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Introduction

Eosinophilic oesophagitis (EoE) is a chronic inflammatory condition thought to be caused by the ingestion of food antigens, with the subsequent development of an eosinophil rich inflammatory infiltrate, oesophageal narrowing, and clinical symptoms of dysphagia and food bolus impaction[1, 2]. Diagnosis requires endoscopy and biopsy demonstrating ≥15 eosinophils per HPF[2]. [3] Dietary therapy with complete avoidance of culprit food groups (e.g. dairy, wheat, eggs, or others) can result in clinical and histological remission, while food reintroduction can cause recurrence, providing strong evidence that EoE is food antigen driven[4, 5]. What has not been clearly established however is if food antigens act locally (in the oesophagus) to cause immune activation and the subsequent inflammatory infiltrate, or if they instead are processed elsewhere (e.g. the small bowel), with secondary recruitment and trafficking of eosinophils to the oesophagus [6, 7].

The role of the oesophagus as an immune organ has not been clear. With its short length, squamous mucosa with a tight epithelial barrier, and rapid transit time for food and liquid boluses (< 10 seconds),[8, 9] the oesophagus could appear to lack the characteristic physiological properties that facilitate digestion and interaction between the gastrointestinal immune compartment and food antigens, as is found in the small intestine[9]. Prior attempts characterising the inflammatory infiltrate in EoE have uniformly demonstrated increased numbers of CD3 lymphocytes, in addition to large numbers of eosinophils and mast cells[10-14]. That IgG, but not IgE deposition is florid in the oesophageal tissue of patients with active EoE, has led to the concept of EoE being a unique Th2 mediated condition, distinct from classical food allergy[15]. Additionally, the

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role of langerhans cells (a type of dendritic cell thought to be a major antigen presenting cell the gastrointestinal tract) is debated, and recent in- vivo studies involving oesophageal food antigen exposure have not been conclusive[10, 15-17]. Therefore, there is no 'smoking gun' that has provided unequivocal evidence of localised immune activation in the oesophagus.

We hypothesised that antigen presentation occurs locally in the oesophagus in patients with EoE, that langerhans cells present that antigen, and we therefore aimed to perform a comparative study of the density of cell surface proteins (specifically those known to be implicated in antigen presentation, and/or early immune activation in the gastrointestinal tract, or disordered in related conditions such as coeliac disease or crohn's disease – see methods and discussion) in oesophageal tissue prior to and after food challenge induced disease recurrence. A secondary aim was to consider if stains for the identified inflammatory cells could be used as novel tissue biomarkers to differentiate active EoE, inactive EoE, gastroesophageal reflux disease (GORD) and healthy controls, thus suggesting utility for diagnosis and disease monitoring.

Methods

Study design and research subjects

We analysed samples from patients seen at University of North Carolina between July 2011, and December 2016, with an established diagnosis of EoE, who participated in a prospective cohort study of EoE diagnosis and treatment, as described previously[18, 19]. To be eligible for the present study, patients had to be adult (age 18-80) with an established diagnosis of EoE per consensus guidelines at the time of the design of the parent study, who attained histological remission (<15 eosinophils per high power field [eos/HPF]) using elimination diet, and who underwent food reintroduction and had histologically proven disease recurrence (≥ 15 eos/HPF) after at least 6 weeks of daily ingestion of a putative food trigger[2, 20-23]. Excluded were patients previously determined to have proton pump inhibitor responsive EoE (PPI-REE), patients who did not respond to initial treatment with dietary elimination, who had GI bleeding, active anticoagulation, known oesophageal cancer, prior oesophageal surgery, known oesophageal varices or medical instability or comorbidities precluding enrollment. Also excluded were patients taking systemic or swallowed topical corticosteroids, or immunosuppressant medications (e.g. azathioprine, methotrexate, or biological therapies) for 6 months prior to or during the period of dietary elimination and food reintroduction. Subjects with EoE were compared to patients with gastroesophageal reflux disease (GORD - defined clinically as per Montreal guidelines and with 24-hour pH studies, and after endoscopy excluded other causes, including EoE), and controls with upper

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gastrointestinal symptoms but a normal oesophagus both endoscopically and histologically. This study was approved by the UNC Institutional Review Board.

Histologic assessment and characterization of the immunophenotype

Oesophageal sampling was carried out using standard large capacity forceps as per protocol, with biopsies taken from the proximal (approximately 15 cm above the gastroesophageal junction [GEJ] and distal (3 cm above the GEJ) oesophagus. Oesophageal biopsies were fixed in formalin and paraffin embedded and stained with haematoxylin and eosin. Oesophageal eosinophil counts were quantified by expert histopathologists using our previously established methods to determine the overall peak eosinophil count (eos/hpf area = 0.24mm^2).[24, 25] Additional processing of samples for immunohistochemistry (IHC) involved retrieval of archived paraffin embedded tissue blocks, slide mounting, and routine deparaffinisation using the Bond Rx fully automated research stainer (Leica Biosystems Ince, Norwell, MA). Specifically, slides were dewaxed in Bond™ Dewax solution (AR9222) and hydrated in Bond Wash solution (AR9590). Epitope retrieval for all targets were done for 30 min or 20min at 100 C in Bond-epitope retrieval solution 1 pH6.0 (AR9661) or solution 2 pH9.0 (AR9640) respectively. The epitope retrieval was followed with 5 min endogenous peroxidase blocking using Bond peroxide blocking solution (DS9800).

