

Dear Editorial Board of the Journal of Pediatric Gastroenterology and Nutrition,

We are pleased to enclose our revised recent retrospective study that arose from the Clayton *et al* article in Gastroenterology (2014) that predominantly looked at adult eosinophilic esophagitis patients. This led us to do a retrospective study looking at our pediatric cohort which resulted in fairly robust findings as you will see in the enclosed manuscript.

Grant Support:

BC Children's Hospital Department of Pathology Microgrant

Disclosure and Conflict of Interest:

This manuscript has been presented as an abstract to the Society for Pediatric Pathology Fall Meeting (September 2017) in Denver CO.

J.W.B. has provided consulting services for Qu Biologics but has no conflict of interest. None of the other authors have a financial interest to disclose nor conflict of interest.

We appreciate your time and consideration in our submission.

Sincerely,

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ACCEPTED

Title: Pediatric Eosinophilic Esophagitis is Associated with Increased Lamina Propria Immunoglobulin G4-Positive Plasma Cells.

Short Title: IgG4 in Pediatric Eosinophilic Esophagitis

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Author Contributions:

J. Bush conceived the study and contributed to study design, data collection, and manuscript preparation. N. Mohammad contributed to data collection and manuscript preparation. V.

Avinashi, E. Chan, B.A. Vallance, and E. Portales-Casamar contributed to study design and/or manuscript preparation.

Abstract:

Introduction: Eosinophilic esophagitis (EoE) is considered a Th2-mediated food allergy disease that leads to submucosal esophageal fibrosis and strictures. Recent studies focused on adults with EoE identified a strong association with elevated esophageal IgG4 immunostaining. Our study aimed to determine the association of IgG4 with EoE in pediatric patients.

Methods: Using our local EoE research registry, we identified 41 adequate biopsies from EoE patients. We used 10 age and sex-matched patients with no diagnostic abnormalities at endoscopy or on biopsy. Using a monoclonal antibody to IgG4, we determined the maximum density of IgG4-positive plasma cells (IgG4-PC) per high-power field (hpf). Using a semi-quantitative assessment, we also graded the non-cellular staining of the lamina propria and epithelium.

Results: Our EoE cohort consisted predominantly of males with an average age of 5.9 years and 63% had a documented IgE-based food allergy. Median peak eosinophilia was 40 eosinophils/hpf and the median IgG4-PC density was 39/hpf in the active esophagitis patients, compared to a median of 0 IgG4-PC/hpf in the non-EoE patients ($p=0.0001$). EoE patients with a food allergy showed a significantly higher IgG4-PC density (44.5/hpf) than those without a food allergy (8/hpf) ($p=0.0385$). There was no significant association between IgG4-PC density and peak eosinophilia ($r^2=0.0011$).

Conclusions: We demonstrate that active esophagitis in pediatric EoE patients is associated with elevated levels of IgG4-positive plasma cells, which was more significant in EoE patients with a documented food allergy. Our study also adds to the growing literature that EoE may involve more than just an exaggerated Th2 immune response.

Key Words:

eosinophilic esophagitis, IgG4, pediatric, allergy

What is Known:

1. Eosinophilic Esophagitis (EoE) is associated with Immunoglobulin G4 (IgG4) in adults.
2. IgG4 may differentiate EoE from reflux disease in adults.
3. The association of IgG4 in pediatric EoE patients is not well characterized.

What is New:

4. We studied the most robust pediatric cohort with IgG4 immunostaining to date.
5. IgG4-positive plasma cells are significantly associated with pediatric EoE patients.
6. Further testing to compare food specific IgG4 serology, histopathologic findings, and clinical food allergy by skin testing may further elucidate the role of IgG4 in EoE.

Introduction:

Eosinophilic esophagitis (EoE) has classically been considered an atopy/allergy-mediated disease with the repetitive antigen suggested to be food related, based on the partial success of the six-food trigger empiric elimination diet¹, directed elimination diet, and elemental diet². Given the predominance of eosinophils in EoE, the postulated pathophysiology centers around Th2-based immune responses with IgE and interleukin-13 playing key roles in driving the esophageal inflammation³. However, recent genome-wide association studies⁴ and mRNA transcription studies⁵ have identified underlying molecular pathways that may play a role in disease pathogenesis, however they remain only partially explored.

