Received: 2 March 2024 | Revised: 31 May 2024 | Accepted: 3 July 2024

DOI: 10.1111/all.16275

Allergy EUROPEAN JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY EAACI WILEY

ORIGINAL ARTICLE

Food Allergy and Gastrointestinal Disease

Biomarkers for a less invasive strategy to predict children with eosinophilic esophagitis

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Funding information
Astma- och Allergiförbundet; Mjölkdroppen; Stiftelsen Lars Hiertas Minne; Cayman Biomedical Research Institute; Åke Wibergs foundation; Magnus Bergvalls foundation; Hjärt-Lungfonden

Abstract

Background: Noninvasive biomarkers for diagnosing and monitoring eosinophilic esophagitis (EoE) are currently lacking. This study evaluates 20 biomarkers in serum and saliva, aiming to assess their diagnostic potential in pediatric EoE patients and healthy individuals.

Methods: Blood and saliva from children undergoing upper endoscopy were analyzed for biomarkers, including absolute eosinophil count (AEC), eosinophil-derived neurotoxin (EDN), total and specific IgG_4-antibodies ($sIgG_4$), specific IgE-antibodies (sIgE) and 15-hydroxyeicosatetraenoic acid (15(S)-HETE). Some patients participated twice, forming a longitudinal cohort. The ability to use the biomarkers to predict the EoE diagnosis was evaluated.

Results: Analysis from 105 children divided into active EoE, remission, and healthy, revealed elevated levels of serum biomarkers (AEC, EDN, 15(S)-HETE, $sIgG_4$, and sIgE) in active EoE compared to healthy individuals. A combination of biomarkers (AEC, EDN, sIgE to egg white and wheat) and symptoms showed an AUC of 0.92 in distinguishing between the three groups. We further showed that optimal cutoff values for these biomarkers could discriminate between active EoE and healthy with a sensitivity of 88% and a specificity of 100% in distinguishing EoE (active and in remission) from healthy. Longitudinally, levels of EDN, $sIgG_4$ to Bos d 4, Bos d 5, Bos d 8, gliadin, and birch, and sIgE to milk decreased in patients progressing from active EoE to remission ($p < .05$).

Conclusions: This study identified novel biomarkers associated with EoE and proposes a panel, together with symptoms, for effective discrimination between active EoE, EoE in remission, and healthy individuals. The findings may contribute to a less invasive diagnostic method and may be a potential surveillance tool for pediatric EoE patients.

Abbreviations: 15(S)-HETE, 15-Hydroxyeicosatetraenoic acid; AEC, absolute eosinophil count in peripheral blood; ALOX15, 15-lipoxygenase; AUC, area under the curve; EDN, Eosinophil-derived neurotoxin; EGD, esophagogastroduodenoscopy; EoE, Eosinophilic esophagitis; eos, eosinophils; hpf, high power field; IgE-ab, IgE-antibodies; IgG4-ab, IgG4-antibodies; PAD, pathological-anatomical diagnosis; RF, Random Forest; ROC, receiver operating characteristic.

Caroline Nilsson and Jesper Säfholm were co-authors with equal contributions.

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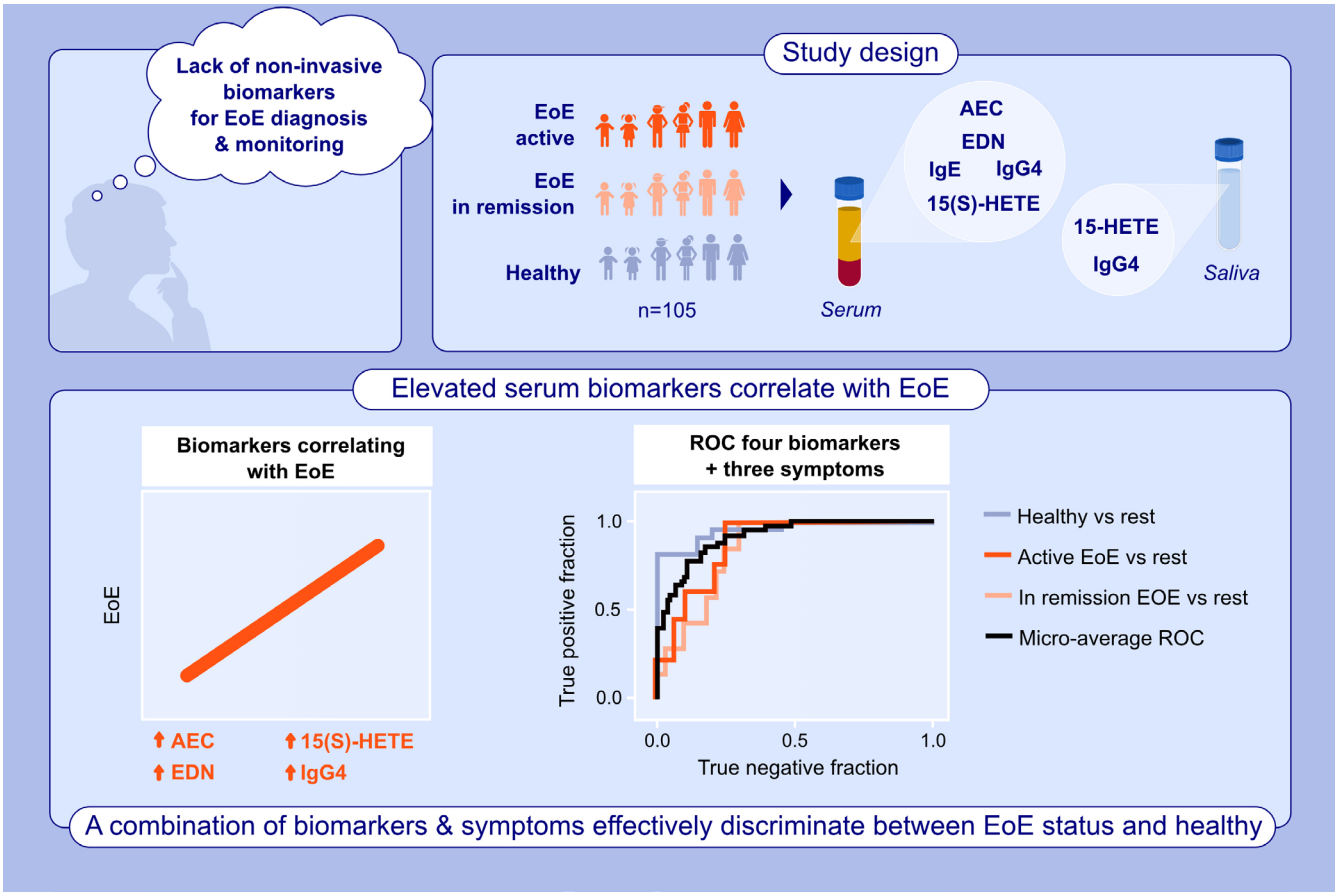
KEYWORDS
15(S)-HETE, cutoff values, IgE, IgG_4, panel



GRAPHICAL ABSTRACT

This study evaluates 20 biomarkers in serum and saliva, aiming to assess their diagnostic potential in pediatric EoE patients and healthy individuals. We identified serum biomarkers associated with EoE: AEC, EDN, 15(S)-HETE, sIgG4, and sIgE. Symptoms along with elevated levels of AEC, sIgE to egg white/wheat, differentiate between EoE status and healthy.

