Low expression of ICAM-1 in blood eosinophils in patients with active eosinophilic esophagitis

Short title: ICAM-1 in patients with eosinophils esophagitis.

Pérez-Lucendo I[1], Gómez Torrijos E[2], Donado P[3], Melero R[1], Feo-Brito F[2], Urra JM[1]

[1]Immunology, Hospital General Universitario de Ciudad Real, Spain.

[2]Allergology Medicine, Hospital General Universitario de Ciudad Real, Spain.

[3]Pediatric Medicine, Hospital General Universitario de Ciudad Real, Spain.

Corresponding author

Pérez-Lucendo, Irene

E-mail: **ireneplucendo@gmail.com**

Abstract

Background: Eosinophilic esophagitis (EoE) is a chronic and isolated inflammation of the esophagus, defined by an important infiltration of eosinophilic leukocytes. Only the histopathological study determines the diagnosis and evolution of the disease. Therefore, patients must undergo a large number of esophageal biopsies, with the risk involved in the procedure and the necessary resources.

Objective: The presence of active circulating eosinophils, quantifiable through the expression of specific proteins of cellular activation in their membrane, could be a concordant parameter with the histopathological findings that are currently the only valid parameters in EoE studies.

Methods: The activity of peripheral blood eosinophils from patients with EoE was analyzed by identifying five surface molecules (CD69, IL-5Rα, CD44, ICAM-1, CD63) expressed by the active eosinophils by flow cytometry. The results were compared with the infiltrate of eosinophils present in patient's esophageal biopsies.

Results: ICAM-1 significantly reduced in patients with active EoE compared to no active EoE patients, allergic patients and healthy controls. In these patients, an inverse correlation between the number of eosinophils present in the esophageal biopsy and the percentage of ICAM-1 expression in peripheral blood eosinophils was observed. For the rest of the molecules studied no difference was observed.

Conclusion: The expression of ICAM-1 in blood eosinophils could be a non-invasive marker useful for the diagnosis and assessment of patients with EoE.

Key words: Eosinophilic esophagitis. Eosinophils. Flow cytometry. ICAM-1.

doi: 10.18176/jiaci.0489

Resumen

Contexto: La esofagitis eosinofílica (EoE) es una inflamación crónica y aislada del esófago, caracterizada por una infiltración importante de eosinófilos. El diagnóstico y evolución de la enfermedad se realiza únicamente por estudios histopatológicos. Además, los pacientes se someten a un elevado número de biopsias esofágicas con el riesgo que implica el procedimiento y los recursos utilizados.

Objetivo: Comprobar si la presencia de eosinófilos activos circulantes, mediante la cuantificación de la expresión de proteínas específicas presentes en la membrana de eosinófilos activados, concuerda con los hallazgos histopatológicos aceptados como únicos parámetros válidos para estudios de EoE.

Métodos: Se analizó la actividad de los eosinófilos de sangre periférica de pacientes con EoE mediante la identificación de cinco moléculas de superficie (CD69, IL-5Rα, CD44, ICAM-1, CD63) expresadas en los eosinófilos activos por citometría de flujo. Los resultados se compararon con el infiltrado de eosinófilos presentes en biopsias esofágicas de los pacientes.

Resultados: Se observó que el marcador ICAM-1 está significativamente reducido en pacientes con EoE activa en comparación con pacientes con EoE inactiva, pacientes alérgicos y controles sanos. En estos pacientes, se observó una correlación inversa entre el número de eosinófilos presentes en la biopsia esofágica y el porcentaje de la expresión del ICAM-1 en eosinófilos de sangre periférica. El resto de los parámetros estudiados no presentaban diferencias.

Conclusión: La expresión de ICAM-1 en eosinófilos de sangre periférica podría comportarse como un marcador no invasivo útil en el diagnóstico y seguimiento de pacientes con EoE.

Palabras clave: Esofagitis eosinofílica. Eosinófilos. Citometría de flujo. ICAM-1.

doi: 10.18176/jiaci.0489

Introduction

Eosinophilic esophagitis (EoE) is a chronic and isolated inflammation of the esophagus, defined by an important infiltration of eosinophilic leukocytes. Although the first cases of adults diagnosed of EoE, with dysphagia as a form of presentation, were described as early as 1975, it is not until 1995 that Kelly and Sampson define the EoE as a different pathology of the gastroesophageal reflux, with esophagitis, with a non-response to the conventional treatments, and that is characterized by an improvement to an elementary diet(1)(1)(1)(1)[1]. EoE is a more frequent disease in children than in adults and in a greater proportion in the male sex. The form of presentation varies with age, abdominal paint, dysphagia and impacted foods are the most frequent symptoms[2]. The histological diagnosis is defined by a cellular infiltration of the squamous epithelium equal to or greater than 15-20 eosinophils per high-power field.