Biomarkers were chosen to consider antigen presentation, via Langerhans cells specifically (CD1a) or by antigen presenting cells more generally (CD40, CD80, HLA-DR), early immune activation of T cells (CD28 and CD69) and the lymphocyte marker CD3, based on previous research that indicated potential significance[10-14]. In addition, two potential markers of intrinsic epithelial immune function were selected, based on prior studies in inflammatory bowel disease and coeliac disease. Thus, CXCR3 (a chemokine receptor, that may bind gluten in intestinal epithelial cells in patients with coeliac disease) and CD138 (a heparin sulfate proteoglycan cell surface receptor, which may be increased in coeliac disease, and demonstrates variable expression related to disease activity in Crohn's disease and ulcerative colitis, and is also used as a marker for plasma cells) were used[26, 27]. Specifically, to consider antigen presentation, incubation with to CD1a (Mouse,1:100, Leica Biosystems, PA0235), HLA-DR (Mouse, 1:4000, Santa Cruz Biotechnology, 307602), CD40 (Mouse, 1:1000, Atlas Antibodies, AMAb90905) and CD80 (Rabbit, 1:400, Abcam,Ab134120) was performed. To consider early immune activation of T cells, primary murine anti-human antibodies to CD28 (Mouse, 1:25, Novus Biologicals, NP2-32817) and CD69 (Mouse, 1:25, Fisher/Invitrogen, 14-0699-82) antibodies were used, and the general lymphocyte marker primary rabbit anti-human antibodies to CD3 (1:2000 Cell Marque,

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PA0553) was also used. Markers of epithelial immune function were antihuman murine antibodies to CD138 (1:50, Cell Marque,138M-15) and CXCR3 (1:2000, Abcam). To cross validate the eosinophil count from the haematoxylin and eosin (H and E) preparation, eosinophil basic protein (EBP, Mouse, 1:50, AbD Serotec) All primary antibodies were incubated for 60 minutes, except for HLA-DR (30 minutes). HLA-DR immunohistochemistry used the Bond Polyer Refine Detection kit, using a polymer-based horseradish peroxidase (HRP)-linked secondary antibody (10 min) followed with 15 min TSA (Tyramide Signal amplification). The secondary antibody was followed with 3,3-Diaminobenzidine (DAB), and then hematoxylin counterstain. Dual staining of slides for CD1a/CXCR3, CD69/CD3, CD40/CD138 and CD28/CD80 were counterstained with Hoechst 33258 (# H3569) and mounted with ProLong® Diamond Antifade Mountant (#P36961) Life Technologies (Carlsbad, CA). Finally, positive and negative controls (no primary antibody) and single stain controls were done when one primary antibody was omitted to confirm that cross reactivity between the antibodies did not occur.

For assessment of completed IHC/IF slides, scanning and conversion to digital photomicrographs using a Leica Biosystems platform, viewing with Aperio ImageScope, and semi- automated analysis via Definiens architect platform and tissue Studio FL software for IF, or Aperio Positive Pixel Count algorithm (version 9.1, Aperio Technologies) for IHC, were performed in order to quantify staining intensity (pixels/mm^2 and cells/mm^2). Staining of individual biomarkers defined as 'intense' according to the analysis were then compared among patients with EoE (following dietary manipulation) and to controls.

Statistical Analysis

Summary statistics were used to characterise cases and controls, and categorical variables were compared using chi square. Mean and standard deviations (SD) were calculated for individual biomarkers and a two-sample t-test was used to compare continuous variables between patients with eosinophilic esophagitis (before and after diet induced disease recurrence) and controls. Only pairwise comparisons were made. These initial comparisons served to characterise the inflammatory milieu in cases vs controls and assess for antigen presenting cells. Then, receiver operating characteristics (ROC) curves were constructed and the area under the ROC curve (AUC) was calculated to determine diagnostic utility of the different stains. The sample size was governed by availability of subjects clearly responding to food elimination, and then relapsing with food reintroduction.

Results

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Characteristics of the study subjects and dietary therapy

In this analysis, we included 14 cases of EoE who achieved histologic remission after dietary elimination and subsequently had recurrence of EoE following food reintroduction, 6 GORD controls, and 5 healthy controls. Demographic features and eosinophil count pre and post dietary induced disease recurrence are shown in Table 1. Compared to patients with EoE (mean age 42), those with GORD were older and less often atopic (mean age 58 years, atopy in 17 %) whilst the healthy controls were of similar age (mean age 38 years), but again atopy was less frequent. The duration of food reintroduction prior to endoscopy that demonstrated disease recurrence varied (range 6 to 10 weeks, median 6 weeks and 5 days), with a minimum of 6 weeks according to our clinical practice.