The group of IgG4-related disorders (IgG4-RD) has been defined with strict histologic criteria proposed by an international consensus statement⁶; nearly a decade after IgG4 was first reported to be associated with autoimmune pancreatitis⁷. Recognition of IgG4-RD is important, as the natural history of these disorders is that of progressive fibrosis, with therapies generally focusing on steroids. The immune mechanisms underlying IgG4-RD, such as sclerosing cholangitis and pancreatitis, are traditionally considered within the Th2 pathway⁸. However, recent studies have suggested that the Th17 regulatory pathway may have some role in IgG4-RD, such as in sclerosing cholangitis⁹ and Sjogren's syndrome¹⁰.

A recent study found a significant association with IgG4 in adults with EoE when evaluating tissue biopsies by IgG4 immunohistochemistry (IHC) as compared to control esophageal biopsies¹¹. Although such results on their own do not suggest this is a “new” form of IgG4-RD¹², the natural history of EoE mimics IgG4-RD in some respects given the gradual but

inevitable development of submucosal fibrosis¹³. However, EoE does not demonstrate the obliterative phlebitis often seen in IgG4-RD.

The role of IgG4 in pediatric EoE has yet to be thoroughly investigated. The purpose of this study was to determine the density of IgG4-positive plasma cells (IgG4-PC) in the esophageal biopsies of patients diagnosed with eosinophilic esophagitis as compared with those without EoE. A secondary measure was determining whether there was a relationship between the number of IgG4-PC and concomitant food allergy or subsequent eosinophil counts.

Materials and Methods:

A retrospective review of patients enrolled between 2012 and 2015 in the British Columbia Children's Hospital Eosinophilic Esophagitis Registry was performed. After a review of all enrolled patients, the most proximal biopsy with sufficient lamina propria and active esophagitis in each patient was selected for the study set. The biopsies were not limited to treatment naïve endoscopies. Clinical and endoscopic information on these patients was obtained from the patients EoE registry chart and electronic medical record. Food allergy was defined by a pediatric allergist in the EoE Registry as an immediate reaction to a food in combination with a positive skin prick test and/or specific IgE blood testing. Ten patients, matched to the median age and sex distribution in our study set, were identified who underwent endoscopy for abdominal pain, did not demonstrate mucosal eosinophilia, and were not diagnosed at the time of biopsy with eosinophilic esophagitis, celiac disease, or chronic inflammatory bowel disease. Two of these patients were subsequently diagnosed with celiac disease, and were kept in for analysis. Biopsies were obtained per the local institution protocol of two tissue biopsies at each of the

three esophageal levels in the EoE patients; two tissue biopsies at one esophageal level was obtained in the non-EoE group.

Biopsies were reviewed by a pediatric pathologist for assessment of peak eosinophil counts and lamina propria fibrosis; peak eosinophilia in the subsequent biopsy was also recorded as a measure of complete ($<1/\text{hpf}$), partial ($1\text{--}15/\text{hpf}$), and no response ($>15/\text{hpf}$). Lamina propria fibrosis was graded by routine hematoxylin and eosin staining in a semi-quantitative manner using the eosinophilic esophagitis histology scoring system¹⁴. All biopsies were given a blinded, randomized study number prior to being stained for IgG4 (Cell Marque, clone MRQ-44 pre-dilute) after optimizing antigen retrieval conditions on tonsillar tissues. The number of IgG4-positive plasma cells was calculated per high-power field (hpf, 400x, 0.196 mm^2) independently by two pathologists in a hotspot manner. Discrepancies were then reviewed and resolved between the two pathologists. Semi-quantitative assessment of non-cellular IgG4 staining in the lamina propria and epithelial compartments was scored from 0-2, with “0” being none to focal punctate or stippled IgG4 staining, “1” being diffuse staining in less than 50% of interstitial or epithelial staining, and “2” being greater than 50% of interstitial or epithelial staining, often with a thickened appearance. Intravascular IgG4 staining was considered present if a dense endothelial band pattern was observed.

