showed differences in mucosal DNA methylation profiles compared to controls (45). Moreover, DNA methylation differences have also been found in responder and non-responder patients (46).

Other non-invasive biomarkers

Exhaled nitric oxide

Fractional exhaled nitric oxide (FeNO) is a biomarker of eosinophilic asthma (47). However, considering the common atopic etiology, FeNO was also measured in a prospective study of 11 non-asthmatic subjects with active esophagitis before and after treatment, without any supporting role in the management of EoE (Table 6) (48). Moreover, FeNO did not help distinguish EoE from GERD (48). Therefore, no studies have shown a potential role of FeNO in EoE diagnosis and monitoring (49).

Metabolomics

Only one study assessed the metabolomic profile in patients with EoE. However, Moye et al. showed that plasma urea cycle metabolites (dimethylarginine, putrescine, and N-acetylputrescine) are elevated in children with EoE, and their levels are modified by proton pump inhibitor treatment (50).

Table 5. Epigenetic biomarkers.

Author, year	Population	Study	Biomarkers	Outcome
Lu et al, 2012 (43)	29 Children and adolescents	Case-control study	miRNAs	The expression levels of the most upregulated miRNAs (miR-21 and miR-223) and the most downregulated miRNA (miR-375) were strongly correlated with esophageal inflammation
Bhardwaj et al, 2020 (44)	44 Adults	Case-control study	Salivary miR-4668-5p	The expression of miR-4668 is higher in EoE vs. non-EoE subjects, suggesting its potential role as a non-invasive biomarker
Strisciuglio et al, 2021 (45)	20 Children and adolescents	Case-control study	Mucosal DNA methylation profile	Analyses revealed striking disease-associated differences in mucosal DNA methylation profiles in children diagnosed with EoE compared to controls
Jensen et al, 2020 (46)	36 Children and adults	Case-control study	DNA methylation profile	EoE patients that respond versus do not respond to treatment have differences in their methylation profile

DNA, deoxyribonucleic acid; RNA, ribonucleic acid.

Table 6. Other non-invasive biomarkers

Author, year	Population	Study	Biomarkers	Outcome
Leung et al, 2013 (48)	11 Children and adults	Prospective study	FeNO	No supporting role for FeNO determination in the management of EoE
Lanz et al, 2012 (49)	55 Children and adolescents	Case-control study	FeNO	Measurement of FeNO does not help identify EoE from GERD
Moye et al, 2019 (50)	24 Children and adolescents	Prospective case-control study	Plasma metabolomics profile	Notable candidate biomarkers include dimethylarginine, putrescine, and N-acetylputrescine
Cunnion et al, 2016 (51)	75 Children and adults	Case-control study	Urinary 3-BT	Median normalized 3-BT levels were increased 93-fold in patients with EoE compared to controls

BT, bromotyrosine; FeNO, Fractionated exhaled nitric oxide; GERD, gastroesophageal reflux disease.

3-Bromotyrosine (3-BT) is a chemical marker of eosinophil activation and is high in patients with asthma. Cunnion et al. found that 3-BT levels were increased 93-fold in patients with EoE compared to controls, providing proof of concept testing urine by a mass spectrometry method (Eosinophil Quantitated Urine Kinetic, EoQUIK) can provide a non-invasive tool to evaluate eosinophil degranulation in EoE (51).