Tissue biomarkers of immune activation/antigen presentation

Among patients with EoE, a significant increase in HLA-DR biomarker density and CD40 was observed following food reintroduction, and there was a trend toward increased CD80, and a decrease in CD1a (Figure 1 and Table 2). Compared to those with GORD, HLA-DR was significantly increased in EoE cases both in remission and following food reintroduction (whilst CD40, CD80 and CD1a were not significantly increased (Tables 2). Finally, compared to healthy controls, HLA-DR was significantly increased in EoE cases both in and following food reintroduction, p=0.047 and p=0.001 respectively. CD40, CD80 and CD1a were not significantly increased (Table 2)

Tissue biomarkers of T cell activation

Among patients with EoE, a significant increase in CD3 and CD28, was evident following food reintroduction (Figure 1 and Table 2). Similarly, there was a trend toward an increase in CXCR3, but a trend toward a decrease in CD69 after food reintroduction (see Table 2). Compared to patients with GORD, patients with EoE had significantly increased expression of CXCR3 both when in remission and after food reintroduction (see Table 2). Following food reintroduction, CD3 was significantly increased in patients with EoE compared to GORD and CD28 was similarly increased. Finally, compared to healthy controls, patients with EoE in remission had significantly decreased expression of CD3 and CD69 and following food reintroduction CD3, CD28 and CD69 were significantly increased (Table 2).

CD 138

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In EoE cases, there was a significant increase in CD138 following food reintroduction (Table 1). Compared to those with GORD, CD138 was indistinguishable when EoE was in remission, but expression was significantly elevated post food reintroduction Table 2). Finally, compared to healthy controls, CD138 was indistinguishable when EoE was in remission but significantly elevated following food reintroduction (Table 2). Given these prominent differences between CD138 in EoE cases and controls, we also assessed the utility of this biomarker to distinguish active EoE from both GORD and healthy controls. In this analysis, the AUC was 0.836 (Figure 2).

Discussion

Oesophageal epithelial cells may have inherent immune function that is only beginning to be appreciated in EoE. In this study, analysing samples from EoE patients and controls that were previously prospectively obtained and banked, we aimed to assess esophageal antigen presentation and immune activation as well as to potentially identify novel biomarkers of disease actively. We have several notable findings. Firstly, antigen presentation was considered following food reintroduction in patients with EoE, with the intent of determining if local (esophageal) antigen/immune compartment contact was responsible for disease recurrence. The expression of HLA–DR (a cell surface receptor typically thought to bind peptides and present these to T -cell receptors) was significantly increased in patients with EoE following food exposure. This is in keeping with previous studies that have demonstrated increased expression in active compared to treated disease (albeit in smaller cohorts of patients treated heterogeneously e.g. with corticosteroids or proton pump inhibitors)[12, 13]. Even in remission, patients with EoE had significantly greater expression of HLA-DR than healthy controls or those with GORD. This may indicate that patients with EoE are predisposed to present relevant food antigens, as previously suggested[12, 14, 28]. Similarly, CD40 (which is typically expressed on antigen presenting cells and binds to CD154 on T cells) was significantly increased following food exposure. In contrast, Langerhans cells, which are typically considered to be APC's and are defined by immunostaining to CD1a, did not increase following food-induced disease recurrence, and expression was no greater in EoE compared to controls. Previous research has been contradictory in relation to CD1a expression in EoE. Lucendo et al demonstrated no difference in EoE compared to control, whilst Teitelbaum et al showed a decrease with topical corticosteroid treatment[10, 11]. Finally, CD80 (typically expressed on antigen presenting cells) was not significantly increased following food antigen exposure in patients with EoE and was paradoxically decreased compared to healthy controls (but not those with GORD). Confusingly, the

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corresponding receptor for the CD80 ligand on T cells is CD28, which did significantly increase following food induced disease recurrence. This strongly suggests that antigen presentation to T cells has occurred (albeit it cannot be specified if this occurred within the esophagus), and arguably also indicates that an alternative co-stimulatory receptor may be implicated (for example CD86). Therefore, a reasonable conclusion to draw is that antigen presentation does not depend on langerhans cells , and may occur either via typical antigen presentation cells (e.g. CD40 to CD154 on T cells) or via atypical antigen presentation cells, specifically by eosinophils or epithelial cells[13, 29, 30]. Previously, Mulder et al similarly demonstrated an absence of CD80 expression in patients with EoE, but increased expression of CD86, whilst Le Carlson et al found increased CD80 (at odds to our data)[13, 14].