The comorbidity of EoE with the presence of atopy is very frequent, which suggests that the recruitment of eosinophils in the esophagus may be the response to environmental antigens in genetically predisposed individuals[3]. High levels of IgE have also been found in tissues of patients with EoE, but its role in the pathogenesis is unclear[4]. Treatments are based on food restriction[5], empirical or directed if data from skin tests or IgE determinations are available and pharmacological with proton pump inhibitors or corticosteroids[6].

At present, we have not a specific non-invasive blood markers of the disease[7,8]. Although high levels of specific IgE and peripheral eosinophilia can be found, these are not present in all patients and, when are shown, it is generally mild[9]. Only the histopathological study, based on the number of eosinophils present in esophageal biopsy (>15), determines the diagnosis and evolution of the disease. Diagnosis and treatment response monitoring in EoE requires endoscopically and histological examination of the esophagus. Therefore, patients undergo a large number of esophageal biopsies, with the risk involved and the required resources.

Easy and noninvasive methods would be highly desirable. Therefore, we hypothesize that parameters reflecting not only the amount of eosinophils but also their state of activation in peripheral blood could be easily attainable and it would represent a noninvasive marker to screen for diagnosis and monitor treatment response in EoE. The presence of active circulating eosinophils, quantifiable through the expression of specific proteins of cellular activation in their membrane, may be a concordant parameter with the histopathological findings that are currently the only valid parameters in EoE studies.

doi: 10.18176/jiaci.0489

Material and Methods

The activity of peripheral blood eosinophils from patients with EoE was analyzed by identifying on the surface, molecules expressed by the activated eosinophils by flow cytometry. Samples for peripheral blood were collected in tubes containing EDTA-K, and 100µL were pretreated and analyzed by the flow cytometer FACScan (Becton Dickinson®). The results were compared with the infiltrate of eosinophils present in patient's esophageal biopsies. Esophageal biopsies are collected from the distal, medium and proximal part of esophagus. The largest number of eosinophils observed in the tissue is the one reported in the study. Both samples histological and blood were collected at the same moment.

Patients

The study has 43 individuals classified into four groups: healthy controls (n = 15), allergic patient controls (n = 9), active EoE patients (eosinophils present in esophageal biopsy >15) (n = 12) and inactive EoE patients (n = 7). All patients classified as EoE currently or previously had specific symptoms of EoE and in some of the histopathological studies performed or in the current one they presented more than 15 eosinophils. At the time of the study, none of the 19 EoE patients were being treated with corticosteroids. Nor was any undergoing treatment with proton pump inhibitors (PPI), although in 4 of them the treatment with PPI had been suspended due to adverse effects. All patients were on a food elimination diet or under study to begin it. In patients with EoE, the blood samples were obtained simultaneously with the endoscopy to take the histological samples of the esophagus. Patients with active EoE present allergy reactions to grass, olive, cereals, nuts and eggs, while inactive EoE had allergy to grass, cow-milk, eggs, nuts and fish as shown in table 1. The allergic controls correspond to patients with a recent allergy diagnosis and without prior treatment, these patients present symptoms like asthma or rhino conjunctivitis. In addition, patients of allergy group present allergy reactions to grass (55,5 %), olive (11,1%), hymenopterous (22,2%), cereals (11,1%), peach (11,1%), and fish (11,1%). Healthy controls, individuals with any pathology or suspected allergy were excluded of the study.

The study has been approved by the ethic committee of Hospital General Universitario of Ciudad Real in accordance with the Declaration of Helsinki, and the patients signed an informed consent.

