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Eosinophils in the oesohageal mucosa: Clinical, pathological and epidemiological relevance in children.

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ABSTRACT

Objectives: Eosinophilic oesophagitis (EO) shows eosinophilic infiltration of the mucosa and can present with symptoms indistinguishable from gastro-oesophageal reflux disease (GORD). We describe the clinical, endoscopic and histopathological features of all cases of histological EO presenting during 2007- 2008 with a 2 year follow-up. The incidence of paediatric EO and the features of a subgroup with features of both GORD and EO ("overlap" syndrome-OS) are described.

Design: Biopsies with ≥ 15 eosinophils/HPF were reviewed. Other histological features sought included: microabscesses, dilated intercellular spaces, basal cell hyperplasia; papillary elongation, etc. OS was defined as the co-existence of clinical and histological features of EO and GORD (abnormal pH study) which improved with PPI.

Setting: Tertiary care.

Participants: all cases with ≥ 15 eosinophils/HPF entered the study

Results: 24 cases of EO were identified, 13 males and 11 females. The incidence of paediatric oesophageal eosinophilia in our region was 9 per 100 000 children. 11/24 patients (46%) presented with some form of allergy, 6 with poor feeding/food aversion, 5 with dysphagia and 4 with vomiting. After follow- up, 56.5% were confirmed to have EO; 30.5% responded to treatment for GORD and were categorised as OS, 9% developed eosinophilic gastroenteritis and 4% did not have further upper gastrointestinal symptoms.

Conclusions: Accurate diagnosis of EO, especially the differentiation from GORD, requires appropriate clinico-pathological correlation. A significant proportion of patients with eosinophilia in the mucosa also have GORD ("OS"). These patients improve after treating the underlying GORD.

The study was registered as a Service Evaluation with the Trust (number SE74).

Article summary

Article focus:

1. To estimate the incidence of oesophageal eosinophilia in the paediatric population of our region
2. To describe the clinical and endoscopic appearances at presentation.
3. To appraise the natural history after 2 -4 years follow up and to recognize the clinical features of those cases that showed an overlap with gastro oesophageal reflux

Key messages:

1. During follow-up 56.5% cases had eosinophilic oesophagitis confirmed; 30% improved with proton pump inhibitor treatment (overlap syndrome); 9% developed eosinophilic gastroenteritis and in 4% symptoms did not recur.

2. In 3/13 (23%) patients with abnormal pH study, the failure of PPI treatment and response to oral steroids/diet placed them in the category of EO.

3. The incidence of eosinophilia in the oesophagus in our region is 9/100 000 children while that of EO is 4.5/100 000 children.

Strengths and limitations of this study:

1. This study defines the occurrence, prevalence and clinical, endoscopic and histologic presentation of oesophageal eosinophilia in the paediatric population in our region.
2. After 2-4 years follow –up approximately 2/3 patients were diagnosed as classical EO and 1/3 as the so- called overlap syndrome (GORD + OE) highlights the importance of keeping longitudinal data on these patients
3. The retrospective nature of the study prevented that all cases received the same clinical approach (i.e. number of biopsies taken and/or performance of pH studies).