Abbreviations: AEC, absolute eosinophil count; EDN, eosinophil-derived neurotoxin; EoE, eosinophilic esophagitis; ROC, receiver operating characteristic; sIg, specific immunoglobulin; 15(S)-HETE, 15-hydroxyeicosatetraenoic acid.

1 | INTRODUCTION

Eosinophilic esophagitis (EoE) is a chronic type 2 (T2)-inflammatory condition that affects the esophagus and can manifest in early childhood.[1] It is distinguished by an abnormal accumulation of eosinophils in the esophageal lining, presenting a spectrum of symptoms including slow eating, dysphagia, and vomiting or nausea.[2] Early diagnosis by histological analysis and appropriate management strategies, which often involve dietary modifications and/or medications, are essential to alleviate symptoms and prevent long-term complications.[3]

Today, the diagnosis, monitoring, and evaluation of treatments require repeated tissue biopsies of the esophagus. This is particularly challenging for children because they must undergo general anesthesia for an esophagogastroduodenoscopy (EGD). Thus, there is a great need for less invasive methods to diagnose and monitor EoE in pediatric patients.

Numerous efforts have been undertaken to identify a distinctive factor for predicting EoE, primarily focusing on peripheral blood. However, as of today, no specific predictive marker has been found. Eosinophil-derived neurotoxin (EDN) and absolute eosinophil count (AEC) in peripheral blood are described as important markers in EoE[4,5] but they cannot alone distinguish EoE from healthy individuals.[6] Others have suggested an association between elevated levels of specific IgG_4 ($sIgG_4$) and EoE[7,8] and a recent study depicts that the S enantiomer of 15-hydroxyeicosatetraenoic acid (15(S)-HETE) is elevated in EoE,[9] adding to the complexity. In addition, several studies lack comparable reference groups with atopic and/or allergic individuals[10–12] making it difficult to predict the disease, as EoE has a high comorbidity with other immune-driven diseases (e.g., asthma, food allergy and allergic rhinitis[13]). Also, most studies only compare healthy individuals with active EoE, and do not take into consideration patients that are currently in remission.

Given the current lack of knowledge and the need for less invasive methods to diagnose and monitor EoE, we aimed to identify biomarkers associated with EoE in a large and well-defined cohort of children. The specific aims were (i) to explore whether a panel of biomarkers, combined with symptoms, could distinguish between active EoE, EoE in remission, and healthy individuals, and (ii) to follow changes in biomarkers longitudinally.

2 | METHODS & MATERIALS

2.1 | Study participants

This cohort study with prospective data recruited children up to the age of 18 years undergoing EGD due to suspected EoE, follow-up of known EoE, or other upper gastroenterological issues. Some children repeatedly performed EGD. The participants were recruited at Sachs Children and Youth Hospital, Stockholm, between February 2019 and October 2021. All EGD examinations were performed under general anesthesia. From each patient, four to six biopsies were extracted from different locations of the esophagus as recommended by international guidelines[14] and sent for regular pathologic-anatomic diagnosis (PAD). Saliva and blood was collected prior to anesthesia and the collection occured between 8 am to noon. All children were fasting according to routine, with no food for 6 h and only clear liquids (water or juice, not milk) allowed up to 2 h before the examination and then sample collections.

All patients responded to standardized questions (Appendix) regarding allergic, asthma, eczema, or other diseases as well as current medications. In addition, participants also answered whether they excluded anything from their diet.

2.2 | Blood analyses

Venous blood were collected in vacutainer tubes (Becton, Dickinson, UK ref nr 368,498, 369,623 and 367,862). Analysis of peripheral AEC was performed the day of sampling at the Karolinska University Laboratory (Stockholm, Sweden). For analyses of other biomarkers, the tubes were left at room temperature (RT) for 60 min, centrifuged at 900 rpm at RT for 10 min, whereafter serum and plasma were collected and stored at −80°C pending further analyses. Samples were batch analyzed by ELISA using commercial kits according to manufacturer's instructions for: EDN (ImmunoCAP™ EDN Assay Kit, Article # 10–9545-05, Thermo Fisher Scientific, Uppsala, Sweden) (measuring range; 2–200 µg/mL), 15(S)-HETE ELISA kit (Catalog # 534721, Cayman Chem, Ann Arbor, MI, USA) (detection limit (range); 78 pg/mL (78–10,000 pg/mL)). Specific IgE-antibodies (sIgE-ab) specific for milk, egg white, wheat, birch pollen, and timothy pollen were measured by ImmunoCap® (Thermo Fisher Scientific, levels >0.1 kU_A/l were considered positive). IgG_4-antibodies (IgG_4-ab) specific for peanut (peanut-extract and Ara h 2), milk proteins (Bos d 8, Bos d 5, Bos d 4), egg white, soy, birch pollen, and gliadin from wheat were measured by ImmunoCAP $sIgG_4$ (Thermo Fisher Scientific, levels >0.07 mg/L were considered positive). Total IgG_4 ($tIgG_4$) was measured using research modification of the ImmunoCap assay as for IgG_4-ab with the difference that the ImmunoCAP IgA/IgG Calibrator tests (AGcal) were able to bind all immunoglobulins were used instead of specific allergen tests.

2.3 | Saliva analyses

The patients provided saliva samples before anesthesia by holding a synthetic swab in the mouth until it was soaked with saliva, which took approximately 1 min and contained between 0.37–2 mL. The swab was then placed in a saliva tube (Salivette, Sarstedt, ref nr. 1,534,500) and centrifuged at 3000 rpm, at RT for 5 min, and stored at −80°C pending analyses. For analysis of 15(S)-HETE and $tIgG_4$ in saliva, the same procedure and manufacturers instructions as for serum were used.

2.4 | Ethical approval

The study was approved by the Regional Ethical Review Board of Stockholm (D-nr 2017/744–31). Informed written consent was obtained from patients. For children less than 15 years of age, both guardians gave approval before inclusion in the study.

2.5 | Statistics

Demographic information and baseline characteristics are presented as numbers and percentages. Comparisons among the groups, active EoE, EoE in remission and healthy individuals, were conducted using ANOVA, Pearson's Chi-squared test, and Kruskal-Wallis rank sum test when applicable. The association between EoE groups (active vs. healthy, active vs. remission) and biomarkers was assessed through multinomial logistic regression. The selection of adjusted covariates was based on principal component regression (Figure S1).