Eosinophils Flow Cytometry

Monoclonal antibodies were used to identify the eosinophils by flow cytometry. Anti-human

Siglec-8 to identify the eosinophils in the cytometer and the following surface markers to identify the active eosinophils; CD69, alfa subunit of IL-5 receptor (IL5RA), CD44, ICAM-1 and CD63. The eosinophils were identified using both, the expression of the surface protein Siglec-8 on cell populations and cellular complexity (SSC) as shown in Figure 1S (Supplementary material). The number of eosinophils analyzed per sample is 500. Over the selected eosinophil cells, the expression of the activation markers was studied with conjugated monoclonal antibodies to calculate the percentage of those were in active state. For the characterization of the eosinophils, the PE-anti-human Siglec-8 antibody, monoclonal mouse IgG1 clone 837535 (R & D Systems, CA, USA) was used, and for the evaluation of the active eosinophils, FITC-conjugated monoclonal antibodies against the previously described inducible cell surface proteins were used: FITC-anti-human CD69 (monoclonal IgG1, κ, clone FN50), FITC-anti-human IL-5Rα (monoclonal IgG1, clone 26815;R & D Systems, CA, USA), FITC-anti-human CD44 (mouse IgG2b, κ, clone C26), FITC-anti-human-ICAM-1 (mouse IgG1, κ, clone HA58), FITC-anti-human CD63 (mouse IgG1, κ, clone H5C6). All these FITC-monoclonal antibodies except IL5Rα proceed from BD Biosciences Pharmingen (San Diego, CA, USA).

Statistical analysis

The statistical analysis was carried out using the statistical program SPSS v21®. The Kruskal-Wallis test was performed to analyze for each cell activation marker differences in the means analyzing the four groups simultaneously. Significant results indicate that the four groups under study do not behave in a homogenous way. Mann-Whitney U test was performed comparing mean differences between two groups with respect to each cell marker. The Spearman test was used to verify the correlation between the activation markers and the number of eosinophils present in the biopsy. To assess the potential of the activation markers in the diagnosis and follow-up of the patients, ROC curve studies were performed. The ROC curve has been calculated from the data on the specific website for the calculation of ROC curves of the JHONS HOPKINS University School of Medicine(10). Considering 0.5 as no discrimination capacity and 1 as optimal. A $p<0.05$ was considered statistically significant. GraphPad Prism was employed for obtaining the figures (GraphPad, San Diego, CA, USA). All results are expressed as mean ± standard error.

doi: 10.18176/jiaci.0489

Results

The mean age of the 43 patients analyzed in the present study was 40,44 years (± 11,33). Four were children under 12 years. In terms of distribution by sex, 33 were males and 10 females. Table 1 summarizes the demographic data of the defined four groups of patients. As show table 1, the most common IgE mediated allergies both, in the allergic control groups and patients with EoE (active or inactive), are pollen from grasses, olive and foods such as eggs and nuts (peanuts).

The number of blood eosinophils (expressed in eosinophils/μL) in peripheral blood not present any difference between the groups studied. The average number of eosinophils in peripheral blood in each group is detail in the table 2.

The results of the eosinophil surface cell markers of each group expressed in percentages, are summarized in the table 3. No significant differences were found for any of the groups studied, with the exception of ICAM-1, which was significantly reduced in patients with the active EoE compared to the rest of the groups analyzed (Figure 1). For this parameter the healthy controls, the allergic patients and patients with stable EoE behave in a similar way. The difference in the percentage of blood eosinophils with expression of ICAM-1 between patients with active and inactive EoE were, 39,2 ± 30,1% for no active patients versus 15,7 ± 7,3% for the active EoE patients, with a statistical significance of 0,028 (Examples of cytometry plots in Figure 2S Supplementary material).

Additionally, as shown Figure 2, in the group of patients with EoE (n=15), an inverse correlation between the number of eosinophils present in the esophageal biopsy and the percentage of ICAM-1 expression in peripheral blood eosinophils was observed (coefficient of correlation Rho of Spearman -0,501; p =0,048). All patients belonging to the active EoE group were diagnosed by esophageal biopsy, except four of these patients who were only diagnosed by clinical criteria, without submit esophageal biopsy data.

Finally, to verify the discrimination capacity between patients with active EoE versus non-active EoE of the expression of ICAM-1 in eosinophils, a ROC curve analysis was performed. The area value under the curve for ICAM-1 of 0,784 (Figure.3), unlike the rest of the activation markers analyzed that varied between 0,511 and 0,567. The optimal cut-off point to differentiate EoE activity was 21.5% of eosinophils with ICAM-1 expression. Values below this cut-off point indicate disease activity with a sensitivity of 0.75 and a specificity of 0.86.