Introduction

Gastro-oesophageal reflux (GOR) is a condition in which an abnormal reflux of gastric contents occurs into the oesophagus. It can be asymptomatic, but when it causes symptoms, it is called gastro-oesophageal reflux disease (GORD). Histologically, the mucosa of the distal oesophagus shows basal cell hyperplasia, papillary elongation and intraepithelial eosinophils (usually <15/high power field) [1].
Since Winter et al. [2] suggested that the presence of intraepithelial eosinophils in the distal oesophageal mucosa is a highly specific diagnostic criterion for GORD, several reports during the following years have identified adult and paediatric patients who failed to respond to acid blockade treatment and showed high numbers of intraepithelial eosinophils in the oesophageal mucosa. These patients presented with a variety of symptoms including poor weight gain, food refusal, dysphagia, vomiting and allergic symptoms [3-,5].
After being initially reported in 1978 in an adult patient with severe achalasia [6], Attwood et al. [7] were the first to identify eosinophilic oesophagitis (EO) as a newly recognized clinico-pathological entity in young adults, predominantly males, presenting with dysphagia in the presence of a normal barium swallow, normal endoscopy and normal oesophageal acid exposure on 24 hour pH monitoring.
EO is an emerging clinic-pathologic condition characterised by severe eosinophilia restricted to the oesophagus in patients were GORD has been excluded by normal pH monitoring and failure to respond to high dose proton pump inhibitor therapy [8,9,10]. It is a chronic interleukin (IL)-5 driven inflammatory disorder in which the aetiology seems to be linked to a combination of allergic and immunologic responses [11, 12]. The immune responses in EO are characterized by enhanced production of T helper cell (Th)-2 cytokines as a result of the interplay between genetic predisposition, environmental exposure, allergic sensitization, eosinophils, mast cells and cytokines [10, 12].
The last decade witnessed a rise in the diagnosis of this entity in both adults and children [14-16]. More recently, new clinical, endoscopic, immunologic and histological features have emerged alongside pioneer microarray genetic studies aimed to provide a more thorough understanding of the pathophysiological mechanisms involved in the development of EO [10,17,18]. The problem faced by paediatric pathologists and clinicians when first confronted to an oesophageal biopsy with intraepithelial eosinophilia is the uncertainty about what the underlying cause could be: eosinophilic oesophagitis, gastro-oesophageal reflux, allergy or eosinophilic gastroenteritis. These lead us to seek correlation between the histological features

at a first biopsy with oesophageal eosinophilia (≥ 15 eosinophils/high power field) presenting at our institution between 2007 and 2008 and the final clinical diagnosis after a 2 -4 year follow. We also sought to define the incidence of oesophageal eosinophilia in the paediatric population of South Yorkshire, a north of England county.

MATERIAL AND METHODS

All oesophageal biopsies with ≥ 15 eosinophils/ high power field (HPF) received in our department between 1st of January 2007 and 31st December 2008 were retrieved from our files and retrospectively reviewed by one of the authors as part of a service evaluation project (MC). The eosinophil count was performed on the HPF with highest concentration of intraepithelial eosinophils (ocular magnification of 10 x, lens magnification 40 x, microscopic field: 0.196 mm^2 Nikon microscope).

Histologically, EO is defined by the presence of at least 15 eosinophils/ HPF in the oesophageal mucosa in the absence of involvement of other parts of the gastrointestinal tract (eosinophil counts in the rest of the gastrointestinal tract biopsies were within the normal ranges published by DeBrosse et al. [19]). Other histological features sought in our cohort included: microabscesses (groups containing > 4 eosinophils), dilated intercellular spaces (DIS), basal cell hyperplasia (≥30% of the mucosal thickness); papillary elongation (≥70 % of the mucosal thickness), increased number of "squiggle cells" (> 6/HPF) and epithelial cell vacuolation (presence of clear vacuoles in the cytoplasm). If any of the biopsies included the lamina propria, the presence or absence of fibrosis was assessed.

The clinical notes were reviewed to obtain the demographic, clinical and endoscopic features of the cohort at presentation and after a 2 year follow-up. All endoscopy procedures were performed using Olympus XP240 or XP260 scopes. "Overlap" syndrome is defined by the presence of clinical and histological features of EO together with GORD (abnormal pH study).

Our institution is the only specialist paediatric gastroenterology centre in the region. Therefore, the incidence of oesophageal eosinophilia n our region was calculated based on the population of children in our catchment area (data obtained from the United Kingdom's office of national statistics - 2009 figures) [20].

The study was registered as a Service Evaluation with the Trust (number SE74).

RESULTS

Twenty four cases fulfilled the criteria for the histological diagnosis of EO (3 other cases were excluded as the patients were diagnosed with eosinophilic gastroenteritis at presentation).

The demographic, clinical and endoscopic characteristics of our cases are shown in consecutive order in Table 1. The cases corresponded to 13 males and 11 females. The average age was 6 years (range: 6 months-15 years). Six patients presented with poor feeding/food aversion, 5 with dysphagia and 4 with vomiting. Clinical and laboratory tests performed either before or after the index biopsy, revealed that 11/24 (46%) children had some form of allergy: 6 patients had either eczema, asthma or both (cases 1,2,9,11,15 and 18) and 4 cases improved with dairy free diet in keeping with cow milk protein allergy (cases 6,14,17,19). Another patient (case 5), although did not have clinical or histologic features of Coeliac disease, the symptoms improved after exclusion diet. Twenty two of our patients (91%) had a trial of Proton Pump Inhibitors (PPI), either before or after the biopsy results became available, without relief of their symptoms. A pH study was performed in 67% of our patients (16/24). In 7 of the 24 (29%) children (cases 4, 5, 8, 9, 10, 12, 18), EO was associated with GORD, fulfilling the criteria for the so-called “overlap syndrome”.