We also demonstrated that the cell surface heparin sulfate proteoglycan CD138 (syndecan-1) was similarly low in EoE patients in remission after dietary elimination, GORD patients, and healthy controls, but increased significantly in EoE following food reintroduction and resultant disease recurrence. This is a novel finding, suggesting that this biomarker has promise in tracking disease activity. When considering the test characteristics of CD138, the ROC analysis shows very good diagnostic utility (AUC=0.84) and supports further study of this biomarker for diagnosis and monitoring of treatment of EoE. However, our current small sample size precludes generating definite cut-points for diagnosis. Moreover, given serum syndecan-1 has been found to be increased in patients with Crohn's disease, a logical future extension of our research is to assess the serum of EoE patients before and after dietary induced disease recurrence[27]. The observation that syndecan-1 expression is the same in healthy controls, those with GORD, and patients with quiescent EoE, may suggest that this cytostructural protein is induced as a consequence of the inflammatory milieu, as opposed to a cause, although this hypothesis should be more rigorously tested, ideally with upstream assays of candidate genes or protein expression. Whilst expression of epithelial CD138 is increased in Crohn's disease and in lupus nephritis, variable findings have been reported in coeliac disease, and asthma[21, 26]. Murine models of asthma suggest an inherent deficiency of CD138 leads to unchecked lymphocyte recruitment[31]. Further research is required to explain the significance of this cell surface protein in EoE. It is acknowledged that CD138 is also expressed by, and even used as a marker for plasma cells (of immediate relevance to EoE, in accordance with the prolific IgG deposition in oesophageal tissue), but that in this case the expression of this biomarker was diffuse and intense on oesophageal epithelial cells, in common with other gastrointestinal conditions such as Crohn's disease[32].

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A T cell rich inflammatory infiltrate with an increase in CD3 and CD28, was unequivocally demonstrated in our study, with a significant increase in these biomarkers with food reintroduction, which is in keeping with previous research [14, 28, 33]. Notably, CD69 (thought to indicate very early T cell activation, for example within days) was not increased, at odds with findings elsewhere[14]. CXCR3 expression did not increase measurably with food reintroduction and was unchanged compared to healthy controls. This is at odds to the hypothesis that that receptor directly binds gluten or other antigens (at least in the esophagus)[34]. Previous consideration of CXCR3 on eosinophils showed variable expression in response to IL-5, but tissue expression has not to our knowledge been considered in *in-vivo* studies[35]. Interestingly, those with GORD had decreased expression of CXCR3 (compared to either EoE or healthy control). This could indicate a unique immunophenotype applies to GORD (which has been considered an 'immune disorder' in its own right) and warrants more rigorous enquiry with a larger patient population[36].

Several limitations in methodology are acknowledged. First, in respect to the primary objective of demonstrating antigen presentation and inflammation following food reintroduction, we were not able to directly assess food antigens themselves. Visualisation of food antigens either by utilising a specific antibody to one or more food proteins (e.g. casein or gluten) was considered but not performed due to a lack of suitable assays, the diversity of food antigens that induced recurrence and would be required, and because electron microscopy was not feasible in this study. Future research could address these shortcomings. Second, automated co-localisation of biomarkers (to then enable analysis and commentary of cellular function) could not be performed, and the semi-quantitative methodology may introduce additional error. Third, the study groups were small, so some of the negative findings could be due to type II error, and the positive findings need to be confirmed in a large set of subjects. That CD40, for example was not significantly elevated when EoE was active (compared to controls) may be indicative of a small sample size (see Table 2). The small bowel is often considered a major site of antigen presentation in the gastrointestinal tract, yet we did not obtain duodenal biopsies in this study. This represents a conscious decision based on previous findings elsewhere demonstrating benign duodenal biopsies in patients with EoE, and a pragmatic decision based on oesophageal biopsies that were taken to guide dietary management[4, 37]. Another limitation was that pre dietary treatment biopsies were not analysed, preventing analysis of three (instead of two) specimens on the same patient, and limiting assessment of the degree of treatment effect. However, this concern is lessened because these patients achieved complete remission defined by the eosinophil count. Finally, discerning pathogenic cause and effect via immunohistochemistry is difficult, and future studies should

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consider additional methodologies including candidate gene and protein expression assays. However, the strengths of the study include comprehensive immunophenotyping of well characterized cases and controls from whom samples were previously prospectively collected, rigorous data collection and analysis, and a set of EoE patients in both remission and with active disease after food reintroduction.

In conclusion, antigen presentation does not significantly involve langerhans cells in patients with EoE. Future study would ideally involve visualisation of food antigens following endocytosis and cell surface presentation, perhaps utilising food antigen specific antibodies and electron microscopy. The cell surface receptor heparin sulfate proteoglycan CD138 increases in concert with eosinophilic infiltration in patients with EoE following food induced recurrence and appears to be a promising biomarker of EoE disease activity.