Statistical analysis was performed using SPSS software (IBM, version 24). Tests of normalcy determined the data to be non-parametric; Mann Whitney U test, Fisher’s exact test, and Pearson correlation were utilized with a significance being considered when $p < 0.05$ (two-tailed) with interquartile ranges (IQR) given for median values.

The study was approved by the Institutional Research Ethics Board (ID# H16-00115). The Eosinophilic Esophagitis Registry was approved under a separate study number.

Results:

From the registry of 109 patients, a total of 41 patients (38%) diagnosed with eosinophilic esophagitis were identified who had biopsies that possessed sufficient lamina propria for evaluation and displayed active (≥ 15 eosinophils/hpf) esophagitis. Three patients did not have serum IgE or skin prick testing to document a food allergy. Of the 38 tested patients, 24 (63.2%) had a food allergy with a reaction to at least one food (Figure 1), while 36.8% did not have a food allergy by serum IgE or skin prick testing. Endoscopic results were available in 37 of 41 patients. The clinical and endoscopic findings are summarized in Table 1. Treatment after biopsy was wide-ranging and included food-elimination diet in addition to single or combination therapy using a proton pump inhibitor (35% of cases), fluticasone (35% of cases), and/or budesonide (20% of cases). Food-elimination alone was used in the minority of cases (22.5%), and not part of therapy in 9.7%. Of the 41 EoE patients, 34 had a follow up endoscopy that occurred on average 13.2 months after the study biopsy.

The esophageal distribution of biopsied material that met inclusion criteria resulted in 22 proximal biopsies (53.7%), 13 mid biopsies (31.7%), and 6 distal biopsies (14.6%). Peak tissue eosinophilia had a median of 40 eosinophils/hpf (IQR: 28-60), with a median decrease of 22 eosinophils/hpf (IQR: -4.5-40) fewer in the next biopsy. The median number of IgG4-positive plasma cells (Figure 2) in EoE patients with active esophagitis was 39/hpf (IQR: 9-52), significantly elevated ($p=0.0001$) compared with a median of 0/hpf (IQR: 0-0) in the non-EoE patients (Figure 3). IgG4-PC did not show a correlation with concurrent peak tissue eosinophilia

($r^2=0.0011$), or to changes in eosinophilia in the next biopsy ($r^2=0.0054$), or to the greatest improvement in tissue eosinophilia regardless of time to re-biopsy ($r^2=0.0004$). Median number of IgG4-PC was significantly greater ($p=0.0414$) in EoE patients who had a food allergy (44.5/hpf, IQR: 23.5-59) than those who did not (8/hpf, IQR: 4-44) (Figure 3). Peak eosinophilia was not significantly different between the allergy (40/hpf, IQR: 27.25-60) and no allergy (33.5, IQR: 30-62) groups.

Within the group of EoE patients, using the semi-quantitative assessment of IgG4 staining revealed a significant association between the number of IgG4-positive plasma cells and denser non-cellular staining in the lamina propria (Kruskal-Wallis $p<0.0001$) and epithelium (Kruskal-Wallis $p=0.0007$) (Figure 3). Twelve (29%) patients showed Grade 2 staining, 10 (24%) patients had Grade 1 staining, and 19 (46%) had no lamina propria granular deposits. Epithelial deposits were more common, being seen in 19 (46%) with Grade 2 staining, 11 (27%) with Grade 1 staining, and 11 (27%) with no epithelial staining. Tissue eosinophilia was not significantly associated with either semi-quantitative measure. Lamina propria fibrosis was also not associated with the semi-quantitative grading of non-cellular IgG4. Interestingly, the more robust density of IgG4-positive plasma cells (median 46.5/hpf, IQR: 32-65) showed a significant association ($p<0.0001$) with intravascular IgG4 staining (N=24, 59%) as compared to the density of IgG4-positive plasma cells in those without (N=17, 41%) intravascular staining (median 5/hpf, IQR: 3-9) (Figures 2 and 3). No intravascular staining was observed in the non-EoE group. Neither peak tissue eosinophilia nor the presence of food allergy was significantly associated with this intravascular staining pattern, and there was no intravascular IgG4 staining in the non-EoE patients. Among active EoE patients with a follow up biopsy, there was no significant difference (Kruskal-Wallis $p=0.5684$) in the index biopsy IgG4-PC density between those that

showed complete response (N=6, 17.7%; median 33.5/hpf, IQR: 11-53), partial response (N=8, 23.5%; median 21/hpf, IQR: 8-21), or no response (N=20, 58.8%; median 42.5/hpf, IQR: 11-70).

