

# Relationship between Disease Activity and Absolute Eosinophil Count and Serum IgE Level in Pediatric Patients with Eosinophilic Esophagitis

## Eozinofilik Özefajitli Çocuk Hastalarda Hastalık Aktivitesi ile Mutlak Eozinofil Sayıları ve IgE Düzeyleri Arasındaki İlişki

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Eosinophilic esophagitis, absolute eosinophil count, serum IgE

### Anahtar kelimeler

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### Abstract

**Introduction:** Clinical improvement does not reflect mucosal healing in the evaluation of response to treatment in eosinophilic esophagitis (EoE), and thus repeated endoscopies and eosinophil count on esophageal biopsy are still needed. Given that endoscopy is an invasive, risky, and costly method, noninvasive biomarkers that could practically indicate inflammation are needed to evaluate the treatment response.

**Materials and Methods:** The study included pediatric patients aged 0-18 years diagnosed with EoE. Age, gender, presenting complaints, comorbid allergic diseases, absolute eosinophil count (AEC), serum total IgE, and specific IgE (sIgE) levels were recorded retrospectively. All endoscopic examinations were performed by the same two experienced pediatric gastroenterologists. Biopsy samples were re-evaluated by two experienced pathologists.

**Results:** The study included 30 patients comprising 25 (83.3%) boys and 5 (16.7%) girls with a mean age of  $6.93 \pm 4.47$  (range, 2-16) years. Esophageal eosinophilic density established no significant correlation with total IgE level ( $p=0.75$ ), while it was correlated with AEC ( $p=0.005$ ,  $r=0.248$ ). Both IgE (1843.1 kU/L vs. 420.8 kU/L,  $p<0.05$ ) and AEC (1073.8/ $\mu$ L vs. 436.3/ $\mu$ L,  $p<0.05$ ) were found to be significantly higher in patients with eosinophilic microabscess. In ROC analysis, AEC was found to have a predictive value in the diagnosis of EoE (AUC: 0.609, 95% CI: 0.51-0.71,  $p=0.022$ ) at a cut-off value of 395/ $\mu$ L, with a sensitivity, specificity, PPV, and NPV of 58.1%, 64.2%, 47.5%, and 53.5%, respectively.

**Conclusion:** Although AEC appears to be a usable parameter in the follow-up of the patients, it is not sufficient as a biomarker alone for the prediction of EoE.

### Öz

**Giriş:** Eozinofilik özefajitte (EoE) tedaviye yanıtı değerlendirmede, klinik iyileşme mukozal iyileşmeyi yansıtılmamaktadır. Bu yüzden hala tekrarlayan endoskopiler yapılmakta ve biyopsi örneklerinden eozinofil sayımımaktadır. Endoskopinin invaziv, riskli ve pahalı bir yöntem olduğu düşünülürse tedaviye yanıtı değerlendirmek için noninvaziv ve inflamasyonu iyi yansitan bir biyobelirtece ihtiyaç vardır.



**Gereç ve Yöntem:** Çalışmaya EoE tanısı almış 0-18 yaş arası pediatrik hastalar dahil edildi. Yaş, cinsiyet, başvuru şikayetleri, eşlik eden alerjik hastalıklar, mutlak eozinofil sayısı (AEC), serum toplam IgE ve spesifik IgE (slgE) düzeyleri retrospektif olarak kaydedildi. Tüm endoskopik incelemeler aynı iki deneyimli pediatrik gastroenterolog tarafından yapıldı. Biyopsi örnekleri iki deneyimli patolog tarafından yeniden değerlendirildi.