Random Forest (RF) models were constructed to evaluate the predictive performance of all biomarkers in distinguishing between the patient groups. RF function, available in the R package randomForest, was employed with the generation of 10,000 trees. Receiver operating characteristic (ROC) analysis, allowing for the calculation of the area under the curve (AUC), was performed based on RF modeling. Cutoff value for each parameter was based on the threshold level in RF when the sum of sensitivity and specificity was the largest. For longitudinal

data, the Wilcoxon matched-pairs signed-rank test was employed. Statistical significance was defined as a p-value <.05. The analyses were performed using GraphPad Prism version 10.1.0 (GraphPad Software, San Diego, California, USA) and R version 4.3.1.

3 | RESULTS

3.1 | Patient characteristics

A total of 162 children agreed to participate in the study. Of those, 26 were excluded due to celiac disease ($n=23$), helicobacter associated disease ($n=2$), and achalasia ($n=1$). A total of 31 children were diagnosed with reflux esophagitis. The median age at inclusion for the remaining 105 participants was 12.7 years (2.4–17.9). The patients were divided into groups according to diagnosis by pathologists.

- Active EoE, $N=34$. Children diagnosed by standard criteria[14,15] ≥15 eosinophils per high power field (hpf) on at least one esophageal biopsy and accompanying esophageal symptoms.
- EoE in remission, $N=18$. Children with a previous EoE diagnosis whose current biopsies had less than 15 eosinophils/hpf.
- Healthy, $N=53$. Children with normal histopathology in esophagus, stomach, and duodenum.

Age and BMI were similar between patients in all three groups. Male sex dominated both the active EoE and EoE in remission compared to the healthy group ($p<.05$). The medical history showed a higher proportion of children in the EoE remission group eliminating milk from their diet compared to EoE active and healthy children ($p<.05$). No differences were reported for asthma, eczema or allergy to furred pets between the groups, however children with EoE, both active and in remission, were more likely to have food and pollen allergies compared to healthy children ($p<.05$) (Table 1).

3.2 | Comparison of biomarkers in active EoE, EoE in remission, and healthy individuals

We evaluated some known biomarkers for eosinophilic inflammation, AEC and EDN and also some less known, 15(S)-HETE, sIgG$_4$, tIgG$_4$, and sIgE, presented in Table 1. In both active EoE and in EoE in remission the levels for AEC and EDN were increased compared to healthy individuals ($p<.05$) and for AEC the active EoE group had higher levels compared to EoE in remission ($p<.05$). Also, the levels of 15(S)-HETE were increased in both active EoE and EoE in remission compared to healthy individuals ($p<.05$). Regarding both total and sIgG$_4$, all levels were increased in active EoE compared to healthy individuals ($p<.05$). For EoE in remission sIgG$_4$ to Bos d 8, peanut, soy, and birch were increased compared to healthy individuals ($p<.05$). Children with EoE, both active and in remission, had higher levels of sIgE to all the investigated allergens compared to healthy individuals, Table 1. There were no significant differences between the groups concerning the analyses in saliva for tIgG$_4$ and 15(S)-HETE.

3.3 | Association between biomarkers and active EoE, EoE in remission, and healthy individuals

To investigate a possible association of biomarkers between the groups, a multinomial logistic regression was performed. The model was adjusted for milk elimination, food allergy, pollen allergy, age and sex based on a principal component analysis (Figure S1) and the relationship between the EoE groups and confounders. Between active EoE and healthy individuals, significant associations were found for AEC, EDN, sIgG$_4$ to Bos d 4, Bos d 5, Bos d 8, Ara h 2, and to egg white and sIgE to milk ($p<.05$) (Figure 1; Table 2). Significant associations were also found for 15(S)-HETE in both EoE in remission and healthy individuals compared to active EoE ($p<.05$) (Figure 1; Table 2). No associations were found in saliva analyses (Table 2).

3.4 | The usefulness of single or combined biomarkers for the prediction of EoE

Next, we assessed the receiver operating characteristics (ROC) of biomarkers to distinguish between active EoE, EoE in remission and healthy individuals. The single biomarker with the best ability to discriminate among the three groups was sIgG$_4$ Bos d 4, and yielded an AUC of 0.82. When including all biomarkers, micro-average AUC was over 0.88 indicating a good discrimination between the groups (Figure 2A). By reducing the included biomarkers from 20 to the four most significant in the RF analysis; AEC, EDN, sIgE to egg white and wheat, a similar AUC of 0.87 was obtained (Figure 2B). If the four biomarker-model was additionally supplemented with three clinical symptoms (dysphagia, stomach pain and vomiting/nausea), an AUC of 0.92 was obtained indicating an excellent discrimination between the groups (Figure 2C). RF analysis and AUC of individual parameters is found in the Figure S2 and Table S1.

3.5 | Distinguishing between active EoE, reflux esophagitis, and healthy individuals using cutoff values of biomarkers

ROC analysis provided cutoff values for the top biomarkers. The optimal cutoff values were for AEC, >0.35 10^9/l (AUC of 0.95), for EDN >52 µg/mL (AUC of 0.93), for sIgE wheat >0.25 kU$_A$/l (AUC of 0.86) and finally for sIgE egg white >0.17 kU$_A$/l (AUC of 0.78).

The established cutoff levels for the combination of AEC, EDN, and sIgE wheat or sIgE egg white, yielded an AUC of 0.9, demonstrating an accuracy of 0.875 (95% confidence interval: 0.68–0.97). Combining the tresholds for three biomarkers, AEC, EDN, and sIgE wheat; or AEC, sIgE wheat, and sIgE egg white, exhibited a sensitivity of 80% and a specificity of 100%, a 100% positive predictive value

TABLE 1 Characteristics of children in the groups EoE active, EoE in remission, and in healthy, at the first visit.