Genetic risk loci

Eosinophilic esophagitis is a multifactorial disease. Although recent evidence suggested a fundamental pathogenetic role of the environmental factors, several studies have also reported that genetic predisposition is a significant risk factor in the development of EoE (52). Different studies, including candidate-gene identification and genome-wide association studies (GWAS), have identified gene *loci* that have been associated explicitly with EoE (53). These gene *loci* are categorized into four major groups: 1) genes involved in Type 2 (T2) inflammation, 2) epithelial barrier dysfunction, 3) enhanced fibrosis, and 4) altered immune response (54). The main genes are TSLP, calpain 14 (CAPN14), CCL26, EMSY, LRRC32, STAT6, and ANKRD27 (Table 7). Additional studies founded mutations within the filaggrin gene and the promoter region of TGFB1 (55, 56). TSLP is released by activated epithelial cells and plays a fundamental role in promoting T2 differentiation (57). Levels of TLSP are increased in patients with atopic diseases, including EoE (58). CAPN14 is a cysteine protease and plays a fundamental role in the integrity of the esophageal epithelial barrier. Furthermore, its expression is only limited to the esophageal mucosa (59). However, CAPN14 expression was almost 4-fold increased in EoE patients compared to controls. Higher levels of CAPN14 expression are associated with the downregulation of DSG-1, filaggrin, and zonulin, which are pivotal proteins of the epithelial barrier (59). CCL26 gene, which encodes for EOT3, is the most highly overexpressed esophageal transcript in patients with EoE and is critical in disease pathogenesis (60). STAT6 is essential for T2 development and is a signaling intermediate for IL-4 and IL-13 post-IL-4 receptor alpha (IL-4Ra) engagement (53). LRRC32 is a TGF-beta binding protein, and EMSY is involved in transcriptional regulation (53). In this context, the Cincinnati Children's Hospital researchers developed a specific diagnostic panel comprising a 96-gene quantitative PCR array to identify patients with EoE, monitor the disease and response to therapy, and improve the diagnosis and treatment (61).

Conclusion

EoE is an emerging disease affecting patients at any age and is currently considered one of the upper GI tract disorders with a relevant burden on patients and the healthcare systems (6). To date, the GI endoscopy is the gold standard for the diagnosis and follow-up of patients with EoE. Therefore, there is a critical need for non-invasive biomarkers to replace such invasive monitoring. Although this review showed promising non-invasive biomarkers, none of these has

been incorporated into guideline recommendations. Despite several signs of progress in understanding EoE pathogenesis, we have more to learn as we strive to improve diagnostic modalities, discover more effective and patient-targeted therapeutic strategies, and develop more accurate disease monitoring systems. We are hopeful that the growing number of genetic, molecular expression, and immunologic analyses, in conjunction with increased differentiation of clinical phenotypes and biomarker supported endotypes, will help us explain differing therapeutic responses, predict clinical response, guide individual therapies, and improve patient outcomes. The future of EoE is exciting from both a diagnostic and therapeutic standpoint. Therefore, further research is required to confirm phenotypes and histological or serological biomarkers to provide a novel endotype classification based on different cytokine or genetic signatures.