doi: 10.18176/jiaci.0489

Discussion

Given the burden of invasive testing with endoscopy and biopsy for diagnosis and monitoring of EoE, a non-invasive biomarker-based blood test would be of immense value. Recently, several studies have been published as non-invasive diagnostic methods for EoE, in which different eosinophil degranulation markers were analyzed from patients´ serum, which are elevated in the esophagus of patients with EoE, by solid-phase sandwich immunoassay. These studies concluded that there were no significant differences between both groups, active and no active EoE patients[1]. In the present study, we analyzed the potential for five eosinophil activation markers. Previously some studies had shown that the eosinophils present in patients with EoE had a phenotype of activation markers that differed from healthy individuals and patients with other pathologies[11]. In a similar study, the possible modification of the peripheral blood eosinophil phenotype in patients with active EoE after corticosteroid treatment was investigated, for this purpose, surface marker such as CD18, CD44 or CD54 (ICAM-1) were analyzed. Low levels of CD18 on the eosinophils surface generating poor binding with ICAM-1 was concluded[12]. However, in this study do not find decrease in CD54 as we described in our study, and on the contrary, they observe decrease in expression of CD44. The discrepancy of results can be explained because in their study they do not differentiate EoE activity in the patients, and the behavior may be different in patients with active disease or not. Otherwise in our work, surface marker expression was analyzed by flow cytometry using non-fractionated leukocytes in order to avoid spurious activation caused by immunomagnetic purification of eosinophils. To date, only the peripheral activation of eosinophils by means of morphological parameters has been studied, which requires great expertise and is difficult to standardize[13]. Our findings indicate that blood eosinophil phenotypes could be used to distinguish patients with EoE. Activated eosinophils are effector cells with proinflammatory and destructive capabilities. Eosinophils with activation phenotypes are observed in the specimens of esophagus of patients with EoE, and deposition of eosinophil products is clearly seen in these patients' affected tissues[14].

It has already been postulated that the level of peripheral blood eosinophils holds promise as an EoE biomarker, although the incidences of peripheral blood eosinophilia in EoE patients (defined as more than 300/μL) might vary and be particularly influenced by factors such as the role of seasons or concomitant atopic conditions[15][16]. Although in several studies a significant correlation between blood eosinophilia and eosinophilic esophageal tissue infiltration was reported[17], in our study, the total number of eosinophils found in the peripheral blood was not different among the groups studied. Other works have also described the lack of correlation between blood eosinophils and those present in the esophagus[13].

doi: 10.18176/jiaci.0489

Eosinophils can in turn rapidly upregulate adhesive functionality to bind an array of counter ligands which can lead to further activation of downstream cytoplasmic signaling pathways that influence cellular responses that play a role in eosinophil survival and activation[18]. The finding that ICAM-1 expression was decreased in active EoE subjects is consistent with the concept that a pre-activation of circulating Eosinophils occurs in Eosinophilic Esophagitis. These biological properties of circulating eosinophils may, at least partially, explain the substantial migration of these activated cells into target tissue that occurs during acute EoE. Cytokines and chemokines are able to pre-activate eosinophils and increase adhesion molecule expression and cell homing. Several studies in severe asthma have observed transient or stable downregulation of key adhesion molecules on blood eosinophils, which may reflect a high degree of ongoing extravasation in which eosinophils with the highest expression of activated integrins may be efficiently and continuously removed from the circulation[19]. Activation of eosinophils appears to be a reliable indication of what will happen when these cells migrate to esophageal tissue. It is possible that because of their more activated state, eosinophil degranulation will occur in the target tissue, resulting in esophageal tissue damage in EoE patients. Induction of ICAM-1 and HLA-DR, has been previously described as a response to eosinophil-endothelial interaction and is thought to be a consequence of trans endothelial migration in general[20]. Other works have also found a negative co-relationship between active disease and adhesion molecules or chemokine receptor[21].

For the rest of the parameters analyzed in the present work, we did not find any difference between the different groups studied nor between active and non-active EoE patients. Possibly these activation markers are expressed at the esophageal level mediated by locally produced factors such as periostin, facilitating the infiltration of eosinophils in the esophagus[22]. Therefore, no changes of its expression in blood eosinophils are observed

Actually, gastrointestinal endoscopy and histopathological examinations of biopsy specimens are considered the only method available to diagnose EoE, as well as for monitoring the activity of EoE despite this method is severe for the patients[23]. For this reason, we have proposed, like a non-invasive method, to study the activity of eosinophils in peripheral blood. Several promising minimally invasive biomarkers for EoE have emerged; however, few are able to differentiate EoE from other atopic diseases. The most commonly reported biomarkers were peripheral blood eosinophils, blood and string eosinophil granule proteins, and eosinophil surface or intracellular markers[7]. Regarding our study and in view of the results discussed previously, we can observe that there is significant difference in the expression of the ICAM-1 marker between patients with active and inactive EoE, observing a decrease in the expression of this marker in patients with active EoE.

doi: 10.18176/jiaci.0489

Acknowledgment

We would like to express our very great appreciation to Isabel Ródenas for her enthusiastic encouragement in the technical aspects of the work.