Characteristics at first visit	EoE active, $n=34$	EoE in remission, $n=18$	Healthy, $n=53$	p-value
Age (y), mean (SD)	11.8 (4.2)	12.4 (4.6)	12.7 (4.8)	.675
Male sex, no. (%)	26 (76.5)	16 (88.9)	24 (45.3)	<.05[b,c]
BMI (kg/m2), mean (SD)	13.7 (4.1)	13.7 (4.1)	14.4 (4.6)	.691
Symptoms at EGD				
Vomting and/or nausea, no. (%)	7 (20)	0	26 (49)	<.05[b,c]
Dysphagia, no. (%)	15 (44)	2 (11)	13 (25)	<.05[a]
Abdominal pain, no. (%)	4 (12)	0	27 (51)	<.05[b,c]
Macroscopic findings				
Normal, no. (%)	2 (6)	15 (83)	53 (100)	<.05[a,b]
Furrows, no. (%)	22 (65)	0	0	<.05[b]
Exudate, no. (%)	23 (68)	2 (11)	0	<.05[b]
Edema, no. (%)	12 (35)	1 (6)	0	<.05[b]
Stricture, no. (%)	3 (9)	0	0	<.05[b]
Histology, max eos/hpf, mean (range)	48 (15–172)	2 (0–10)	0.1 (0–4)	<.05[b]
Medical history				
Milk elimination from diet, no. (%)	10 (29.4)	14 (77.8)	1 (1.9)	<.05[a-c]
Asthma as a current disease, no. (%)	14 (41)	7 (40)	12 (23)	.145
Eczema, no. (%)	8 (24)	5 (28)	10 (19)	.704
Fur animal allergy, no. (%)	11 (32)	4 (22)	8 (15)	.165
Food Allergy*	20 (59)	8 (44)	7 (13)	<.05[b,c]
Pollen Allergy*	22 (65)	9 (50)	11 (21)	<.05[b,c]
Serum analysis, mean (SD)				
AEC 109/L	0.54 (0.3)	0.37 (0.5)	0.23 (0.3)	<.05[a,b]
EDN, µg/L	102 (71.8)	96.2 (78.4)	49.2 (73.0)	<.05[b,c]
15 (S)-HETE, pg/ml	2784 (680)	2548 (770)	2097 (849)	<.05[b,c]
IgG4 total, mg/L	679 (434)	595 (539)	496 (468)	<.05[b]
IgG4 Bos d 4, mg/L	34.9 (50.4)	3.2 (4.5)	4.2 (17)	<.05[b]
IgG4 Bos d 5, mg/L	37.4 (56.3)	12.2 (26.7)	5.3 (10.5)	<.05[b]
IgG4 Bos d 8, mg/L	26.2 (36.9)	4.6 (6.5)	2.3 (4.8)	<.05[b,c]
IgG4 gliadin, mg/L	4.9 (6.5)	2.3 (4.7)	0.9 (2.6)	<.05[b]
IgG4 peanut, mg/L	2.5 (3.2)	1.8 (2.8)	0.2 (0.4)	<.05[b,c]
IgG4 Ara h 2, mg/L	0.3 (0.5)	0.2 (0.4)	0.04 (0.1)	<.05[b]
IgG4 soy, mg/L	4.7 (21.8)	1.4 (2.7)	0.2 (0.6)	<.05[b,c]
IgG4 egg white, mg/L	8.4 (9)	5.9 (6.2)	4.3 (7.1)	<.05[b]
IgG4 birch, mg/L	1.8 (3.1)	1.1 (1.3)	0.7 (2.3)	<.05[b,c]
IgE milk, kU/L	1.4 (2.8)	4.6 (16.7)	0.1 (0.3)	<.05[b,c]
IgE egg white, kU/L	6.7 (23.7)	6.3 (23.4)	0.09 (0.2)	<.05[b,c]
IgE wheat, kU/L	1.2 (1.6)	2.2 (6.6)	0.1 (0.4)	<.05[b,c]
IgE thimoty, kU/L	11.2 (24.5)	13.5 (28.5)	1.3 (3.8)	<.05[b,c]
IgE birch, kU/L	27.9 (37.5)	25.7 (38.6)	11.3 (29.0)	<.05[b,c]
Saliva analysis, mean (SD)				
15 (S)-HETE, pg/ml	2437 (883) ($n=31$)	2483 (1042) ($n=17$)	2122 (1159) ($n=50$)	.064
IgG4 total, mg/L	1.5 (1.4) ($n=30$)	1.4 (1.8) ($n=15$)	1.1 (1.7) ($n=43$)	.095

*Food and pollen allergy = reported symptoms and IgE-sensitization (IgE > 0.1 kU/L) to the same allergen.

[a]EoE Active vs. EoE in Remission.

[b]EoE Active vs. Healthy.

[c]EoE in Remission vs. Healthy.

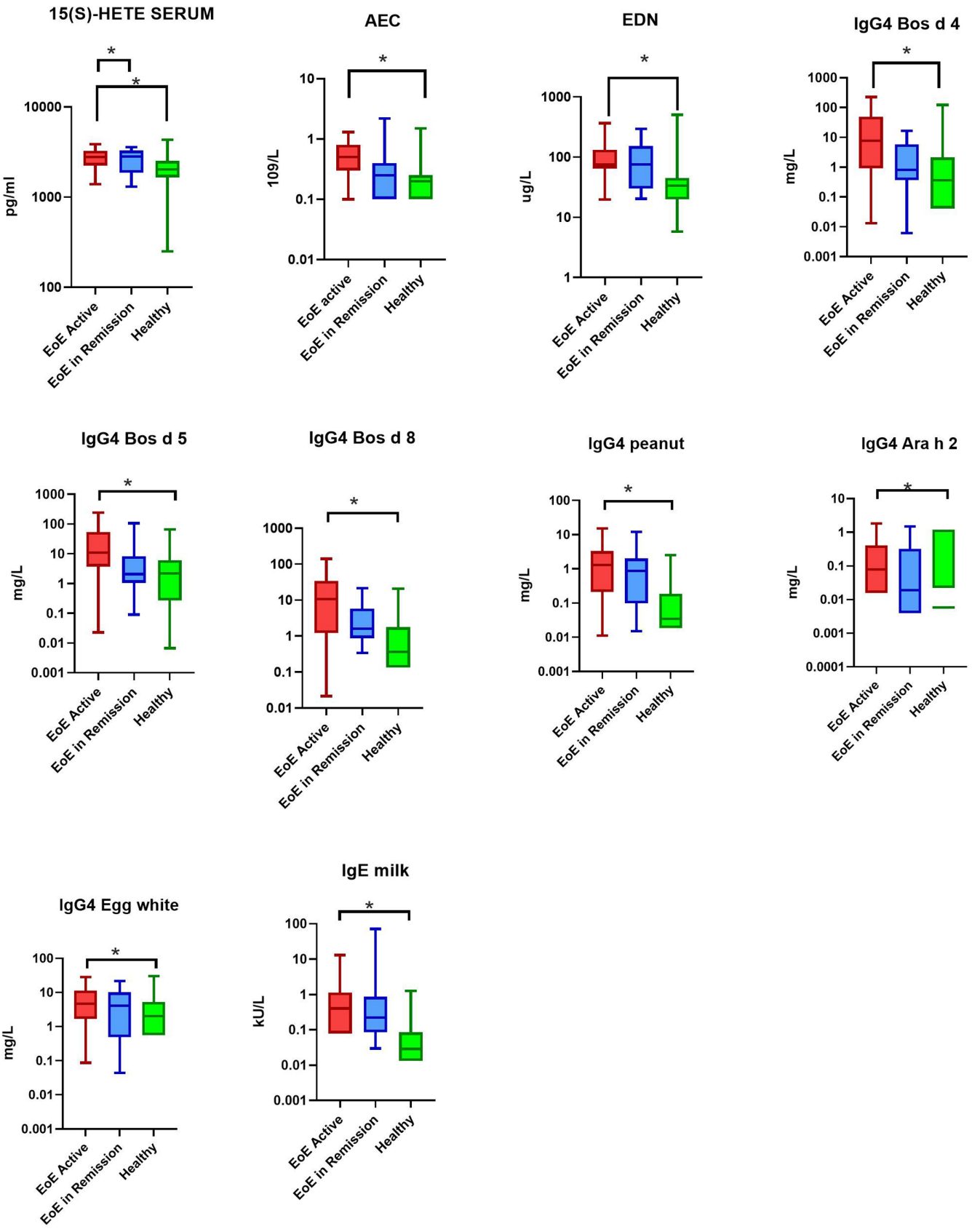


FIGURE 1 Bar graphs derived from the significant results in Table 2 depict the differences in immunological markers among children with active EoE, EoE in remission, and healthy individuals following adjustments of confounding factors. Data represents mean ± SD. *$p < .05$.