**Bulgular:** Çalışmaya 25 (%83.3)' i erkek ve 5 (16.7)' i kız, yaş ortalaması  $6.93 \pm 4.47$  yıl (2-16 yaş) olan toplam 30 hasta alındı. Özefajial eozinofil yoğunluğu ile total IgE düzeyleri arasında korelasyon görülmezken ( $p=0.75$ ), AEC ile korele olduğu tespit edildi ( $p=0.005$ ,  $r=0.248$ ). Eozinofilik mikroabsesi olan hastaların IgE (1843.1 kU/L vs 420.8 kU/L,  $p < 0.05$ ) ve AEC (1073.8 / $\mu$ L vs 436.3 / $\mu$ L,  $p < 0.05$ ) düzeyleri belirgin olarak daha yüksek bulundu. ROC analizi ile yapılan değerlendirme sonucunda AEC değerinin, EoE' i öngörmekte tanışal değeri olduğu görüldü (AUC: 0.609, %95CI:0.51-0.71,  $p=0.022$ ). Bu değer için önerilen sınır AEC değeri 395 / $\mu$ L olup, sensitivite %58.1, spesifitesi %64.2, PPV%47.5, NPV %53.5 olarak bulundu.

**Sonuç:** AEC hastaların takibinde kullanılabilir bir parametre gibi görünse de, tek başına biyobelirteç olarak EoE'yi öngörmekte yeterli değildir.

## Introduction

Eosinophilic esophagitis (EoE) is a chronic, progressive immune-mediated disease characterized by antigen-driven type 2 inflammation (1). Its incidence is gradually increasing and its prevalence in children has been reported as 34.0 per 100,000 population (2). Although it affects individuals of all ages, it is more common in infants and young children. Young children mostly present to clinics with non-specific findings such as nausea, eating disorders, and growth retardation, whereas adolescents typically present with complaints of dysphagia and food retention due to progressive fibrosis (3). The diagnosis is usually made by the presence of a minimum of 15 intraepithelial eosinophils in at least one high-power field (hpf) in biopsy samples taken from the esophageal mucosa (4). Common treatment options include drug therapy, removal of dietary allergens, and esophageal dilation in cases with esophageal stricture (5).

Literature suggests that clinical improvement does not reflect mucosal healing in the evaluation of response to treatment in EoE, and thus repeated endoscopies and eosinophil count on esophageal biopsy are still needed (6). Given that endoscopy is an invasive, risky, and costly method, noninvasive biomarkers that could practically indicate inflammation are needed to evaluate the treatment response.

The aim of this study was to evaluate the utility of Absolute Eosinophil Count (AEC) and serum IgE level in the treatment and follow-up of pediatric patients with EoE.

## Materials and Methods

The study included pediatric patients aged 0-18 years who applied to Karadeniz Technical University Medical School Pediatric Gastroenterology outpatient clinic with various complaints and were diagnosed with EoE between January 1, 2010 and December 31, 2023. For the diagnosis

of EoE, a minimum of two biopsies were taken from the upper third (proximal), middle third, and lower third (distal) segments of the esophagus (7). Diagnosis was made based on the presence of a minimum of 15 intraepithelial eosinophils in at least one hpf in biopsy samples (4).

Age, gender, presenting complaints, comorbid allergic diseases, AEC, serum total IgE, and specific IgE (slgE) levels (milk, egg, gluten, hazelnut) were recorded retrospectively from clinical notes. Hypereosinophilia was defined as AEC  $> 500/\mu\text{L}$  (8).

Skin prick testing (SPT) was conducted on the volar aspect of the forearm using standardized commercial allergen extracts and a 1-mm single-use lancet, in strict accordance with the guidelines established by the European Academy of Allergy and Clinical Immunology (EAACI). Histamine dihydrochloride (10 mg/mL) and physiological saline were employed as positive and negative controls, respectively. A test was considered positive if the mean wheal diameter was  $\geq 3$  mm greater than that of the negative control (9). Specific IgE ( $\geq 0.35$  kU/L) was considered positive (9). Total IgE and food allergen-specific IgE (slgE) levels were quantified in serum samples using the ImmunoCAP system (Thermo Fisher Scientific, Uppsala, Sweden).

All endoscopic examinations were performed by the same two experienced pediatric gastroenterologists and were evaluated macroscopically. Biopsy samples taken during endoscopy were re-evaluated by two experienced pathologists blinded to the clinical and laboratory characteristics of the patients.