References

1. Philpott H, Nandurkar S, Thien F, Gibson PR, Royce SG. Eosinophilic esophagitis: a clinicopathological review. *Pharmacol Ther* 2015;**146**:12-22.
2. Dellon ES, Gonsalves N, Hirano I, et al. ACG clinical guideline: Evidenced based approach to the diagnosis and management of esophageal eosinophilia and eosinophilic esophagitis (EoE). *Am J Gastroenterol* 2013;**108**(5):679-92; quiz 693.
3. Dellon ES, Liacouras CA, Molina-Infante J, et al. Updated International Consensus Diagnostic Criteria for Eosinophilic Esophagitis: Proceedings of the AGREE Conference. *Gastroenterology* 2018;**155**(4):1022-1033 e10.
4. Philpott H, Nandurkar S, Royce SG, Thien F, Gibson PR. A prospective open clinical trial of a proton pump inhibitor, elimination diet and/or budesonide for eosinophilic oesophagitis. *Aliment Pharmacol Ther* 2016;**43**(9):985-93.
5. Lucendo AJ, Arias A, Gonzalez-Cervera J, et al. Empiric 6-food elimination diet induced and maintained prolonged remission in patients with adult eosinophilic esophagitis: a prospective study on the food cause of the disease. *J Allergy Clin Immunol* 2013;**131**(3):797-804.
6. Katzka DA. Eosinophilic Esophagitis: Leaky Gullet or Leaky Gut? *Am J Gastroenterol* 2017;**112**(7):1072-1073.
7. Philpott H, Dellon ES. A Penetrating Look at Eosinophilic Esophagitis Pathogenesis: Direct Antigen Exposure in the Esophagus? *Gastroenterology* 2017;**153**(2):605-606.

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8. O'Shea KM, Aceves SS, Dellon ES, et al. Pathophysiology of Eosinophilic Esophagitis. *Gastroenterology* 2018;**154**(2):333-345.

9. Gelberg HB. Comparative anatomy, physiology, and mechanisms of disease production of the esophagus, stomach, and small intestine. *Toxicol Pathol* 2014;**42**(1):54-66.

10. Teitelbaum JE, Fox VL, Twarog FJ, et al. Eosinophilic esophagitis in children: immunopathological analysis and response to fluticasone propionate. *Gastroenterology* 2002;**122**(5):1216-25.

11. Lucendo AJ, Navarro M, Comas C, et al. Immunophenotypic characterization and quantification of the epithelial inflammatory infiltrate in eosinophilic esophagitis through stereology: an analysis of the cellular mechanisms of the disease and the immunologic capacity of the esophagus. *Am J Surg Pathol* 2007;**31**(4):598-606.

12. Patel AJ, Fuentebella J, Gernez Y, et al. Increased HLA-DR expression on tissue eosinophils in eosinophilic esophagitis. *J Pediatr Gastroenterol Nutr* 2010;**51**(3):290-4.

13. Mulder DJ, Pooni A, Mak N, Hurlbut DJ, Basta S, Justinich CJ. Antigen presentation and MHC class II expression by human esophageal epithelial cells: role in eosinophilic esophagitis. *Am J Pathol* 2011;**178**(2):744-53.

14. Le-Carlson M, Seki S, Abarbanel D, Quiros A, Cox K, Nadeau KC. Markers of antigen presentation and activation on eosinophils and T cells in the esophageal tissue of patients with eosinophilic esophagitis. *J Pediatr Gastroenterol Nutr* 2013;**56**(3):257-62.

15. Davis BP, Rothenberg ME. Mechanisms of Disease of Eosinophilic Esophagitis. *Annu Rev Pathol* 2016;**11**:365-93.

16. Marietta EV, Geno DM, Smyrk TC, et al. Presence of intraepithelial food antigen in patients with active eosinophilic oesophagitis. *Aliment Pharmacol Ther* 2017;**45**(3):427-433.

17. Warners MJ, Terreehorst I, van den Wijngaard RM, et al. Abnormal Responses to Local Esophageal Food Allergen Injections in Adult Patients With Eosinophilic Esophagitis. *Gastroenterology* 2018;**154**(1):57-60 e2.

18. Dellon ES, Speck O, Woodward K, et al. Markers of eosinophilic inflammation for diagnosis of eosinophilic esophagitis and proton pump inhibitor-responsive esophageal eosinophilia: a prospective study. *Clin Gastroenterol Hepatol* 2014;**12**(12):2015-22.

19. Dellon ES, Rusin S, Gebhart JH, et al. Utility of a Noninvasive Serum Biomarker Panel for Diagnosis and Monitoring of Eosinophilic Esophagitis: A Prospective Study. *Am J Gastroenterol* 2015;**110**(6):821-7.

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20. Liacouras CA, Furuta GT, Hirano I, et al. Eosinophilic esophagitis: updated consensus recommendations for children and adults. *J Allergy Clin Immunol* 2011;**128**(1):3-20 e6; quiz 21-2.

21. Kim KJ, Kim JY, Baek IW, Kim WU, Cho CS. Elevated serum levels of syndecan-1 are associated with renal involvement in patients with systemic lupus erythematosus. *J Rheumatol* 2015;**42**(2):202-9.

22. Reed CC, Wolf WA, Cotton CC, et al. Optimal Histologic Cutpoints for Treatment Response in Patients With Eosinophilic Esophagitis: Analysis of Data From a Prospective Cohort Study. *Clin Gastroenterol Hepatol* 2018;**16**(2):226-233 e2.