TABLE 2 Association between biomarkers and active EoE, EoE in remission, and healthy individuals after adjustments.

	Active EoE vs Healthy, OR (CI)	*p* value*	Active EoE vs EoE in remission, OR (CI)	*p* value*
Serum analysis				
AEC	0.03 (0.003–0.3)	<.001	0.65 (0.06–3)	.7
EDN	0.99 (0.98–0.9997)	<.05	1.002 (0.99–1.01)	.7
15 (S)-HETE	0.999 (0.9986–0.9993)	<.001	0.999 (0.9987–0.9996)	<.05
IgG4 total	0.9998 (0.999–1.001)	.7	0.999 (0.998–1.001)	.3
IgG4 Bos d 4	0.97 (0.94–0.99)	<.05	0.95 (0.9–1.02)	.2
IgG4 Bos d 5	0.93 (0.899–0.97)	<.001	0.988 (0.97–1.01)	.3
IgG4 Bos d 8	0.85 (0.78–0.94)	<.001	0.93 (0.88–1.02)	.1
IgG4 gliadin	0.85 (0.7–1.02)	.09	0.88 (0.77–1.03)	.1
IgG4 peanut	0.22 (0.08–0.65)	<.05	0.89 (0.7–1.1)	.4
IgG4 Ara h 2	0.005 (0.0002–0.23)	<.05	0.5 (0.90–6.9)	.6
IgG4 soy	0.6 (0.3–1.1)	.1	0.98 (0.9–1. 04)	.5
IgG4 egg white	0.93 (0.87–0.998)	<.05	0.97 (0.90–1.06)	.5
IgG4 birch	1.1 (0.87–1.3)	.6	0.84 (0.5–1.4)	.5
IgE milk	0.06 (0.007–0.4)	<.05	1.02 (0.9–1.2)	.8
IgE egg white	0.1 (0.01–1.1)	.06	0.999 (0.97–1.02)	.9
IgE wheat	0.4 (0.14–1.7)	.2	1.06 (0.89–1.3)	.6
IgE thimoty	0.95 (0.9–1.04)	.3	0.999 (0.98–1.03)	.9
IgE birch	1.0 (0.990–1.03)	.3	0.999 (0.98–1.02)	.9
Saliva analysis				
15 (S)-HETE	0.9995 (0.999–1.00002)	.06	1.000 (0.9999–1.0007)	.2
IgG4 total	0.98 (0.7–1.4)	.90	0.88 (0.65–1.7)	.7

*Mulinomial logistic regression adjusted with milk elimination, pollen allergy, food allergy, sex, and age.

(PPV) and a 75% negative predictive value (NPV) in distinguishing active EoE from healthy individuals in our cohort. Currently, EDN is not readily accessible for routine lab testing, rendering the combination excluding EDN the most practical and usable in practice.

As it can be difficult in the clinic, and important to distinguish the group with reflux esophagitis from the group with EoE, we also calculated cutoff values for the 31 children diagnosed with reflux esophagitis. This group was then merged with the healthy group and we then obtained the following cutoff values to distinguish healthy (reflux now included) from children with active EoE: AEC >0.23 10^9/l, sIgE wheat >0.12 kU_A/l and sIgE egg white >0.12 kU_A/l. The combination of these tresholds yield a sensitivity of 96%, specificity 56%, PPV 86%, and NPV 83%.

3.6 | Longitudinal analysis of treatment response

Among the patients diagnosed with EoE, 20 children were examined on multiple occasions, as the disease was monitored, using repeated EGDs. The median time between visits was 4 months. During this time, a total of 11 patients changed from active to remission due to successful treatments. The serum levels of EDN, $tIgG_4$, $sIgG_4$ to Bos d 4, Bos d 5, Bos d 8, gliadin, and to birch pollen, and sIgE to milk decreased significantly from active to remission ($p < .05$), Figure 3. No differences were found in saliva analyses. Furthermore, in the nine patients who did not enter remission at the second EGD, no significant differences were detected in any biomarker compared to the first visit.

4 | DISCUSSION

In this prospective cohort study of 105 children with active EoE, EoE in remission and healthy, we have identified biomarkers, both single and combinations, associated to EoE diagnosis. Elevated levels of single serum biomarkers (AEC, EDN, 15(S)-HETE, both total and $sIgG_4$, and sIgE) were seen in active EoE compared to healthy individuals. AEC, EDN, sIgE to egg white, and wheat in combination and together