This work was supported by Instituto de Salud Carlos III (ISCII) co-founded by FondoEuropeo de Desarrollo Regional – FEDER for the Thematic Networks and Co-operative Research Centers: ARADyAL (RD16/0006/0028).

Conflict of interest

The authors certify that they have NO affiliations with or involvement in any organization or entity with any financial interest in the subject matter or materials discussed in this manuscript.

doi: 10.18176/jiaci.0489

References

1. Kelly KJ, Lazenby AJ, Rowe PC, Yardley JH, Perman JA, Sampson HA. Eosinophilic esophagitis attributed to gastroesophageal reflux: improvement with an amino acid-based formula. Gastroenterology. 1995 Nov;109(5):1503–12.
2. Gómez Torrijos E, Sánchez Miranda P, Donado Palencia P, Castro Jimenez A, Rodriguez Sánchez J, Mendez Díaz Y, et al. Eosinophilic Esophagitis: Demographic, Clinical, Endoscopic, Histologic, and Atopic Characteristics of Children and Teenagers in a Region in Central Spain. J Investig Allergol Clin Immunol. 2017 Apr 10;27(2):104–10.
3. Ricker J, McNear S, Cassidy T, Plott E, Arnold H, Kendall B, et al. Routine screening for eosinophilic esophagitis in patients presenting with dysphagia. Therap Adv Gastroenterol. 2011 Jan 14;4(1):27–35.
4. Mulder DJ, Justinich CJ. Understanding eosinophilic esophagitis: the cellular and molecular mechanisms of an emerging disease. Mucosal Immunol. 2011 Mar 12;4(2):139–47.
5. Gómez Torrijos E, Moreno Lozano L, Extremera Ortega A, González Jimenez O, Mur Gimeno P, Borja Segade J, et al. Eosinophilic Esophagitis: Personalized Treatment With an Elimination Diet Based on IgE Levels in Children Aged <16 Years. J Investig Allergol Clin Immunol. 2019 Apr 23;29(2):155–7.
6. Gómez Torrijos E, Donado Palencia P, Sanchez Miranda M, Moreno Lozano L, Extremera Ortega A, Borja Segade J, et al. Eosinophilic Esophagitis: Treatment With Different Doses of Omeprazole in Children Under 16 Years. J Investig Allergol Clin Immunol. 2018 Jun 25;28(3):191–2.
7. Hines BT, Rank MA, Wright BL, Marks LA, Hagan JB, Straumann A, et al. Minimally invasive biomarker studies in eosinophilic esophagitis. Ann Allergy, Asthma Immunol. 2018 Aug;121(2):218–28.
8. Liacouras CA, Furuta GT, Hirano I, Atkins D, Attwood SE, Bonis PA, et al. Eosinophilic esophagitis: updated consensus recommendations for children and adults. J Allergy Clin Immunol. 2011 Jul;128(1):3-20.e6; quiz 21–2.
9. Dellon ES, Rusin S, Gebhart JH, Covey S, Higgins LL, Beitia R, et al. Utility of a Noninvasive Serum Biomarker Panel for Diagnosis and Monitoring of Eosinophilic Esophagitis: A Prospective Study. Am J Gastroenterol. 2015 Jun 17;110(6):821–7.
10. ROC Analysis: Web-based Calculator for ROC Curves [Internet]. [cited 2020 Jan 22]. Available from: http://www.rad.jhmi.edu/jeng/javarad/roc/JROCFITi.html
11. Doménech Witek J, Jover Cerdà V, Gil Guillén V, Doménech Clar JB, Rodríguez Pacheco R. Assessing eosinophilic cationic protein as a biomarker for monitoring patients with eosinophilic esophagitis treated with specific exclusion diets. World Allergy Organ J. 2017 Dec 23;10(1):12.
12. Ishihara S, Shoda T, Ishimura N, Ohta S, Ono J, Azuma Y, et al. Serum Biomarkers for the Diagnosis of Eosinophilic Esophagitis and Eosinophilic Gastroenteritis. Intern Med. 2017 Nov 1;56(21):2819–25.
13. Johnsson M, Bove M, Bergquist H, Olsson M, Fornwall S, Hassel K, et al. Distinctive blood eosinophilic phenotypes and cytokine patterns in eosinophilic esophagitis, inflammatory bowel disease and airway allergy. J Innate Immun. 2011;3(6):594–604.
14. Teitelbaum JE, Fox VL, Twarog FJ, Nurko S, Antonioli D, Gleich G, et al. Eosinophilic esophagitis in children: immunopathological analysis and response to fluticasone propionate. Gastroenterology. 2002 May;122(5):1216–25.
15. Botan V, Dos Santos Borges TK, Rocha Alves ÉA, Claudino Pereira Couto S, Bender Kohnert Seidler H, Muniz-Junqueira MI. Enhanced activation of eosinophils in peripheral blood and implications for eosinophilic esophagitis diagnosis. J Gastroenterol Hepatol. 2017 Jul;32(7):1318–27.
16. Virchow JC. Eosinophilic esophagitis: asthma of the esophagus? Dig Dis. 2014;32(1–2):54–60.
17. Jensen ET, Shah ND, Hoffman K, Sonnenberg A, Genta RM, Dellon ES. Seasonal variation