A total of 10 sections of 4-6 mm thickness were taken from formalin-fixed paraffin-embedded esophageal tissue samples and Hematoxylin-Eosin (H&E) staining was used to evaluate the histological morphology of the samples. Histological examination of the samples was conducted using an Olympus BX51 microscope at x40 magnification (resulting in an area of microscopic field of  $0.238 \text{ mm}^2$ ). Five fields of

view were counted from the area with peak eosinophilic density and the average of these five fields was taken. Presence of basal cell hyperplasia (BCH), polymorphonuclear leukocyte (PNL), and eosinophilic microabscess formation was evaluated in all sections (Figure 1).

### Statistical Analysis

Data were analyzed using SPSS 26.0 for Windows (Armonk, NY: IBM Corp.). Descriptives were expressed as frequencies (n) and percentages (%) for categorical variables and as mean  $\pm$  standard deviation (SD) and minimum-maximum for continuous variables. Normal distribution of variables was evaluated using Kolmogorov-Smirnov test. In independent groups, continuous variables were compared using Mann-Whitney U test since they did not show normal distribution. Correlations were assessed using Spearman's Correlation Coefficient. The diagnostic value of AEC (sensitivity, specificity, positive predictive value [PPV], and negative predictive value [NPV]) in predicting EoE was evaluated by Receiver Operating Characteristics (ROC) curve analysis. A *p* value of  $<0.05$  was considered significant.

The study was conducted in accordance with the principles of the Declaration of Helsinki. An ethics committee approval was obtained from Karadeniz Technical University Scientific Research Ethics Committee (no: 2023/234, date: 07.12.2023).

### Results

The study included 30 patients comprising 25 (83.3%) boys and 5 (16.7%) girls with a mean age of  $6.93 \pm 4.47$  (range, 2-16) years. A total of 147 endoscopy procedures were performed, with an average of  $4.9 \pm 3.01$  (range, 1-12) procedures. Most common presenting complaints included abdominal pain (40%) and dysphagia (30%). On endoscopic examination, esophageal mucosa was normal in 55 (37.4%)

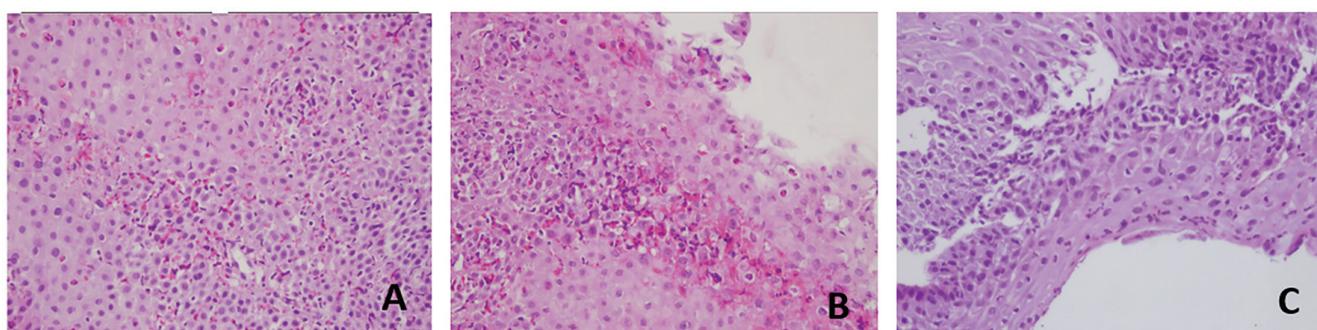
patients, while BCH was detected in 24 (16.3%), PNL in 18 (12.2%), and eosinophilic microabscess in 13 (8.8%) patients (Table 1).