23. Wolf WA, Cotton CC, Green DJ, et al. Evaluation of Histologic Cutpoints for Treatment Response in Eosinophilic Esophagitis. *J Gastroenterol Hepatol Res* 2015;**4**(10):1780-1787.

24. Rusin S, Covey S, Perjar I, et al. Determination of esophageal eosinophil counts and other histologic features of eosinophilic esophagitis by pathology trainees is highly accurate. *Hum Pathol* 2017;**62**:50-55.

25. Dellon ES, Farrell TM, Bozymski EM, Shaheen NJ. Diagnosis of eosinophilic esophagitis after fundoplication for 'refractory reflux': implications for preoperative evaluation. *Dis Esophagus* 2010;**23**(3):191-5.

26. Salvestrini C, Lucas M, Lionetti P, et al. Matrix expansion and syncytial aggregation of syndecan-1+ cells underpin villous atrophy in coeliac disease. *PLoS One* 2014;**9**(9):e106005.

27. Cekic C, Kirci A, Vatansever S, et al. Serum Syndecan-1 Levels and Its Relationship to Disease Activity in Patients with Crohn's Disease. *Gastroenterol Res Pract* 2015;**2015**:850351.

28. Davis BP, Rothenberg ME. Antigen presentation by eosinophils in eosinophilic esophagitis? *J Pediatr Gastroenterol Nutr* 2013;**56**(3):242.

29. Arebro J, Tengroth L, Razavi R, Kumlien Georen S, Winqvist O, Cardell LO. Antigen-presenting epithelial cells can play a pivotal role in airway allergy. *J Allergy Clin Immunol* 2016;**137**(3):957-60 e7.

30. Cianferoni A, Ruffner MA, Guzek R, et al. Elevated expression of activated TH2 cells and milk-specific TH2 cells in milk-induced eosinophilic esophagitis. *Ann Allergy Asthma Immunol* 2018;**120**(2):177-183 e2.

31. Fal AM, Kraus-Filarska M, Miecielica J, Malolepszy J. [Mechanisms of action of nebulized low molecular weights heparin in patients with bronchial asthma]. *Pol Merkur Lekarski* 2003;**15**(90):543-5.

32. Simon D, Cianferoni A, Spergel JM, et al. Eosinophilic esophagitis is characterized by a non-IgE-mediated food hypersensitivity. *Allergy* 2016;**71**(5):611-20.

33. Fuentebella J, Patel A, Nguyen T, et al. Increased number of regulatory T cells in children with eosinophilic esophagitis. *J Pediatr Gastroenterol Nutr* 2010;**51**(3):283-9.

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34. Lammers KM, Lu R, Brownley J, et al. Gliadin induces an increase in intestinal permeability and zonulin release by binding to the chemokine receptor CXCR3. *Gastroenterology* 2008;**135**(1):194-204 e3.

35. Fulkerson PC, Schollaert KL, Bouffi C, Rothenberg ME. IL-5 triggers a cooperative cytokine network that promotes eosinophil precursor maturation. *J Immunol* 2014;**193**(8):4043-52.

36. Dunbar KB, Agoston AT, Odze RD, et al. Association of Acute Gastroesophageal Reflux Disease With Esophageal Histologic Changes. *JAMA* 2016;**315**(19):2104-12.

37. Warners MJ, Vlieg-Boerstra BJ, Verheij J, et al. Esophageal and Small Intestinal Mucosal Integrity in Eosinophilic Esophagitis and Response to an Elemental Diet. *Am J Gastroenterol* 2017;**112**(7):1061-1071.