in detection of oesophageal eosinophilia and eosinophilic oesophagitis. Aliment Pharmacol Ther. 2015 Aug;42(4):461–9.
18. Marlais M, Francis N, Fell J, Rawat D. Blood tests and histological correlates in children with eosinophilic oesophagitis. Acta Paediatr. 2011 Aug;100(8):e75–9.
19. Calderwood DA. Integrin activation. J Cell Sci. 2004 Feb 15;117(Pt 5):657–66.
20. Johansson MW. Eosinophil Activation Status in Separate Compartments and Association with Asthma. Front Med. 2017 Jun 12;4:75.
21. Walker C, Rihs S, Braun RK, Betz S, Bruijnzeel PL. Increased expression of CD11b and functional changes in eosinophils after migration across endothelial cell monolayers. J Immunol. 1993 May 1;150(9):4061–71.
22. Lingblom C, Bergquist H, Johnsson M, Sundström P, Quiding-Järbrink M, Bove M, et al. Topical Corticosteroids Do Not Revert the Activated Phenotype of Eosinophils in Eosinophilic Esophagitis but Decrease Surface Levels of CD18 Resulting in Diminished Adherence to ICAM-1, ICAM-2, and Endothelial Cells. Inflammation. 2014;37(6):1932–44.
23. Blanchard C, Mingler MK, McBride M, Putnam PE, Collins MH, Chang G, et al. Periostin facilitates eosinophil tissue infiltration in allergic lung and esophageal responses. Mucosal Immunol. 2008 Jul 7;1(4):289–96.
24. Posten S, Adamiak T, Jensen M. Pediatric Eosinophilic Esophagitis. S D Med. 2018 Aug;71(8):362–6.

doi: 10.18176/jiaci.0489

Table 1. Demographic data of patients and healthy controls. Age expressed as mean in years ± standard desviation.

	HEALTHY CONTROLS	***ALLERGIC CONTROL***	***EoE ACTIVE***	***EoE INACTIVE***
N	15	9	12	7
Age (years)	40,44 ± 11,33	43,00 ± 19,45	28,61 ± 14,25	26,00 ± 11,17
Maximum age	60	70	54	45
Minimum age	18	10	6	15
Male	11 (73,3%)	8 (88,9%)	9 (75,0%)	5 (74,1%)
Female	4 (26,7%)	1 (11,1%)	3 (25,0%)	2 (28,6%)
Allergy	None	Pollen from grasses (55,5%), olive (11,1%), nuts (11,1%), Hymenoptera (22,2%).	Pollen from grasses (38,5%), olive (23,0%), nuts (15,4%), eggs (7,7%), cereals (15,4%).	Pollen from grasses (30,0%), nuts (20,0%), eggs (20%), cow milk (20,0%), fish (10,0%).

Table 2. Absolute number of eosinophils in peripheral blood. The results of the number of eosinophils are presented as the mean ± standard error of the mean. The units of measurement are number of eosinophils/µL of peripheral blood.

TYPE OF PATIENT	**N**	**NUMBER OF EOSINOPHIL**	**MIN**	**MAX**
HEALTHY CONTROLS	15	320,00 ± 116,2	100	1000
ALLERGIC CONTROL	9	266,67 ± 62,3	100	700
EOE ACTIVE	12	358,33 ± 82,9	100	1100
EOE INACTIVE	7	300,00 ± 117,5	100	800

doi: 10.18176/jiaci.0489

Table 3. Results of surface activation markers in blood eosinophils of each group studied. Results are expressed as percentage on total blood eosinophils ± standard error of the mean. The results in the groups are analyzed by the Kruskal Wallis test.