Discussion:

We report herein the strong association between IgG4-positive plasma cells in the esophageal lamina propria and the diagnosis of pediatric eosinophilic esophagitis, further supporting the recent evidence in the literature of this association in adults. Our study adds to the literature by demonstrating that although there is an increase in IgG4-PC in EoE patients compared to non-EoE patients, the density of IgG4-PC is significantly elevated in EoE patients that have a known food allergy as compared with those that do not. This latter finding suggests future analysis may benefit from a case-control population of non-EoE patients with a documented IgE-mediated food allergy to further elucidate whether an elevated IgG4-PC is due to a food allergy or an as yet unexplained pathway unique to EoE. These future studies are also needed to test for the role that IgG4 may be playing in developing food tolerance rather than part of the pathophysiologic process of EoE. The results of this study build a more robust clinical, endoscopic, and pathologic description of IgG4 in pediatric EoE, which was recently published after this study was completed in the French literature¹⁵.

Interestingly, we were unable to show a correlation between tissue IgG4 and peak eosinophilia. This was surprising as it was anticipated that the relationship between IgG4-PC and eosinophils would be intertwined if they were in a linear relationship pathway. Although a direct relationship may prove true in future studies that can assess IgG4 in a prospective manner with serial counts, the disassociation between IgG4-PC density and peak eosinophilia seen in this study may indicate a more complex pathophysiology.

Our study confirms that in addition to EoE being associated with IgG4, patients with active esophagitis have non-cellular IgG4 in their esophageal epithelium and lamina propria. Previous studies have shown these non-cellular granular deposits to be electron dense, similar to immune complexes, on ultrastructural exam^{11,16}. Epithelial IgG4 was seen in 73% of our cohort and lamina propria IgG4 was seen in 53% of patients, similar to the numbers reported in Zuckerberg *et al*¹⁶, but slightly less epithelial staining than reported by Clayton *et al*¹¹. We feel this epithelial and lamina propria staining is associated with the EoE disease process as our non-EoE patients did not demonstrate any significant non-cellular staining. The reason for the epithelial staining discrepancy is unclear, but may be related to the modality of detection. Our study, and Zuckerberg *et al*¹⁶, used immunohistochemistry while Clayton *et al*¹¹ used immunofluorescence, which may represent greater sensitivity for detecting non-cellular IgG4 or false positive autofluorescence. In addition to this confirmation of non-cellular IgG4 staining in the epithelium and lamina propria, we also report the presence of dense intravascular endothelial staining. This staining pattern appeared analogous to that seen in antibody mediated rejection (AMR) in solid organ transplant pathology which is seen using IHC for the complement product C4d. As with AMR, this dense intravascular staining may represent a false positive such as stasis, but it may also represent subendothelial deposits and thus correlation with ultrastructural studies is needed.

We were unable to draw a statistically significant association between the density of IgG4-PC and therapeutic outcomes. This may be due to the relatively small study size or factors such as poor compliance for medication or food restriction, which is difficult to account for in a retrospective study. Another consideration for the lack of association with outcomes may be that we used a retrospective active esophagitis biopsy and looked forward at therapeutic outcomes.

Future studies using a prospective cohort may better determine if therapeutic outcomes can be predicted by the initial IgG4-PC density or its change over time.

Limitations include the retrospective nature of this study. This is particularly important as not all index biopsies were the patient's first, treatment-naïve biopsy. Of note, all patients were deemed to have "failed" PPI therapy with active eosinophilic esophagitis, thus we have excluded the confounding factor of patients with PPI-responsive EoE. Observer bias was addressed by assigning study numbers blinded to the pathologist; however, the initial selection of cases was not blinded and therefore could have introduced a theoretical risk of remembrance when evaluating randomized biopsies for eosinophil density and IgG4-positive plasma cells. Although the number of non-EoE patients was limited due to budgetary constraints, future studies will require a larger collection of negative controls that may prove difficult to collect in a highly selective, resource-limited hospital practice.