References

1. Licari A, Votto M, D'Auria E, et al. Eosinophilic gastrointestinal diseases in children: a practical review. Curr Pediatr Rev 2020;16:106-114.
2. Licari A, Votto M, Scudeller L, et al. Epidemiology of nonesophageal eosinophilic gastrointestinal diseases in symptomatic patients: a systematic review and meta-analysis. J Allergy Clin Immunol Pract 2020;8:1994-2003.
3. Dellon ES, Liacouras CA, Molina-Infante J, et al. Updated international consensus diagnostic criteria for eosinophilic esophagitis: proceedings of the AGREE conference. Gastroenterology 2018;155:1022-1033.
4. Furuta GT, Katzka DA. Eosinophilic esophagitis. N Engl J Med.2015;373:1640-8.
5. Dellon ES, Hirano I. Epidemiology and natural history of eosinophilic esophagitis. Gastroenterology 2018;154:319-332.
6. Votto M, Castagnoli R, De Filippo M, et al. Behavioral issues and quality of life in children with eosinophilic esophagitis. Minerva Pediatr 2020;72:424-432.
7. FDA-NIH Biomarker Working Group. BEST (Biomarkers, EndpointS, and other Tools) Resource. Silver Spring (MD): Food and Drug Administration (US); Bethesda (MD): National Institutes of Health (US), 2016.
8. Califf RM. Biomarker definitions and their applications. Exp Biol Med (Maywood). 2018;243:213-221.
9. Rodríguez-Sánchez J, Gómez-Torrijos E, De-la-Santa-Belda E, et al. Effectiveness of serological markers of eosinophil activity in monitoring eosinophilic esophagitis. Rev Esp Enferm Dig 2013;105:462-7.
10. Wechsler JB, Ackerman SJ, Chehade M, et al. Non-invasive biomarkers identify eosinophilic esophagitis: a prospective longitudinal study in children. Allergy 2021. Epub ahead of print.
11. Min SB, Nylund CM, Baker TP, et al. Longitudinal evaluation of noninvasive biomarkers for eosinophilic esophagitis. J Clin Gastroenterol 2017;51:127-135.
12. Nguyen T, Gernez Y, Fuentebella J, et al. Immunophenotyping of peripheral eosinophils demonstrates activation in eosinophilic esophagitis. J Pediatr Gastroenterol Nutr 2011;53:40-47.
13. Morris DW, Stucke EM, Martin LJ, et al. Eosinophil progenitor levels are increased in patients with active pediatric eosinophilic esophagitis. J Allergy Clin Immunol 2016;138:915-918.
14. Johansson MW, McKernan EM, Fichtinger PS, et al. dIb-Integrin (CD41) associated with blood eosinophils is a potential biomarker for disease activity in eosinophilic esophagitis. J Allergy Clin Immunol 2020;145:1699-1701.
15. Schwartz JT, Morris DW, Collins MH, et al. Eosinophil progenitor levels correlate with tissue pathology in pediatric eosinophilic esophagitis. J Allergy Clin Immunol 2019;143:1221-1224.
16. Henderson A, Magier A, Schwartz JT, et al. Monitoring eosinophilic esophagitis disease activity with blood eosinophil progenitor levels. J Pediatr Gastroenterol Nutr 2020;70:482-488.
17. Subbarao G, Rosenman MB, Ohnuki L, et al. Exploring potential non-invasive biomarkers in eosinophilic esophagitis in children. J Pediatr Gastroenterol Nutr 2011; 53:651–658.
18. Schlag C, Pfefferkorn S, Brockow K, et al. Serum eosinophil cationic protein is superior to mast cell tryptase as a marker for response to topical corticosteroid therapy in eosinophilic esophagitis. J Clin Gastroenterol 2014;48:600-606.
19. Doménech Witek J, Jover Cerdà V, Gil Guillén V, et al. Assessing eosinophilic cationic protein as a biomarker for monitoring patients with eosinophilic esophagitis treated with specific exclusion diets. World Allergy Organ J 2017;10:12.
20. Cengiz C. Serum eosinophilic cationic protein is correlated with food impaction and endoscopic severity in eosinophilic esophagitis. Turk J Gastroenterol 2019;30:345-349.
21. Wright BL, Ochkur SI, Olson NS, et al. Normalized serum eosinophil peroxidase levels are inversely correlated with esophageal eosinophilia in eosinophilic esophagitis. Dis Esophagus 2018;31:dox139.
22. Wen T, Stucke EM, Grotjan TM, et al. Molecular diagnosis of eosinophilic esophagitis by gene expression profiling. Gastroenterology 2013;145:1289-1299.
23. Lu S, Herzlinger M, Cao W, et al. Utility of 15(S)-HETE as a serological marker for eosinophilic esophagitis. Sci Rep 2018;8:14498.
24. Dellon ES, Rusin S, Gebhart JH, et al. Utility of a noninvasive serum biomarker panel for diagnosis and monitoring of eosinophilic esophagitis: a prospective study. Am J Gastroenterol 2015;110:821-7.