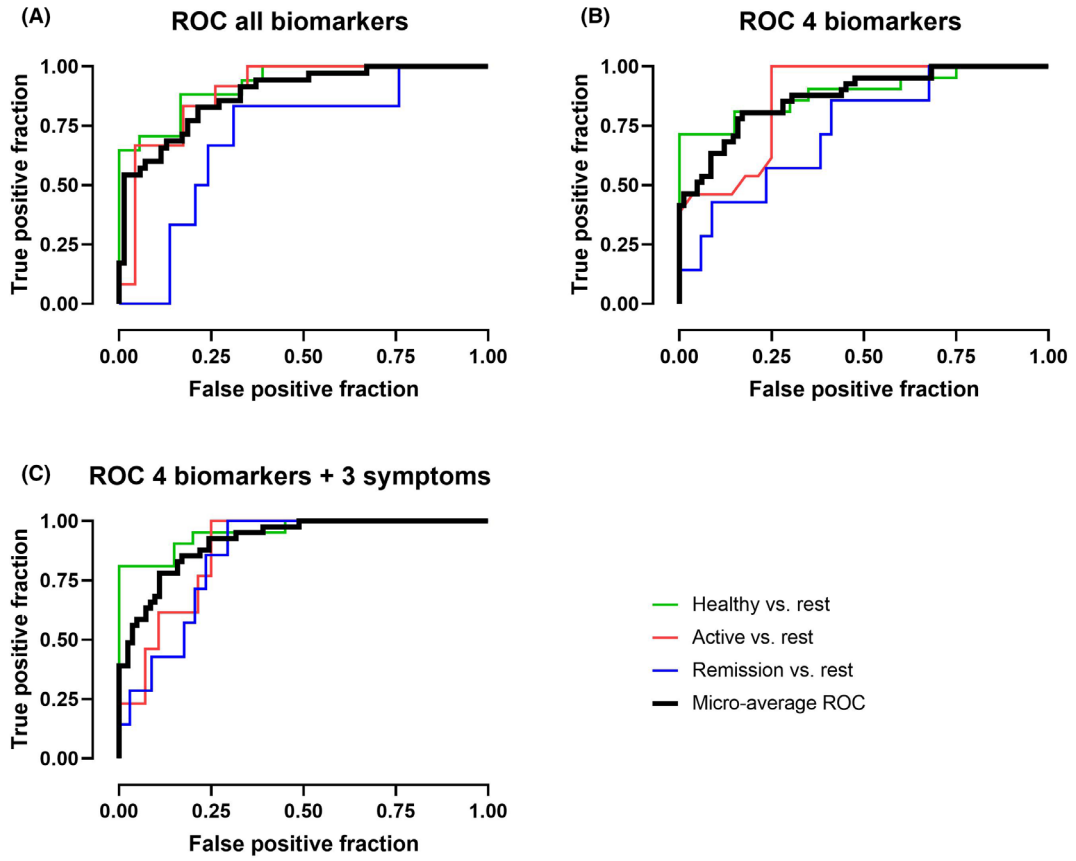


FIGURE 2 (A) Random Forest (RF) analysis for all the biomarkers, (except three values (EDN, sIgE to wheat, and sIgE to timothy) from one patient were excluded from this analysis due to missing values). AUC contains a list of AUC for each group of different classifiers v.s. the rest in the classifier. Micro-average PR-AUC was calculated by stacking all groups together, thus converting the multi-class classification into binary classification. (B) RF analysis for combination of biomarkers including AEC, EDN, sIgE to egg white and sIgE to wheat. These four biomarkers are selected because they are the top four important variable selected in the Random Forest analysis when all the biomarker included in the model. (C) RF analysis for combination of the top four biomarkers AEC, EDN, sIgE to egg white and sIgE to wheat plus three symptoms (dysphagia, stomach pain and vomiting/nausea).

with typical EoE symptoms showed promising potential to differentiate between active EoE, EoE in remission and healthy subjects with an AUC of 0.92. Furthermore, we were able to propose cutoff values for biomarkers, of which the combination of three (AEC, EDN, sIgE to egg white, or wheat) could accurately distinguish active EoE from healthy individuals. Analysis of biomarkers in the subgroup that transitioned from active disease to EoE in remission showed that $sIgG_4$ and sIgE to milk and EDN appeared to be of importance as the level of these biomarkers decreased in the remission group.

Despite the increased understanding of EoE in recent years, several aspects of the condition are still incompletely understood, and reliable biomarkers are lacking. We found that levels of both AEC and EDN were highest in the EoE active individuals followed by EoE in remission and lowest levels were observed in the healthy individuals. These associations have previously been described and are known markers of eosinophilic inflammation.[4,6,16] In accordance with our study, lower levels of AEC and EDN are also known to correlate with disease remission.[17,18] However, these biomarkers are also observed as with elevated levels in other diseases such as asthma and other allergic conditions and are therefore not specific enough to be used as a predictive marker for EoE.[6] Similarly, levels of $sIgG_4$ and sIgE were also increased in EoE patients compared to healthy individuals, which is in accordance with current knowledge.[7,8,19–22] In addition, we analyzed a less explored biomarker in the context of EoE, the lipid mediator 15(S)-HETE, which was found to be elevated in EoE patients compared to healthy individuals, both before and after adjusting for confounders. A recent study suggested the importance of 15(S)-HETE in EoE, however, it did not observe a significant difference between active EoE and controls.[9] This lack of distinction may be attributed to limited sample material, as suggested by the authors. The lipid mediator 15(S)-HETE originates from arachidonic acid which, by the metabolism of the 15-lipoxygenase (ALOX15) or cyclooxygenase enzymes, exerts a range of physiological functions in the body.[23] Genes associated with esophageal narrowing in EoE included ALOX15,[24] which has been found upregulated in esophageal mucosal biopsies of individuals with EoE compared to healthy controls.[25] In addition, metabolites from 15(S)-HETE, such as 5-oxo-15(S)-HETE (lipoxin A_4), are known to be inflammatory modulators in other chronic inflammatory diseases such as asthma and atopic dermatitis,[26,27] but its role in EoE is unclear. 15(S)-HETE can,

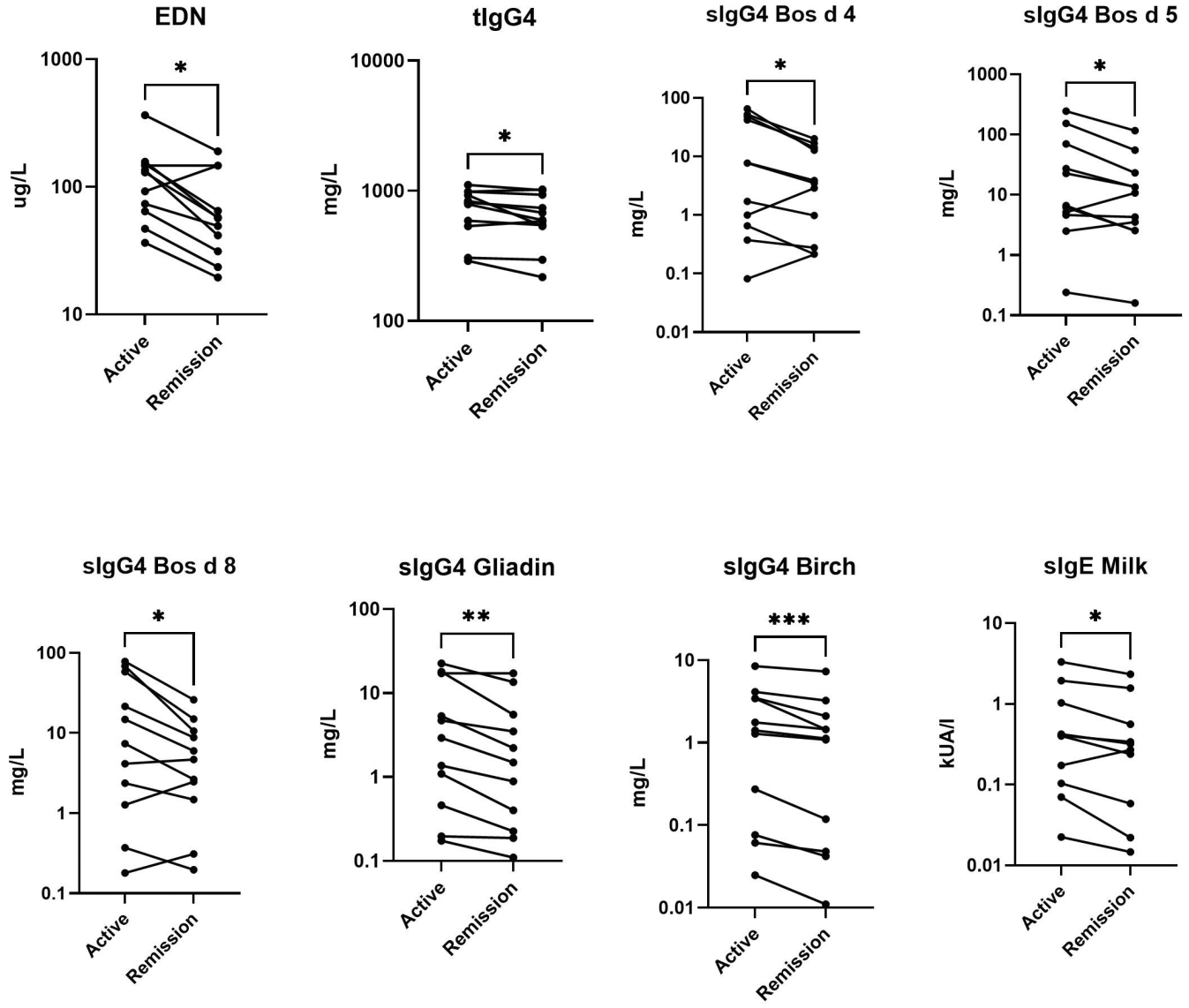


FIGURE 3 Logitudinal analysis of treatment response following EoE patients tramsgressing from active to remissive state. Data represent actual measurement at two separate occasions with a connective line for each different patient. $^{*}p < .05$, $^{**}p < .05$ and $^{***}p < .001$.