The endoscopic findings were described as: normal in 9 cases (38%), furrowing or trachealization in 10 cases (42%), Candida infection (white speckles) was suspected in 2 cases (8%), erythema in keeping with oesophagitis was queried in 2 cases (8%) and no description was recorded in 1 case (4%). See figure 1a.

The histologic features are shown in Table 2 and Figure 1 b-d. A total of 36 oesophageal biopsies were performed in the 24 patients. The biopsy site was labelled as proximal in 10; middle in 2 and distal in 13. No site was recorded in 11 specimens.

No eosinophils were seen infiltrating the oesophageal mucosa in 1/36 biopsies but eosinophils were present in other biopsies from the same patient. The average number of eosinophils in the remaining 34 biopsies was 32 (range 4-57)/HPF. When this figure was analysed per biopsy site, the corresponding average number and range of eosinophils/HPF was: 24.5 (range 4-55)/HPF in the proximal biopsies; 37.5 (range 22-55)/HPF in the middle biopsies; 38 (range 20-57)/HPF in the distal biopsies and 32 (range 16-45)/ HPF in the unknown site samples.

Other features seen in EO were various degrees of DIS; basal cell hyperplasia; papillary elongation and vacuolation of the epithelial cells. Microabscesses in the superficial mucosa were identified in 4 patients (cases 2,9,10 and 17). An interesting finding in the studied cohort was that only 10 cases had an increased number of the

so –called “squiggle” cells (more than 6 per high power field), 3 of which were later confirmed to have overlap syndrome. 8 biopsies contained a small amount of superficial lamina propria. Therefore, the presence or absence of fibrosis could not be assessed.

The clinical management and follow –up is presented in Table 3. One patient was lost from follow-up. The diagnosis of EO was confirmed in 13/23 (56.5%) cases; 7/23 (30%) patients improved with treatment for GORD and were ascribed to the “overlap syndrome”; 2/23 (9%) cases later developed eosinophilic gastroenteritis and in 1/23 (4%) patient upper gastrointestinal symptoms did not recur and was later diagnosed with irritable bowel syndrome. 4/ 13 patients with EO showed no response to PPI and had a normal pH study; 6/13 patients with not response to PPI treatment improved with diet management with or without the addition of topical Budesonide (no pH study had not been performed) and 3/13 patients although with abnormal pH results who did not improve with PPI treatment, responded to oral steroids and /or diet.

Our institution serves a population of 2 million, 250 000 of whom are younger than 16 years of age [20]. During the 2 year study period, 1046 patients had upper GI endoscopies with oesophageal biopsy at our hospital. The incidence of oesophageal eosinophilia in this cohort was calculated to be 2.2%; while that of EO (after further tests, treatment and 2-4 year follow-up) was 1.2%. One hundred and fifty seven (15%) of all patients referred for upper gastrointestinal endoscopy in our institution had features of oesophagitis on histology (data not shown). Therefore, the incidence of oesophageal eosinophilia among all cases with oesophagitis (24/157) was 15% while that of EO (13/157) was 8.2%. We estimate that the incidence of oesophageal eosinophilia in our region is 9/100 000 children while that of EO is 4.5/100 000 children.

DISCUSSION

The presence of eosinophilia in the gastrointestinal mucosa is seen in numerous conditions. The differential diagnosis includes IgE-mediated food allergy, eosinophilic gastroenteritis, allergic colitis, inflammatory bowel disease, hypereosinophilic syndrome, drug reactions, collagen vascular disease, parasitic infections, myeloproliferative disorders and EO [10, 17].

The symptoms of EO are often difficult to distinguish from those of GORD thus posing a management dilemma [21]. These symptoms include vomiting, regurgitation, nausea, epigastric pain, heartburn, food aversion, dysphagia and failure to thrive [8,11,17,22,23], all of which were present in our cases (see Table 1).