Esophageal eosinophilic density established no significant correlation with total IgE level (*p*=0.75), while it was correlated with AEC (*p*=0.005, *r*=0.248). Both IgE (1843.1 kU/L vs. 420.8 kU/L, *p*<0.05) and AEC (1073.8/ $\mu$ L vs. 436.3/ $\mu$ L, *p*<0.05) were found to be significantly higher in patients with eosinophilic microabscess. No significant difference was found between patients with and without BCH with regard to IgE (251.6 kU/L vs. 624.5 kU/L, *p*=0.257) and AEC (712.1/ $\mu$ L vs. 450.3/ $\mu$ L, *p*=0.07) values. Although IgE (1537.3 kU/L vs. 466.9 kU/L, *p*=0.012) was found to be significantly higher in patients with PNL, no significant difference was detected between patients with and without PNL with regard to AEC (785.7/ $\mu$ L vs. 463.4/ $\mu$ L, *p*=0.073).

Esophageal eosinophilic density was significantly higher in patients with eosinophilic microabscess (76.6/hpf vs. 15.6/hpf, *p*<0.05) and BCH (47.4/hpf vs 15.6/hpf, *p*<0.05) compared to patients without, whereas it established no significant difference between patients with and without PNL (29.8/hpf vs. 19.6/hpf, respectively, *p*=0.196).

Both total IgE and AEC were significantly higher in patients with sIgE and/or skin prick test positivity than in patients without (*p*=0.049 vs. *p*=0.036, respectively). However, no significant difference was found between patients with and without sIgE and/or skin prick test positivity with regard to esophageal eosinophilic density (*p*=0.147).

At a cut-off value of  $>500/\mu$ L, the sensitivity, specificity, PPV, and NPV of AEC in the prediction of EoE were 62.7%, 46.3%, 57.5%, and 35.7%, respectively. No significant difference was detected between patients with and without hypereosinophilia with regard to esophageal eosinophilic density and treatment response (*p*=0.717 and *p*=1.00, respectively).

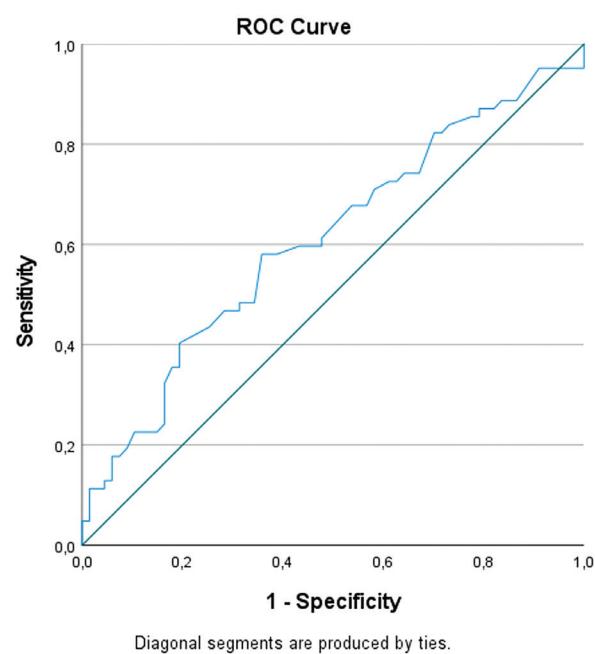


**Figure 1.** Histologic Characteristics of Eosinophilic Esophagitis. 1A. Eosinophilic leukocyte infiltration within stratified squamous epithelium (H&Ex400). 1B. Eosinophilic microabscess within stratified squamous epithelium (H&Ex400). 1C. Polymorphonuclear leukocytes infiltration within stratified squamous epithelium (H&Ex400)