for example in asthma, act proinflammatory by activating the leukotriene B_4 receptor 2 (BLT2),[28] but also anti-inflammatory, through activation of peroxisome proliferator-activated receptor γ (PPAR- γ).[29] One study has described higher levels of PPAR-γ in esophageal biopsies of EoE patients compared to healthy individuals.[30] However, the existing evidence regarding the pro- or anti-inflammatory nature of 15(S)-HETE in different diseases remains inconclusive.

Because EoE has high comorbidity with other immune-driven diseases, for example, asthma and food allergy,[13,31] and both $sIgG_4$ and sIgE levels can vary with age and food intake,[8,19,32] it is important to adjust for potential confounders and consider their possible influence on the investigated biomarkers. In our study, we adjusted for factors that have previously been demonstrated to be important in relation to EoE after a principal component analysis before performing the multinomial logistic regression analysis. After adjustment for milk elimination, pollen allergy, food allergy, age, and sex, the biomarkers AEC, EDN, 15(S)-HETE, $sIgG_4$ to Bos d 4, Bos d 5, Bos d 8, peanut, Ara h2, and egg white, and sIgE to milk still had a positive correlation with active EoE and EoE in remission compared to healthy individuals.

We conducted additional investigations to assess the possibility of creating a predictive biomarker panel. Our findings showed that four biomarkers (AEC, EDN, sIgE for egg white, and wheat) were sufficient to effectively differentiate between active EoE, EoE in remission, and healthy individuals. The inclusion of three symptoms in the analysis further enhanced the predictive capability, resulting in an AUC of 0.92. A previous study has shown that a panel of six plasma biomarkers; galectin-10 (CLC/GAL-10), eosinophil cationic protein (ECP), EDN, Eotaxin-3 and major basic protein-1 (MBP-1) and AEC can distinguish patient with EoE diagnosis from healthy controls with an AUC of 0.90.[5] Another study, where 15(S)-HETE as well as AEC and 12 cytokines were included in a panel, show an AUC of 0.96 to differentiate active EoE from healthy controls.[9]

Given that the biomarkers investigated in our study are common standard method analyses conducted in most hospital laboratories, and that patient symptoms are routinely recorded, our panel emerges as a convenient and minimally invasive method for diagnosing the status of EoE. For potential clinical utility, we then calculated optimal cutoff levels for the biomarkers that found to be most important in the comparison between the groups of active EoE and healthy individuals, and by combining three biomarkers (AEC, sIgE wheat, and sIgE to egg white), we were able to achieve a PPV of 100% and an NPV of 75%. Previous studies have suggested cutoff values for AEC, EDN, and Eotaxin-3, which, when combined, yielded a PPV of 88% and an NPV of 70%, but with a smaller number of patients than in our study.[6] Furthermore, we included the children with reflux esophagitis in the healthy group and then obtained new cutoff levels but still satisfactory values of PPV, 86% and an NPV of 83%. To our knowledge, there is no other study that has achieved the high precision that we have shown in our study, nevertheless, these values have to be validated in separate clinical studies.

The longitudinal part of our study tracked 11 individuals as they transitioned from active EoE to remission, examining biomarker changes at the individual level. Notably, $sIgG_4$ levels decreased, even for specific allergens that had not been eliminated from the diet. A plausible explanation may be that when inflammation in the esophagus decreases, the damaged epithelial barrier begins to heal, resulting in a reduced ability for allergens to cross the esophageal barrier. Consequently, the sIgE and $sIgG_4$ levels may decrease in the blood. This was also in line with our findings of unchanged $sIgG_4$ levels in the patients who continued with an active EoE. The reduction of $sIgG_4$-levels to the milk allergens Bos d 4, Bos d 5, and Bos d 8 in patients with EoE who excluded milk from their diet and came into remission has been shown previously,[8] but without describing how other $sIgG_4$ markers changed. In addition, levels of sIgE to milk also decreased upon remission in our study, possibly due to the combination of milk elimination and barrier restoration. The longitudinal variations of sIgE in EoE patients when they go into remission have, to our knowledge, not been previously studied.

This study's robustness is underscored by the large cohort of well-characterized patients. The inclusion of diagnostic biopsies, confirming the esophageal status of all participants, adds further validity to our results. However, it is essential to acknowledge certain limitations. The different treatments to achieve remission introduces a heterogeneity, and the absence of information on the severity of allergic diseases and allergic rhinitis represent limitations. Moreover, despite the substantial patient population included, this study lacked sufficient power to conduct subgroup analyses on different treatments.

In conclusion, our study has shown a positive correlation between elevated levels of serum biomarkers (AEC, EDN, 15(S)-HETE, and IgG_4) and EoE. In addition, we have proposed an innovative panel of readily available plasma biomarkers (AEC, EDN, and sIgE to egg white, or wheat) with cutoff values, which together with typical EoE symptoms appear to be able to distinguish active EoE from EoE in remission and from healthy individuals.

For longitudinal monitoring of EoE, $sIgG_4$, sIgE, and EDN would be important biomarkers to assess the regression of the esophageal inflammation.

AUTHOR CONTRIBUTIONS

Helena Thulin: Conceptualization, Methodology, Formal analysis, Investigation, Resources, Data Curation, Writing—Original Draft, Writing—Review & Editing, Project administration. **Ladan Mansouri**: Conceptualization, Formal analysis, Writing–Original Draft. **Maria Altman**: Conceptualization, Writing—Original Draft. **Simon Kebede Merid**: Software, Formal analysis, Writing—Original Draft. **Joachim Lundahl**: Conceptualization, Writing—Original Draft. **Caroline Nilsson**: Conceptualization, Methodology, Writing—Original Draft, Writing—Review & Editing, Supervision, Funding acquisition. **Jesper Säfholm**: Conceptualization, Formal analysis, Data Curation, Writing—Original Draft, Writing—Review & Editing, Supervision, Funding acquisition.

ACKNOWLEDGEMENTS

None.