Interestingly, the most common presenting features in our cohort were symptoms related to allergy (11/24). Dysphagia, which is present in only 5 of our cases, has been reported as the most common feature of EO in patients, both adults and children [15,16, 24, 25].

We calculated the incidence of oesophageal eosinophilia among all cases of oesophagitis in our region to be 15%; although the incidence of EO is only 8.2%. This figure is higher than the 6.8% reported by Fox et al [25] and lower than the incidence documented by Lim et al [22]. The incidence of oesophageal eosinophilia in our region is 9/100 000 children, while the incidence of EO is half of this amount. This figure is less than the 2-4 per 10 000 children cited by Noel et al [14] and Rothenberg [17]. However, Straumann et al [14] reported an average annual incidence of 1.438 cases per 100 000 population throughout a 16 year observation period (range 0-6). This wide range of figures probably reflects the different population studied, differences in the diagnostic thresholds or under-recognition of the condition.

As previously seen in both adults and children [9, 14, 15], we have also noticed a marked increase in the number of cases of EO during the last few years (data not shown). As a matter of fact, a few years ago paediatric EO was not offered as a diagnosis at all. Therefore, our calculated incidence may still be the tip of the iceberg in the paediatric population because many oligo-symptomatic or asymptomatic cases may remain undiagnosed [15]. On the other hand, the reported raising incidence of EO may be due to increased recognition by both gastroenterologists and pathologists, and increasing number of endoscopy procedures performed in patients with upper gastrointestinal tract symptoms.

Classically, EO shows a male to female ratio of 3 to 1 [13, 15, 24, 26]. Our cohort failed to show a male predominance when EO and OS were analysed together (11 males : 9 females). However, this became apparent when only cases of EO were analysed (9 males : 4 females). .

The endoscopic appearance of EO is puzzling. Endoscopy is described as showing a ring-like oesophagus (“trachealization”), longitudinal linear furrows, friability or multiple small white papules suggestive of Candida [19,21,24,27]. Interestingly, a study that addressed the correlation between endoscopic and histologic features demonstrated a striking accumulation of eosinophils in those biopsies taken from “white” fungal –looking areas [11]. In a paediatric series, white specks were described in approximately 30% of the cases and have been demonstrated to have a specificity of 95% [22, 24] while linear furrows can be subtle and may easily be missed during routine endoscopy [16]. Consequently, histologically severe EO can

be associated to normal-looking mucosa at endoscopy [23]. Indeed, 9 of our cases had normal endoscopy.

The diagnosis of EO requires histological assessment of the oesophageal mucosa, ideally from the distal, middle and proximal oesophagus. A systematic review of the literature performed recently showed a wide variation of diagnostic histologic criteria such as the number of eosinophils/HPF, eosinophil density in eosinophils/mm^2, and oesophageal biopsy protocols [9]. A study of the histopathological features aiming to derive an optimal number of biopsies needed for diagnosis demonstrated that significant histologic variability exists among biopsy specimens from children with EO [26]. A criterion of >15 eosinophils/HPF in a single biopsy achieved the diagnosis in 73% of patients. The diagnostic sensitivity increased to 97% of patients using 3 biopsy specimens.

Various histological features of oesophageal mucosa have been demonstrated in patients with EO. The hallmark of EO is the presence of > 15-20 eosinophils//HPF with preferential localization of eosinophils near the surface of the epithelium in a background of basal cell hyperplasia and papillary elongation. The number of eosinophils/HPF varies according to different investigators . While some require ≥ 20 eosinophils /HPF [8, 24, 28], others use ≥24/HPF [11] and yet now ≥ 15 eosinophils/HPF are accepted as in keeping with EO [10,22,26,29,30]. Eosinophil microabscesses with degranulation phenomena, if present, are further supportive of this diagnosis [28,31].