**Table 1. Demographic, clinic, endoscopic and histological characteristics of patients**

Parameters	Mean±SD
Age (years)	6.93±4.47
Gender (M) n (%)	25 (83.3)
Allergies (+) n (%)	10 (33.3)
Peak eosinophil count (/ $\mu$ L) Mean±SD	487.6±635.6
Total IgE level (kU/L) Mean±SD	556.9 ±1226.7
<b>Specific IgE (+)</b>	n (%)
Egg white	7 (23.3)
Egg yolk	5 (16.7)
Milk	8 (26.7)
Other food	4 (13.3)
<b>Presenting complaints</b>	n (%)
Abdominal pain	12 (40.0)
Dysphagia	9 (30.0)
Nausea	6 (20.0)
Vomiting	5 (16.7)
Loss of appetite	4 (13.3)
Anemia	3 (10.0)
Malnutrition	3 (10.0)
Constipation	2 (6.7)
<b>Endoscopy</b>	n (%)
Normal	55 (37.4)
Hyperemic	21 (14.3)
Trachealization	16 (10.9)
Linear interpolation	14 (9.5)
Nodular	11 (7.5)
White plaque	10 (6.8)
Erosive	9 (6.1)
Ulcer	7 (4.8)
Edematous	4 (2.7)
<b>Histology</b>	n (%)
Basal cell hyperplasia	24 (16.3)
Polymorphonuclear leukocyte	18 (12.2)
Eosinophilic microabscess	13 (8.8)

In ROC analysis, AEC was found to have a predictive value in the diagnosis of EoE (Area Under ROC Curve [AUC]: 0.609, 95% Confidence Interval [CI]: 0.51-0.71,  $p=0.022$ ) at a cut-off value of 395/ $\mu$ L, with a sensitivity, specificity, PPV, and NPV of 58.1%, 64.2%, 47.5%, and 53.5%, respectively (Figure 2).

**Figure 2.** Diagnostic value of AEC in predicting EoE (AUC: 0.609, %95CI: 0.51-0.71,  $p=0.022$ )

## Discussion

Eosinophilic esophagitis (EoE) is a disease with an increasing incidence, leading to reduced quality of life as well as severe long-term complications. Repeated endoscopic and histological examinations remain essential to evaluate treatment response. The present study evaluated the usability of AEC and serum IgE values in the follow-up of pediatric patients with EoE. Esophageal eosinophilic density established no significant correlation with total IgE, while it was correlated with AEC.

In the literature, there are numerous studies reporting on a correlation between AEC and esophageal eosinophilic density (10-12). The first report of eosinophilic esophagitis in Türkiye was published by Bakırtaş et al. (14) in 2012, and eosinophilia was detected in 4 of 7 patients (57.2%) (13). A study by Furuta et al. (5) reported that AEC decreased significantly after two weeks of budesonide treatment and that AEC was correlated with esophageal eosinophilic density. In another study, Rodríguez-Sánchez et al. (15) observed that AEC decreased in 22 patients who responded to a six-week diet treatment, while it did not decrease in six patients that did not respond to the treatment. In the same study, however, no significant correlation was found between AEC and esophageal eosinophilic density. In a study by Wechsler et al. (16), it was revealed that AEC and peak eosinophil count

(PEC) had a predictive value in the diagnosis of EoE. Schlag et al. (10) evaluated the correlation between esophageal eosinophilic density and several parameters including AEC, serum CCL-17, CCL-18, CCL-26, eosinophil-cationic-protein, and mast cell tryptase levels measured before and after budesonide treatment and reported that AEC showed the highest correlation. In the same study, the authors noted that AEC was also useful for assessing local disease activity and was significantly associated with histological remission.

To our knowledge, there is no established cut-off value for AEC to predict EoE or its prognosis. At a cut-off value of 300/ $\mu$ L, AEC has been shown to have a sensitivity and specificity of 88% and 56% in the prediction of histological remission (10). By contrast, a study by Min et al. (17) reported that a cut-off value of  $>150/\mu$ L had a sensitivity and specificity of 85% and 55%, while it had a sensitivity and specificity of 75% and 64% at a cut-off value of  $>200/\mu$ L, respectively. In our study, the optimal cut-off value was found to be 395/ $\mu$ L, with a sensitivity and specificity of 64.2% and 58.1%, respectively. Moreover, AEC was found to be correlated with esophageal eosinophilic density although the AUC (0.609) and correlation coefficient ( $r=0.248$ ) were remarkably low. Taken together, all these findings implicate that AEC alone is not likely to evaluate the disease severity and replace endoscopy. On the other hand, it is known that esophageal eosinophilic density in EoE patients may vary depending on the seasons (18-19). Likewise, in our study, AEC showed variation between atopic and non-atopic patients. Additionally, AEC decreased after treatment, which we believe may be helpful in treatment response.