FUNDING INFORMATION

Dr Thulin was supported by project grants from Mjölkdroppen, Dr Nilsson was founded by Swedish Asthma and Allergy foundation, Jesper Säfholm was supported by Swedish Heart-Lung Foundation, Magnus Bergvalls foundation, Åke Wibergs foundation, CABRI (Cayman Biomedical Research Institute) and Lars Hiertas minne. ThermoFisher Scientific supplied reagents for analysis of sIgE, IgG_4 and EDN.

CONFLICT OF INTEREST STATEMENT

The authors report no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

1. Attwood S, Sabri S. Historical aspects of eosinophilic esophagitis: from case reports to clinical trials. *Dig Dis*. 2014;32:34-39.
2. Liacouras CA, Spergel JM, Ruchelli E, et al. Eosinophilic esophagitis: a 10-year experience in 381 children. *Clin Gastroenterol Hepatol*. 2005;3:1198-1206.
3. Lucendo AJ, Arias-Gonzalez L, Molina-Infante J, et al. Systematic review: health-related quality of life in children and adults with

eosinophilic oesophagitis-instruments for measurement and determinant factors. *Aliment Pharmacol Ther*. 2017;46:401-409.
4. Marlais M, Francis ND, Fell JM, Rawat DJ. Blood tests and histological correlates in children with eosinophilic oesophagitis. *Acta Paediatr*. 2011;100:e75-e79.
5. Wechsler JB, Ackerman SJ, Chehade M, et al. Noninvasive biomarkers identify eosinophilic esophagitis: a prospective longitudinal study in children. *Allergy*. 2021;76:3755-3765.
6. Konikoff MR, Blanchard C, Kirby C, et al. Potential of blood eosinophils, eosinophil-derived neurotoxin, and eotaxin-3 as biomarkers of eosinophilic esophagitis. *Clin Gastroenterol Hepatol*. 2006;4:1328-1336.
7. Aalberse RC, Platts-Mills TA, Rispens T. The developmental history of IgE and IgG4 antibodies in relation to atopy, eosinophilic esophagitis, and the modified TH2 response. *Curr Allergy Asthma Rep*. 2016;16:45.
8. Schuyler AJ, Wilson JM, Tripathi A, et al. Specific IgG4 antibodies to cow's milk proteins in pediatric patients with eosinophilic esophagitis. *J Allergy Clin Immunol*. 2018;142:139-148.e12.
9. 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.
10. Hines BT, Rank MA, Wright BL, et al. Minimally-invasive biomarker studies in eosinophilic esophagitis: a systematic review. *Ann Allergy Asthma Immunol*. 2018;121:218-228.
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. Straumann A, Kristl J, Conus S, et al. Cytokine expression in healthy and inflamed mucosa: probing the role of eosinophils in the digestive tract. *Inflamm Bowel Dis*. 2005;11:720-726.
13. 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.
14. Lucendo AJ, Molina-Infante J, Arias A, et al. Guidelines on eosinophilic esophagitis: evidence-based statements and recommendations for diagnosis and management in children and adults. *United European Gastroenterol J*. 2017;5:335-358.
15. 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.
16. Subbarao G, Rosenman MB, Ohnuki L, et al. Exploring potential noninvasive biomarkers in eosinophilic esophagitis in children. *J Pediatr Gastroenterol Nutr*. 2011;53:651-658.
17. Schlag C, Miehlke S, Heiseke A, et al. Peripheral blood eosinophils and other non-invasive biomarkers can monitor treatment response in eosinophilic oesophagitis. *Aliment Pharmacol Ther*. 2015;42:1122-1130.
18. Straumann A, Conus S, Degen L, et al. Budesonide is effective in adolescent and adult patients with active eosinophilic esophagitis. *Gastroenterology*. 2010;139(1526–37):1537.
19. Hill DA, Dudley JW, Spergel JM. The prevalence of eosinophilic esophagitis in pediatric patients with IgE-mediated food allergy. *J Allergy Clin Immunol Pract*. 2017;5:369-375.
20. 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.
21. Wong S, Smith G, Ruszkiewicz A, Nguyen NQ. Distinguishing gastroesophageal reflux disease and eosinophilic esophagitis in adults: the role of esophageal mucosal immunoglobulin G4. *JGH Open*. 2020;4:851-855.
22. Wright BL, Kulis M, Guo R, et al. Food-specific IgG(4) is associated with eosinophilic esophagitis. *J Allergy Clin Immunol*. 2016;138:1190-1192.e3.
23. Johnsson AK, Rönnberg E, Fuchs D, et al. COX-1 dependent biosynthesis of 15-hydroxyeicosatetraenoic acid in human mast cells. *Biochim Biophys Acta Mol Cell Biol Lipids*. 2021;1866:158886.
24. Dellon ES, Selitsky SR, Genta RM, Lash RH, Parker JS. Gene expression-phenotype associations in adults with eosinophilic esophagitis. *Dig Liver Dis*. 2018;50:804-811.
25. Matoso A, Allen D, Herzlinger M, et al. Correlation of ALOX15 expression with eosinophilic or reflux esophagitis in a cohort of pediatric patients with esophageal eosinophilia. *Hum Pathol*. 2014;45:1205-1212.
26. Celik GE, Erkekol FO, Misirligil Z, et al. Lipoxin A4 levels in asthma: relation with disease severity and aspirin sensitivity. *Clin Exp Allergy*. 2007;37:1494-1501.
27. Wu SH, Chen XQ, Liu B, Wu HJ, Dong L. Efficacy and safety of 15(R/S)-methyl-lipoxin a(4) in topical treatment of infantile eczema. *Br J Dermatol*. 2013;168:172-178.
28. Yokomizo T, Kato K, Hagiya H, Izumi T, Shimizu T. Hydroxyeicosanoids bind to and activate the low affinity leukotriene B4 receptor, BLT2. *J Biol Chem*. 2001;276:12454-12459.
29. Naruhn S, Meissner W, Adhikary T, et al. 15-hydroxyeicosatetraenoic acid is a preferential peroxisome proliferator-activated receptor beta/delta agonist. *Mol Pharmacol*. 2010;77:171-184.
30. Nhu QM, Hsieh L, Dohil L, et al. Antifibrotic effects of the Thiazolidinediones in eosinophilic esophagitis pathologic remodeling: a preclinical evaluation. *Clin Transl Gastroenterol*. 2020;11:e00164.
31. Durrani SR, Mukkada VA, Guilbert TW. Eosinophilic esophagitis: an important comorbid condition of asthma? *Clin Rev Allergy Immunol*. 2018;55:56-64.
32. Bayram RO, Özdemir H, Emsen A, Türk Dağı H, Artaç H. Reference ranges for serum immunoglobulin (IgG, IgA, and IgM) and IgG subclass levels in healthy children. *Turk J Med Sci*. 2019;49:497-505.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Thulin H, Mansouri L, Altman M, et al. Biomarkers for a less invasive strategy to predict children with eosinophilic esophagitis. *Allergy*. 2024;79:3464-3474. doi:10.1111/all.16275