In addition to the presence of eosinophils, basal cell hyperplasia and DIS have also been reported to be a frequent finding in EO [17,24, 27, 28, 32]. The mechanism of DIS is through loss or rearrangement of intercellular glycoconjugates that “seal” the intercellular spaces, impairing sodium transport and causing water accumulation in the intercellular space [33, 34]. It is possible that eosinophilic infiltration causes mucosa cell damage and increased permeability that render the oesophageal mucosa susceptible to injury by gastric acid [28]. The presence of lamina propria fibrosis has also been described as a feature of EO and can be related to the occurrence of oesophageal stenosis [30,35]. None of our biopsies contained enough lamina propria to allow the assessment of the presence or absence of fibrosis.

Eosinophils are specialised cells that contain granule proteins, cytokines, platelet activating factors and leukotrienes. Their main role is traditionally thought to be combating parasitic infections, although they can be stimulated by a variety of other triggers such as tissue injury, allergens and viruses [17]. Their cytoplasmic granules contain a major basic protein, eosinophil cationic protein, peroxidase and a neurotoxin [36]. These proteins have cytotoxic effects and are thought to be involved

in the pathogenic mechanisms leading to EO. Since eosinophil –derived neurotoxin is associated with ataxia and destruction of Purkinje fibres [36], it is plausible that it can be related to the dysphagia present in many patients with EO.

EO is a IL-5 driven inflammatory disorder of the oesophagus in which the aetiology seems to be linked to a combination of allergic and immunologic responses [11,12]. IL-5 is a cytokine involved in the production, migration, survival and activation of eosinophils, and IL-5 mRNA has been shown to be increased in the biopsies of patients with EO [13].

Allergic disorders are noted to be more common in patients with EO than in those with GORD, and the majority of patients show food and aeroallergen hypersensitivity identified by skin prick tests, food specific radioallergosorbent testing (RAST) or both [10,12,17, 37]. The fact that symptoms of EO improve with orally administered corticosteroids, further support an allergic aetiology [12] Eleven (46%) of our cases of oesophageal eosinophilia and 5/13 (38.5%) with EO had an associated allergic condition including asthma, eczema and cow milk protein allergy. One additional patient, although did not have clinical or histologic features of Coeliac disease, improved with a gluten-free diet.

Previous literature has shown that 50%-75% of patients with EO have a strong history of allergic symptoms including asthma, rhinitis, eczema and food allergy [17, 23, 24, 38, 39] and that this can be reversed by institution of an allergen-free diet. The association between Coeliac disease and EO has been reported in 6 patients [40]. However, the eosinophilic infiltration in the oesophagus did not improve with gluten free diet in these cases [40]. The relevance of these findings suggests the need to refer patients with EO for food allergy evaluation, a practice more commonly seen in paediatric than adult gastroenterology practice [41].

Eotaxins are a group of chemokines that are relatively specific for eosinophils and have a key role in the modulation of eosinophil accumulation in the gastrointestinal tract [17]. All eotaxins act on a selective transmembrane eotaxin CCR3 receptor primarily expressed on eosinophils. The same eotaxin CCR3 receptor is also expressed in gastrointestinal mast cells. Using genetic microarray expression profile analysis, Blanchard et al [18] demonstrated an approximately 50 fold overexpression of the gene of eotaxin-3 in the oesophageal mucosa of patients with EO compared to controls, suggesting a role of eotaxin in the pathogenesis of EO. The level of eotaxin-3 mRNA and protein strongly correlated with the number of eosinophils in the oesophageal mucosa. They also showed that mast cell gene expression is highly increased in EO. This correlates with the description of increased number of mast cells and mast cell degranulation in oesophageal biopsies of patients with EO [17,18,

28, 29]. Kirsch et al [29] also found that the number of IgE-bearing cells, an indicator of an allergic process, is much more in patients with EO compared with GORD. Therefore, counts of mast cells and IgE-bearing cells in the oesophageal mucosa may help to distinguish a subgroup of patients with EO and allergy. Although we had not tested for mast cells in the cases described in this series, we are planning to institute this in future cases.