Another limitation due to the retrospective, cross-sectional, nature of this study was the inability to find a relationship between IgG4-PC density and lamina propria fibrosis, which may have provided insight into the possible pathophysiology of the resultant fibrosis seen in EoE patients. Although tempting to suggest extracellular IgG4 may play a role in fibrosis, further prospective studies of newly diagnosed EoE patients will be needed to elucidate this potential relationship. These studies would allow for controlling various factors such as disease duration, treatment, and treatment compliance, as well as ensuring that tissue biopsies are deep enough to sample the lamina propria consistently. We have recently received a small grant to look at IgG4 in a prospective cohort with one aim to examine this potential role. Prospective studies may also allow for the characterization of non-cellular staining and its drivers, as we currently have shown that there is more extracellular staining with a greater number of IgG4-PC. However, could the

extracellular staining be a marker of evolving chronicity, or could it be a marker that the histologic appearance is an acute event due to a trigger causing release of the immunoglobulin?

Future directions include testing the potential for IgG4 staining to differentiate eosinophilic esophagitis from other entities that lead to tissue eosinophilia including reflux disease, proton pump inhibitor-responsive esophageal eosinophilia, and inflammatory bowel disease in the pediatric population. Although these are often distinguished by other clinical or histologic parameters, IgG4 staining may be able prove beneficial in borderline cases. In adults, it has been suggested that intra-epithelial IgG4 staining is strongly supportive of EoE when the differential diagnosis is reflux esophagitis¹⁶.

Despite efforts to develop methods to evaluate EoE in a less invasive manner¹⁷, an endoscopic procedure with tissue biopsy is still the standard of care for diagnosing and monitoring EoE. Searching for a non- or minimally-invasive biomarker still eludes researchers and clinicians, and the finding of intravascular IgG4 staining in our study is intriguing as it may suggest serum IgG4 as a potential biomarker. This intravascular IgG4 finding may be supportive of a recent report demonstrating a significant difference in food-specific plasma IgG4, but not total IgG4, between EoE patients and controls¹⁸. The presence of epithelial IgG4 staining raises consideration for its potential utility in other minimally-invasive testing modalities, such as the string test¹⁷.

In summary, we have demonstrated that pediatric eosinophilic esophagitis patients have significantly elevated numbers of IgG4-positive plasma cells, with the subset of those patients having a food allergy demonstrating a more robust relationship with the number of IgG4-positive plasma cells.

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References

1. Kagalwalla AF, Shah A, Li BU, et al. Identification of Specific Foods Responsible for Inflammation in Children with Eosinophilic Esophagitis Successfully Treated with Empiric Elimination Diet. *J Pediatr Gastroenterol Nutr* 2011;53:145-149.
2. Kelly KJ, Lazenby AJ, Rowe PC, et al. Eosinophilic esophagitis attributed to gastroesophageal reflux: Improvement with an amino acid-based formula. *Gastroenterology* 1995;109:1503-1512.
3. Davis BP, Rothenberg ME. Mechanisms of disease of eosinophilic esophagitis. *Annu Rev Pathol* 2016;11:365-393.
4. Kottyan LC, Davis BP, Sherrill JD, et al. Genome-wide association analysis of eosinophilic esophagitis provides insight into the tissue specificity of this allergic disease. *Nat Genet* 2014;46:895-900.
5. Blanchard C, Stucke EM, Rodriguez-Jimenez B, et al. A striking local esophageal cytokine expression profile in eosinophilic esophagitis. *J Allergy Clin Immunol* 2011;127:208-217.
6. Deshpande V, Zen Y, Chan JK, et al. Consensus statement on the pathology of IgG4-related disease. *Mod Pathol* 2012;25:1181-1192.
7. Hamano H, Kawa S, Horiuchi A, et al. High serum IgG4 concentrations in patients with sclerosing pancreatitis. *N Engl J Med* 2001;344:732-738.
8. Zen Y, Fujii T, Harada K, et al. Th2 and regulatory immune reactions are increased in immunoglobulin G4-related sclerosing pancreatitis and cholangitis. *Hepatology* 2007;45:1538-1546.