25. Dellon ES, Higgins LL, Beitia R, et al. Prospective assessment of serum periostin as a biomarker for diagnosis and monitoring of eosinophilic oesophagitis. Aliment Pharmacol Ther 2016;44:189-97.
26. Capucilli P, Cianferoni A, Grundmeier RW, et al. Comparison of comorbid diagnoses in children with and without eosinophilic esophagitis in a large population. Ann Allergy Asthma Immunol 2018;121:711-716.
27. Dellon ES, Lin L, Beitia R, et al. Serum autoantibodies against epithelial cell adhesion molecules as disease biomarkers of eosinophilic esophagitis. Clin Exp Allergy 2018;48:343-346.
28. Collins MH. Histopathologic features of eosinophilic esophagitis. Gastrointest Endosc Clin N Am 2008;18:59-71.
29. Wright BL, Doyle AD, Shim KP, et al. Image analysis of eosinophil peroxidase immunohistochemistry for diagnosis of eosinophilic esophagitis. Dig Dis Sci 2021;66:775-783.
30. Saffari H, Leiferman KM, Clayton F, et al. Measurement of inflammation in eosinophilic esophagitis using an eosinophil peroxidase assay. Am J Gastroenterol 2016;111:933-939.
31. Schroeder S, Ochkur SI, Shim KP, et al. Throat-derived eosinophil peroxidase is not a reliable biomarker of pediatric eosinophilic esophagitis. J Allergy Clin Immunol Pract 2017;5:1804-1805.
32. Peterson KA, Gleich GJ, Limaye NS, et al. Eosinophil granule major basic protein 1 deposition in eosinophilic esophagitis correlates with symptoms independent of eosinophil counts. Dis Esophagus 2019;32:doz055.
33. Kim GH, Park YS, Jung KW, et al. An increasing trend of eosinophilic esophagitis in Korea and the clinical implication of the biomarkers to determine disease activity and treatment response in eosinophilic esophagitis. J Neurogastroenterol Motil 2019;25:525-533.
34. Dellon ES, Woosley JT, McGee SJ, et al. Utility of major basic protein, eotaxin-3, and mast cell tryptase staining for prediction of response to topical steroid treatment in eosinophilic esophagitis: analysis of a randomized, double-blind, double dummy clinical trial. Dis Esophagus 2020;33:doaa003.
35. Dellon ES, Speck O, Woodward K, et al. Markers of eosinophilic inflammation for diagnosis of eosinophilic esophagitis and proton pump inhibitor-responsive esophageal eosinophilia: a prospective study. Clin Gastroenterol Hepatol 2014;12:2015-2022.
36. Dellon ES, Chen X, Miller CR, et al. Diagnostic utility of major basic protein, eotaxin-3, and leukotriene enzyme staining in eosinophilic esophagitis. Am J Gastroenterol 2012;107:1503-1511.
37. Smadi Y, Deb C, Bornstein J, et al. Blind esophageal brushing offers a safe and accurate method to monitor inflammation in children and young adults with eosinophilic esophagitis. Dis Esophagus 2018;31.
38. Matoso A, Mukkada VA, Lu S, et al. Expression microarray analysis identifies novel epithelial-derived protein markers in eosinophilic esophagitis. Mod Pathol 2013;26:665–676.
39. Hui Y, Chen S, Lombardo KA, et al. ALOX15 immunohistochemistry aids in the diagnosis of eosinophilic esophagitis on paucieosinophilic biopsies in children. Pediatr Dev Pathol 2017;20:375–380.
40. Clayton F, Fang JC, Gleich GJ, et al. Eosinophilic esophagitis in adults is associated with IgG4 and not mediated by IgE. Gastroenterology 2014;147:602–609.
41. Zukerberg L, Mahadevan K, Selig M, et al. Oesophageal intrasquamous IgG4 deposits: an adjunctive marker to distinguish eosinophilic oesophagitis from reflux oesophagitis. Histopathology 2016;68:968–976.
42. Rosenberg CE, Mingler MK, Caldwell JM, et al. Esophageal IgG4 levels correlate with histopathologic and transcriptomic features in eosinophilic esophagitis. Allergy 2018;73:1892–1901.
43. Lu TX, Sherrill JD, Wen T, et al. MicroRNA signature in patients with eosinophilic esophagitis, reversibility with glucocorticoids, and assessment as disease biomarkers. J Allergy Clin Immunol 2019;129:1064-1075.
44. Bhardwaj N, Sena M, Ghaffari G, Ishmael F. MiR-4668 as a novel potential biomarker for eosinophilic esophagitis. Allergy Rhinol (Providence) 2020;11:2152656720953378.
45. Strisciuglio C, Payne F, Nayak K, et al. Disease-associated DNA methylation signatures in esophageal biopsies of children diagnosed with eosinophilic esophagitis. Clin Epigenetics 2021;13:81.
46. Jensen ET, Langefeld CD, Zimmerman KD, et al. Epigenetic methylation in eosinophilic esophagitis: molecular ageing and novel biomarkers for treatment response. Clin Exp Allergy 2020;50:1372-1380.
47. Votto M, De Filippo M, Licari A, Marseglia A, De Amici M, Marseglia GL. Biological therapies in children and adolescents with severe uncontrolled asthma: a practical review. Biologics 2021;15:133-142.
48. Leung J, Nguyen-Traxler A, Lee EM, et al. Assessment of fractionated exhaled nitric oxide as a biomarker for the treatment of eosinophilic esophagitis. Allergy Asthma Proc 2012;33:519-524.
49. Lanz MJ, Guerrero RA, Gonzalez-Vallina R. Measurement of exhaled nitric oxide in the evaluation for eosinophilic esophagitis in children. Ann Allergy Asthma Immunol 2012;109:81-82.
50. Moye LM, Liu Y, Coarfa C, et al. Plasma urea cycle metabolites may be useful biomarkers in children with eosinophilic esophagitis. Front Pediatr 2019;6:423.
51. Cunnion KM, Willis LK, Minto HB, et al. Eosinophil quantitated urine kinetic: a novel assay for assessment of eosinophilic esophagitis. Ann Allergy Asthma Immunol 2016;116:435-439.
52. Votto M, Marseglia GL, De Filippo M, et al. Early life risk factors in pediatric EoE: could we prevent this modern disease? Front Pediatr 2020;8:263.
53. O'Shea KM, Aceves SS, Dellon ES, et al. Pathophysiology of eosinophilic esophagitis. Gastroenterology 2018;154:333-345.
54. Ruffner MA, Cianferoni A. Phenotypes and endotypes in