CELL MARKER	***HEALTHY CONTROL***	***ALLERGIC CONTROL***	***EoE ACTIVE***	***EoE INACTIVE***	***p***
CD69	24,0±14,9	32,3±24,4	19,7±13,3	22,4±15,5	0,650
IL5RA	20,0±14,7	42,6±29,4	22,2±16,8	21,8±15,6	0,902
CD44	53,6±17,9	63,4±26,3	53,1±22,4	47,6±24,0	0,967
ICAM-1	29,0±20,2	39,2±30,1	15,7± 7,4	36,1±28,4	0,048
CD63	18,4±13,0	45,4±29,1	24,7±16,7	23,4±13,9	0,967

doi: 10.18176/jiaci.0489

Legends of figures

Figure 1. Values of the percentage of expression of ICAM-1 in blood eosinophils in the four groups defined in the study (healthy controls, allergic controls, active EoE patients and non-active EoE patients). Patients with active EoE have lower values than the rest of the groups (ns: no significant differences).

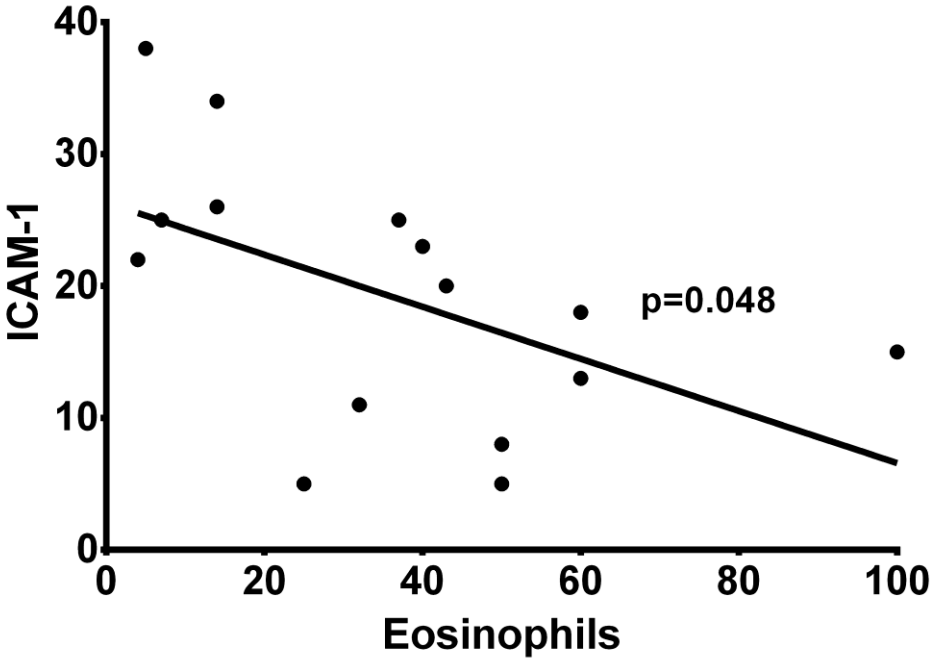
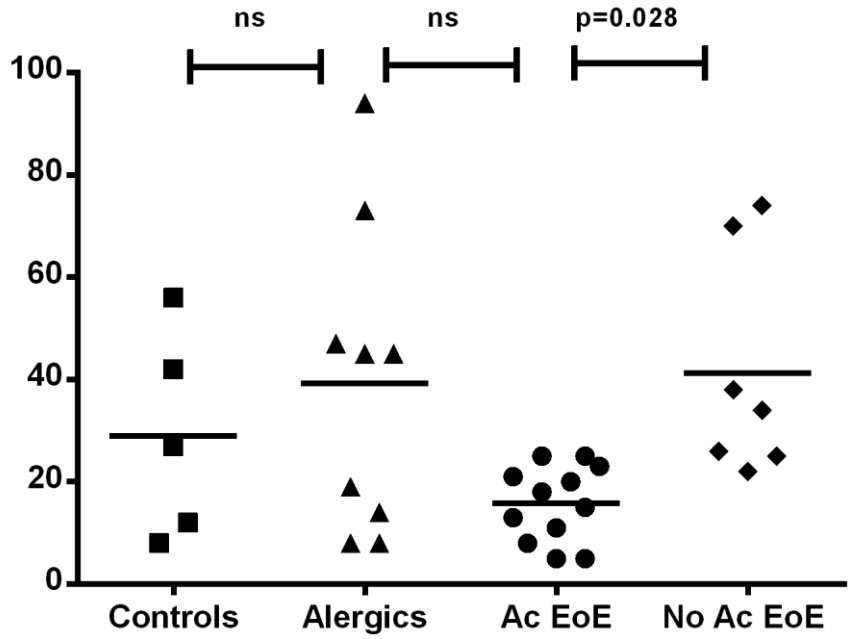
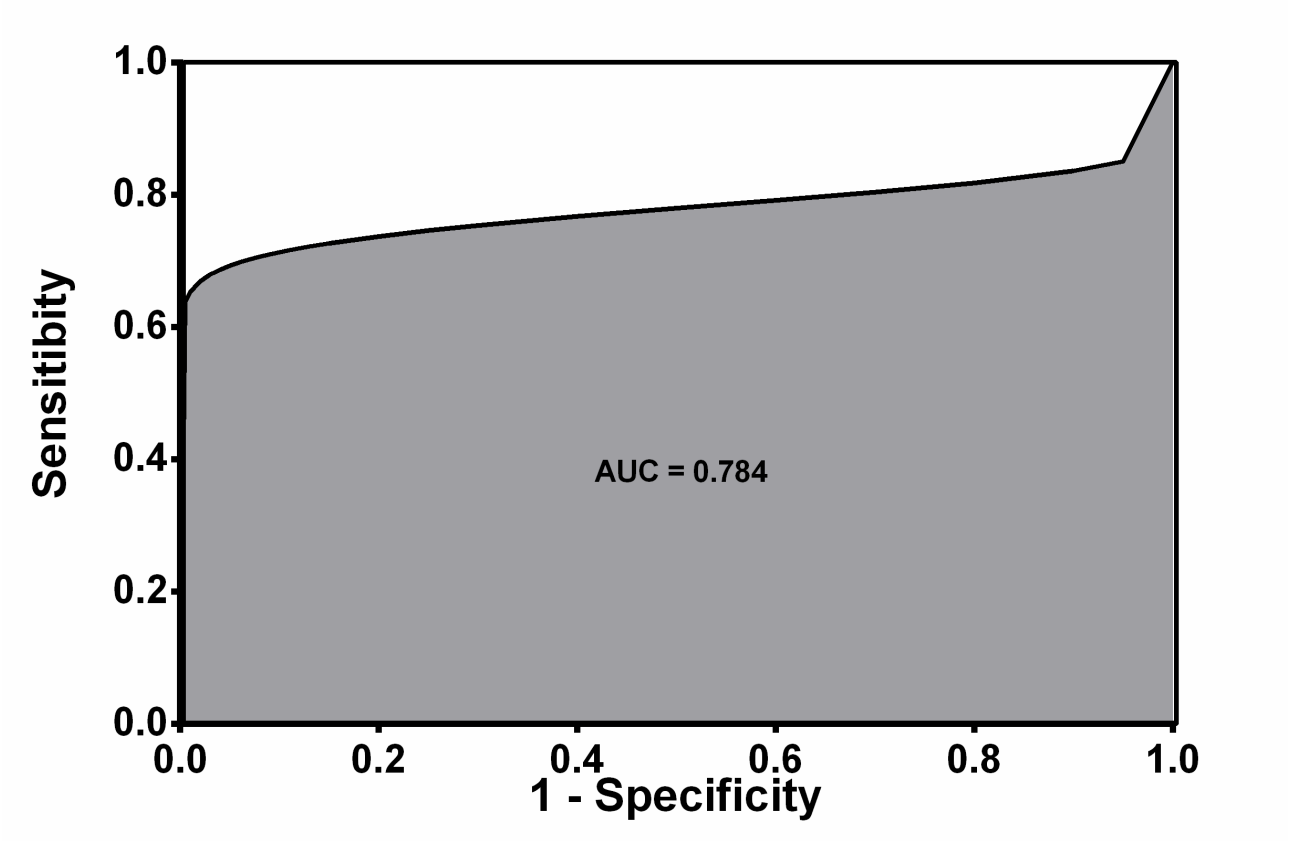


Figure 2. Correlation between the number of eosinophils present in esophageal biopsies and the expression of ICAM-1 in blood eosinophils in n=15 patients with EoE.



doi: 10.18176/jiaci.0489

Figure 3.ROC Curve Analysis on the usefulness of ICAM-1 eosinophilic membrane expression to discriminate between patients with active EoE of patients without disease activity. AUC = area under the curve.



doi: 10.18176/jiaci.0489