9. Abe M, Hiasa Y, Orji M. T helper 17 cells in autoimmune liver diseases. Clin Dev Immunol 2013;2013:607073: Epub 2013 Oct 10.
10. Moriyama M, Tanaka A, Maehara T, et al. T helper subsets in Sjogren's syndrome and IgG4-related dacryoadenitis and sialoadenitis: A critical review. J Autoimmun 2014;51:81-88.
11. 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.
12. Clayton F, Fang JC, Lucendo AJ, et al. Reply: To PMID 24907494. Gastroenterology 2015;148:454.
13. Kagalwalla AF, Akhtar N, Woodruff SA, et al. Eosinophilic esophagitis: Epithelial mesenchymal transition contributes to esophageal remodelling and reverses with treatment. J Allergy Clin Immunol 2012;129:1387-1396.
14. Collins MH, Martin LJ, Alexander ES, et al. Newly developed and validated eosinophilic esophagitis histology scoring system and evidence that it outperforms peak eosinophil count for disease diagnosis and monitoring. Dis Esophagus 2017;30:1-8.
15. Vigier C, Henno S, Willot S, et al. [Eosinophilic esophagitis in children: Evaluation of practices through a multicenter study. Article in French]. Arch Pediatr 2017; 24: 327-335.
16. Zuckerberg L, Mahadevan K, Selig M, et al. Oesophageal intrasquamous IgG4 deposits: an adjunctive marker to distinguish eosinophilic oesophagitis from reflux oesophagitis. Histopathology 2016;68:968-976.
17. Furuta GT, Kagalwalla AF, Lee JJ, et al. The oesophageal string test: A novel, minimally invasive method measures mucosal inflammation in eosinophilic oesophagitis. Gut 2013;62:1395-1405.

18. Wright BL, Kulis M, Guo R, et al. Food-specific IgG4 is associated with eosinophilic esophagitis. *J Allergy Clin Immunol* 2016;138:1190-1192.e3.

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Table 1. Summary of clinical and endoscopic variables between the EoE and non-EoE patient groups.

	EoE (n = 41)	Non-EoE (n = 10)
Clinical Variables		
Sex	73% Male	70% Male
Age of Symptoms	5.9 years	4.4 years
Age of Diagnosis	6.2 years	5.3 years
Presence of Food Allergy	63%	20%*
Findings at Endoscopy		
Normal	2.3%	100%
Furrowing	94.6%	0%
White Plaques	37.8%	0%
Rings	10.8%	0%
Strictures	0%	0%

*Two patients without EoE were diagnosed with celiac disease subsequent to the biopsy included in this study.

Figure 1. Percent of patients with a positive serum IgE or skin-prick testing (total tested, N=38).

As expected, the IgE-mediated food triggers (any positive test, N=24) most commonly seen in our patients (peanut (N=13), egg (N=10), tree nuts (N=10), milk (N=9), sesame (N=6), shellfish (N=4)) are similar to those commonly eliminated in food diet therapy. *Food allergies with less than 10% prevalence: Fish, legumes, cashew, hazelnut, pecan, walnut, kiwi, mustard, avocado, apple, cherry, barley, soy, corn, chickpea, almond, rice, cod, and pistachio.