pH studies are generally considered useful to distinguish patients with EO from those with GORD [10,12,22]. However, we have shown that there is considerable overlap in the clinical and histological features of EO and GORD. Both ends of the spectrum are not so difficult to recognize but sometimes differentiating between these 2 conditions can be challenging [29]. Moreover, the possibility of an "overlap" group showing features of both conditions have been demonstrated [28, 42, 43]. The identification of those patients with overlap syndrome has therapeutic implications, as the addition of acid blockade and prokinetic agents can aid in healing by reducing exposure to acid which adds a further insult to the mucosa. Thirty percent (7/23) of our cases with oesophageal eosinophilia had evidence of GORD that improved with PPI treatment ("overlap syndrome"). This figure is approximately three-quarters of the 40% reported by Remedios et al. in adults with EO [42]. A recent report demonstrated resolution of oesophageal eosinophilia in three children with clinical symptoms as well as endoscopic features of EO following a course of proton pump inhibitor therapy [43], indicating that a large number of eosinophils can be seen in patients with GORD. The underlying proposed mechanism is that either EO causes a dysfunction of the lower oesophageal sphincter or an allergy type reaction of the oesophageal mucosa to reflux contents [16,27,42]. This would explain why as many as 94% of children with EO exhibit reflux symptoms refractory to proton pump inhibitor therapy [43]. In line with this proposal, 3/13 cases that were clinically categorised as EO after 2 years follow –up demonstrated abnormal pH results but their symptoms did not improve with PPI treatment although disappeared or markedly improved with oral steroids and /or diet.

Rothenberg [17] indicated that the presence of 7 to 20 to 24 eosinophils/HPF likely represents a combination of GORD and food allergy, while more than 20 to 24 eosinophils/HPF is characteristic of EO. In our study, the number of intraepithelial eosinophils in the "overlap" group was between 16 to 57/HPF. Results from a recent histomorphological and immunohistological investigation performed in adult patients with EO concluded that the differential diagnosis of EO and GORD cannot be based on counts of eosinophils alone, and that the presence and intensity of secondary

changes such as basal cell hyperplasia, DIS and vacuolization of keratinocytes would be helpful to better delineate these two conditions [28]. If EO is suspected, endoscopy with biopsy and histology is critical to achieve the correct clinicopathologic diagnosis.

In summary, we have presented the clinical, endoscopic histological and epidemiological features of oesophageal eosinophilia in our area. A significant proportion of patients had either EO or EO associated with GORD ("overlap syndrome"). Further studies need to be done in order to delineate the interaction between genetic factors, allergens and eosinophils. This would help to plan interventionist measures that could remedy the perceived worldwide increasing incidence of EO. The fact that after 2-4 years of treatment and follow-up, approximately 2/3 patients were diagnosed as classical EO and 1/3 as the so- called OS (GORD + OE) highlights the importance of keeping longitudinal data on these patients. As a consequence of the study, we have now introduced a register of patients with features of EO, aiming to gather long term follow- up data which could assist in the identification of further histological and/or clinical characteristics that would allow better management of the disease.

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Authors Contribution:

Dr Marta Cohen designed, performed the study (histology) and wrote the paper

Prithviraj Rao: performed the study (clinical and endoscopy) and wrote the paper

Mike Thomson: performed the study (clinical and endoscopy) and wrote the paper

Dr Mudher Al Adnani: performed the study (histology) and wrote the paper

Data Sharing:

Technical appendix and dataset available from the corresponding author at Marta.Cohen@sch.nhs.uk

Consent was not specifically obtained as this was a retrospective study, but the presented data are anonymised and risk of identification is low.

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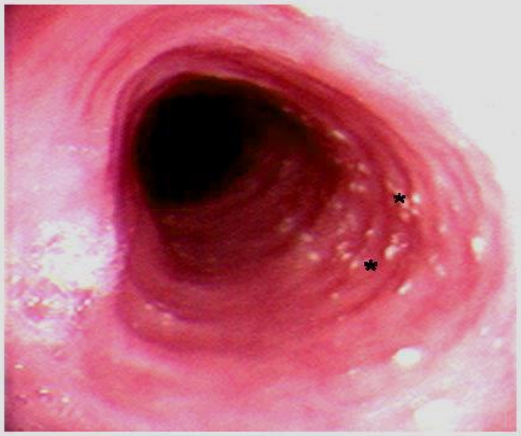
Figure 1

a: Endoscopic appearance of eosinophilic oesophagitis showing “trachealization” of the oesophagus and white speckles (*); b: Biopsy from the middle oesophagus depicting 53 eosinophils/high power field. These were located toward the surface of the mucosa (H&E x 40. Case 2); c: Dilatation of the intercellular spaces (curved arrow), papillary elongation and basal cell hyperplasia were frequent changes present in biopsies with eosinophilic oesophagitis (H&E x 40. Case 4); d: Microabscesses containing more than 4 eosinophils present near the surface of the mucosa (arrow) (H&E x 40. case 10).