Key

Tables

1. Characteristics of the study population

2. Table 2: Immunohistochemical biomarkers from oesophageal tissue in patients with EoE and controls

Figures

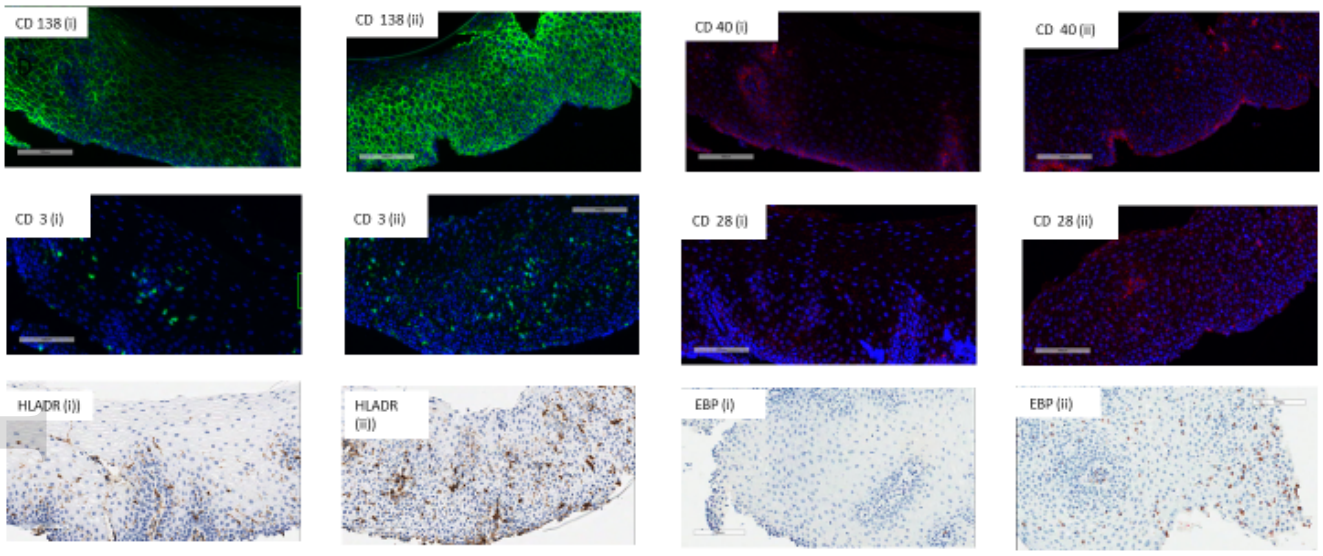
1. Photomicrographs of biomarkers from oesophageal tissue, that demonstrated a significant increase following food reintroduction. (i) remission, (ii) post food trigger induced recurrence

2.Area under the curve (AUC) analysis for CD138 for patients with EoE (post food induced recurrence) compared to those with GORD or healthy control

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Figure 1. Photomicrographs of biomarkers from oesophageal tissue, that demonstrated a significant increase following food reintroduction. (i) remission, (ii) post food trigger induced recurrence



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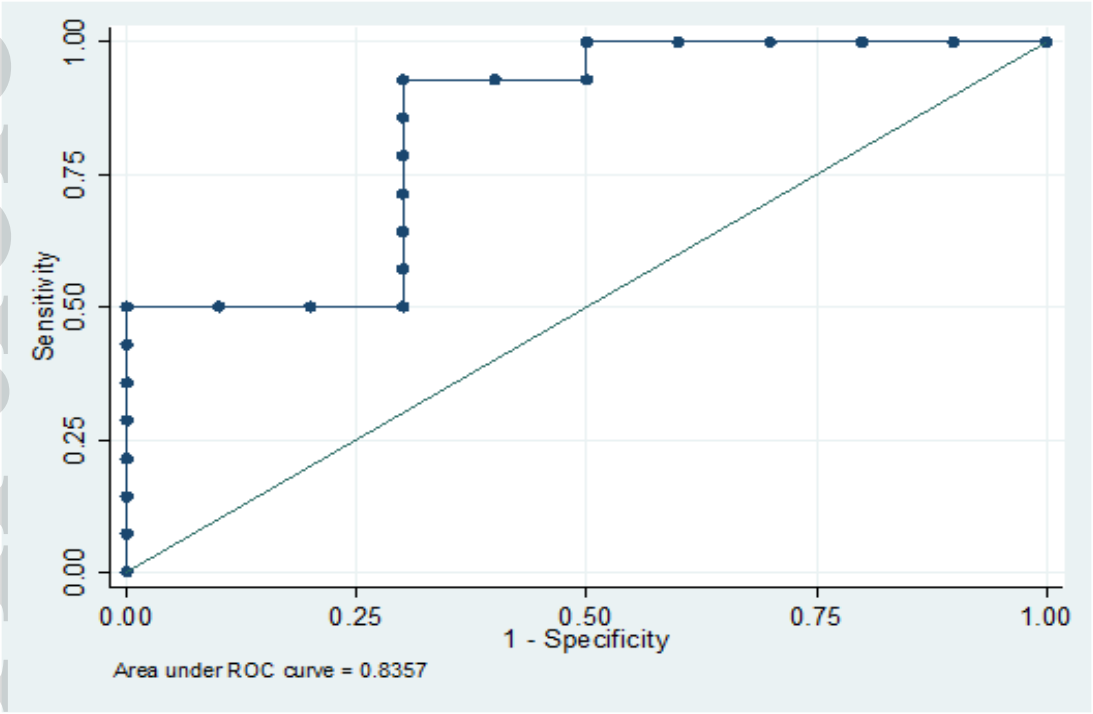


Figure 2: Area under ROC curve for CD138 in patients with active EoE vs GORD or control

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Table 1: Characteristics of the study population