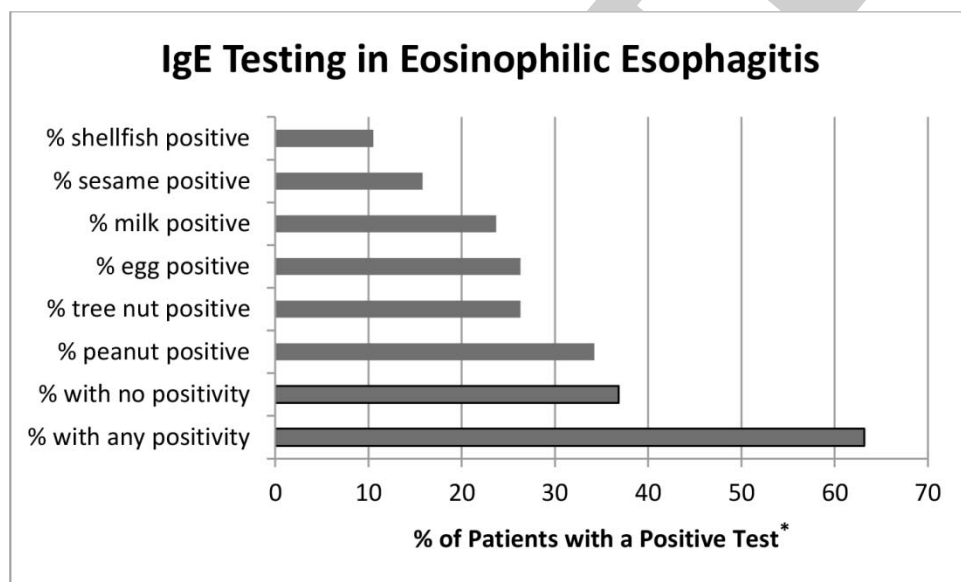


Figure 2. Photomicrographs showing peak eosinophilia with corresponding peak IgG4-PC in EoE. H&E image (A) of high peak eosinophilia and underlying plasma cells with a perivascular concentration (arrow) with corresponding high IgG4-PC count (B, arrow showing IgG4-PC rings). As a comparison, H&E image (C) of a high peak eosinophil count and low IgG4-PC count (D). Some cases showed a dark ribbon of endothelial staining with IgG4 (E, arrows mark vessels with dark endothelial staining). Non-EoE patients demonstrated a near complete absence of staining, including no significant interstitial staining (F). All images are 200x original magnification. EoE: Eosinophilic esophagitis. IgG4: Immunoglobulin G4. H&E: Hematoxylin and eosin. PC: Plasma cells.

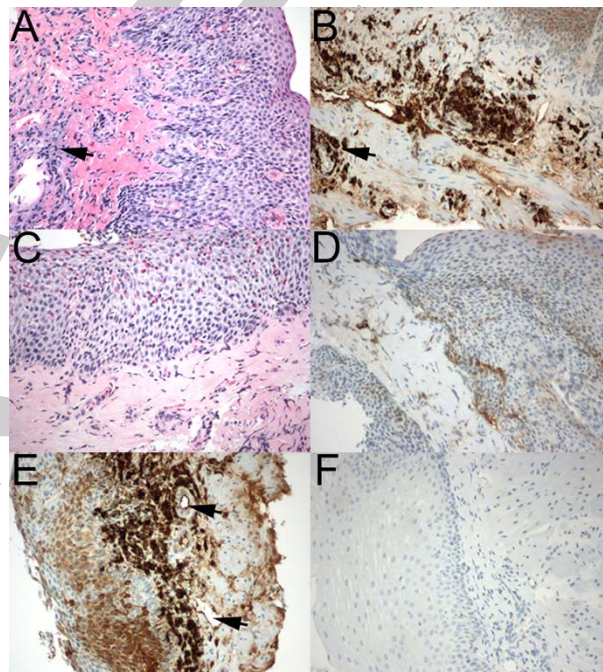


Figure 3. Box and whisker plots showing median, interquartile ranges, and minimum and maximum values. IgG4-PC density is significantly increased in EoE compared with normal controls (A) and in patients with active EoE that have a food allergy compared to those who do not (B). The density of IgG4-PC is significantly increased as the degree of non-cellular IgG4 staining in the lamina propria (C) and epithelium (D) increases. There was no significant difference in IgG4-PC density between grades 1 and 0 in the epithelium. The presence of dense intravascular endothelial staining was strongly associated with an increased IgG4-PC density (E). ns= $p > 0.05$, *= $p \leq 0.05$, ** = $p \leq 0.01$, *** $p \leq 0.001$. EoE: Eosinophilic esophagitis. IgG4: Immunoglobulin G4. PC: Plasma cells.