eosinophilic esophagitis. Ann Allergy Asthma Immunol 2020;124:233-239.
55. Blanchard C, Stucke EM, Burwinkel K, et al. Coordinate interaction between IL-13 and epithelial differentiation cluster genes in eosinophilic esophagitis. J Immunol 2010;184:4033-4041.
56. Aceves SS, Newbury RO, Chen D, et al. Resolution of remodeling in eosinophilic esophagitis correlates with epithelial response to topical corticosteroids. Allergy 2010;65:109-116.
57. Kitajima M, Lee HC, Nakayama T, et al. TSLP enhances the function of helper type 2 cells. Eur J Immunol 2011;41:1862-1871.
58. Hui CC, Rusta-Sallehy S, Asher I, et al. The effects of thymic stromal lymphopoietin and IL-3 on human eosinophil-basophil lineage commitment: relevance to atopic sensitization. Immun Inflamm Dis 2014;2:44-55.
59. Sleiman PM, Wang ML, Cianferoni A, et al. GWAS identifies four novel eosinophilic esophagitis loci. Nat Commun 2014;5:5593.
60. Blanchard C, Wang N, Stringer KF, et al. Eotaxin-3 and a uniquely conserved gene-expression profile in eosinophilic esophagitis. J Clin Invest 2006;116:536-547.
61. Wen T, Rothenberg ME. Clinical applications of the eosinophilic esophagitis diagnostic panel. Front Med (Lausanne) 2017;4:108.

Correspondence:
Received: 1 September 2021
Accepted: 30 September 2021
Prof. Amelia Licari, MD,
Pediatric Clinic, Fondazione IRCCS Policlinico San Matteo
University of Pavia,
Piazzale Golgi 19, 27100 Pavia, Italy
Phone: +390382502818
E-mail: a.licari@smatteo.pv.it