	EoE cases (n = 14)	GORD controls (n = 6)	Normal controls (n = 5)
Age (mean years ± SD)	35.9 ± 14.4	57.3 ± 7.9	38.4 ± 19.8
male (n, %)	12 (86)	4 (67)	4 (80)
White (n, %)	13 (93)	5 (83)	4 (80)
Atopic disease (n, %)			
Asthma	1 (7)	1 (17)	0 (0)
Allergic rhinitis/environmental allergies	7 (50)	1 (17)	0 (0)
Food allergies	4 (29)	0 (0)	1 (20)
Baseline symptoms (n, %)			
Dysphagia	13 (93)	5 (83)	0 (0)
Food impaction requiring endoscopy	1 (7)	0 (0)	0 (0)
Heartburn	3 (21)	6 (100)	0 (0)
Chest pain	2 (14)	1 (17)	2 (40)
Abdominal pain	3 (21)	1 (17)	5 (100)
Type of dietary elimination			
SFED	5 (36)	--	--
Targeted elimination	4 (29)	--	--
SFED + targeted	3 (21)	--	--
4FED	1 (7)	--	--
2FED	1 (7)	--	--
Total EREFS scores (mean ± SD)			
Quiescent endoscopy	1.0 ± 0.9	--	--

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Flare endoscopy	2.2 ± 1.9	--	--
Peak eosinophil counts (mean eos/hpf ± SD)		0.2 ± 0.4	0 ± 0
Quiescent endoscopy	1.6 ± 2.0		
Flare endoscopy	62.6 ± 38.1		

Abbreviations: SFED = six food elimination diet (dairy, wheat, egg, soy, nuts, seafood); 4FED = four food elimination diet (dairy, wheat, egg, soy); 2FED = two food elimination diet (dairy, wheat); 'Targeted' diet refers to the use of allergy tests such as IgE or IgG or cytokine release assays to guide treatment; SD = standard deviation; EREFS = EoE endoscopy reference score, of exudates, rings, edema, furrows, and stricture; eos/hpf = eosinophils per high-power field

Table 2: Immunohistochemical biomarkers from oesophageal tissue in patients with EoE and control

Biomarker (density $mm^{2)/(SD)}$	EoE remission (r)	EoE post food introduction (f)	P value [EoE (r) vs EoE (f)]	Healthy Control (HC)	P value [EoE (f) vs HC]	GORD	P value [EoE (f) vs GORD]
CD1a	203 (140)	128 (117)	.1361	110 (9)	0.497	30 (17)	0.055
			NS		NS		NS

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CXCR3	7060 (2145)	7643 (2536)	P=0.465	7429 (715)	0.719	3728 (1987)	0.002
			NS		NS		
CD3	176 (123)	1012 (501)	P=.0021	349 (305)	0.001	249 (145)	0.001
CD28	5225 (1168)	8056 (1980)	P=.008	6138 (2021)	0.049	5520 (2930)	0.021
CD40	3228 (1304)	5725(3026)	P =.008	3227(1738)	0.068	3872(1543)	0.165
							NS
CD69	921 (680)	643 (1314)	P=.488	2395 (1613)	0.012	1487 (1013)	0.162
			NS				NS
CD80	3037 (1680)	3630 (1679)	P=0.218	5994 (2119)	0.009	5287 (3428)	0.038
			NS				
CD138	5794 (1219)	9036 (1997)	P=.001	5842 (1520)	0.001	5842 (2028)	0.002
HLA-DR	1122 (542)	2766 (1074)	P=.038	552 (408)	0.001	640 (521)	0.001
EBP	1028 (1716)	10458 (2690)	P=.008	346 (221)	0.001	365 (270)	0.001
Eosinophil/HPF$^{(range)}$	3 (0-10)	41 (15-120)		0		0	

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Table A. Correlations between percent change in peak eosinophil count and change in biomarkers, from active EoE to inactive EoE

Biomarker	Median percent change from active to inactive (IQR)	Spearman's R*	p value*
Peak eosinophil count	-90 (-100 – -88)	--	--
CD1	96 (-2 – 297)	0.34	0.25
CXCR3	-18 (-40 – 39)	0.46	0.11
CD28	-42 (-51 – -31)	-0.08	0.81
CD80	-32 (-63 – 142)	0.63	0.02
CD69	472 (-47 – 1488)	0.11	0.73
CD3	-81 (-95 – -75)	0.12	0.70
CD40	-46 (-63 – 5)	0.26	0.40
CD138	-35 (-44 – -24)	0.15	0.63
HLADR	-67 (-75 – -55)	0.13	0.67
EMBP	-99 (-100 – -98)	0.67	0.01

*for correlation between change in peak eosinophil count from active EoE to inactive EoE, and change in biomarker of interest from active EoE to inactive EoE

Table B. Correlations between inactive eosinophil counts and biomarkers, and correlations between active eosinophil counts and biomarkers

	Correlations for inactive eosinophil counts		Correlations for active eosinophil counts	
Biomarker	Spearman's R	p value	Spearman's R	p value
CD1	-0.06	0.86	-0.46	0.11
CXCR3	0.52	0.07	-0.09	0.77
CD28	-0.06	0.85	-0.31	0.30
CD80	0.05	0.86	-0.32	0.29
CD69	0.04	0.90	-0.11	0.73
CD3	-0.02	0.70	-0.06	0.84
CD40	0.68	0.01	0.09	0.77
CD138	0.07	0.81	-0.03	0.93
HLADR	0.20	0.52	-0.16	0.59
EMBP	0.36	0.23	0.27	0.40