Some studies have attempted to improve the sensitivity and specificity of AEC alone by adding several other biomarkers. Among these, a study by Min et al. (17) reported that the use of eosinophil cationic protein (ECP)  $>30$  ng/ml along with AEC  $>200/\mu$ L increased the specificity to 77% while it decreased the sensitivity to 55%. The authors noted that the combined use of AEC and ECP was effective in the diagnosis of EoE. In a study by Thulin et al. (20), AEC was combined with eosinophil-derived neurotoxin (EDN), total and sIgG4, and sIgE to distinguish active EoE, EoE in remission, and healthy individuals. A combination of biomarkers (AEC, EDN, sIgE to egg white and wheat) and symptoms revealed an AUC of 0.92 in discriminating between the three groups. A study by Wechler et al. (16) reported that a combination of six serum biomarkers (galectin-10, ECP, EDN, Eotaxin 3, major basic protein-1 [MBP-1], and AEC) showed an AUC of 0.90, whereas another study indicated that a combination of 12 cytokines, AEC, and 15-hydroxyeicosatetraenoic acid (15(S)-

HETE) had an AUC of up to 0.96 (21). Nevertheless, some other studies found that the use of parameters (ECP, EDN, MBP) in isolation was not sufficient to predict the diagnosis, and thus the studies attempted to predict the diagnosis by using data mining and machine learning techniques by adding some other different parameters (22,23).

In recent studies, these parameters have begun to be evaluated together with clinical findings. In an adult study conducted by Lingblom et al. (24), clinical findings (patients' reported outcomes) were combined with these parameters and the results suggested that this combination could be helpful in treatment monitoring. Nonetheless, there is need for further evidence to substantiate these findings. Moreover, multicenter studies involving more patients are needed to investigate the substitution of endoscopy with clinical, laboratory, endoscopic, and histopathological scoring systems.

Another issue to consider related to EoE is the correlation between histological findings and the prognosis. Hiremath et al. (25) indicated that histological scoring for EoE was correlated with histological findings (eosinophilic microabscess, BCH, eosinophilic inflammation, and dilated intercellular spaces). Choudhury et al. (26) found that esophageal eosinophilic density was higher in patients with BCH and eosinophilic microabscess regardless of the presence of neutrophils or lymphocytes. The authors also showed that esophageal eosinophilic density was correlated with AEC. In our study, although AEC was remarkably high particularly in patients with eosinophilic microabscess, esophageal eosinophilia density was higher in patients with eosinophilic microabscess and BCH. Additionally, esophageal eosinophilia density established a correlation with AEC in patients with eosinophilic microabscess, while there was no such correlation in patients with BCH and PNL. This finding may be due to the small number of patients in our study. Accordingly, further studies are needed on this subject to investigate whether the AEC cut-off value differs depending on the presence or absence of histological findings.

### Study Limitations

Our study was limited in several ways. First, it had a small number of patients and thus active and remission patients could not be evaluated separately. Second, there was no healthy control group. Finally, the number of parameters examined was remarkably small. Given the retrospective nature of our study, further multicenter studies on this subject are needed.

## Conclusion

In conclusion, EoE is a disease with an increasing incidence, requiring endoscopic and histological examination in the diagnosis and follow-up of the patients. Although AEC appears to be a usable parameter in the follow-up of the patients, it is not sufficient as a biomarker alone for the prediction of EoE. There is a need for scoring systems that could be used for evaluating other parameters and clinical findings together with endoscopic and histological findings.

## Ethics

**Ethical Approval:** The study was conducted in accordance with the principles of the Declaration of Helsinki. An ethics committee approval was obtained from Karadeniz Technical University Scientific Research Ethics Committee (no: 2023/234, date: 07.12.2023).

## Footnotes

**Conflict of Interest:** No conflict of interest was declared by the authors.

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