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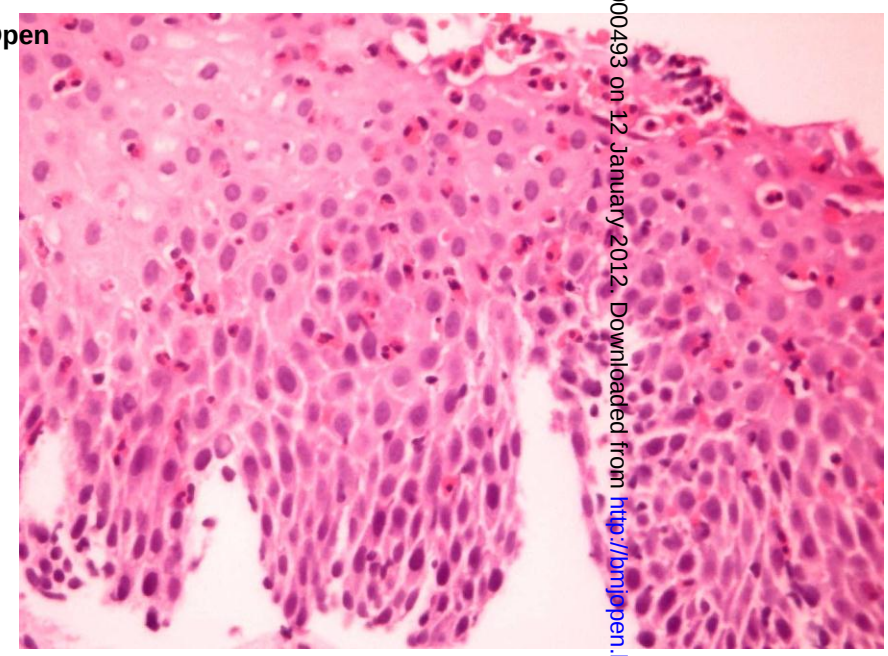

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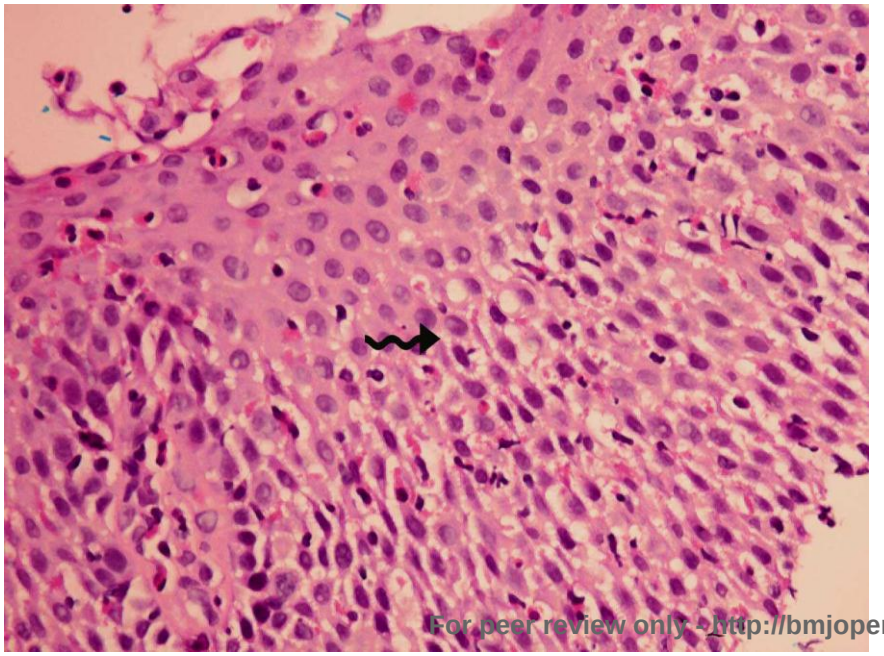

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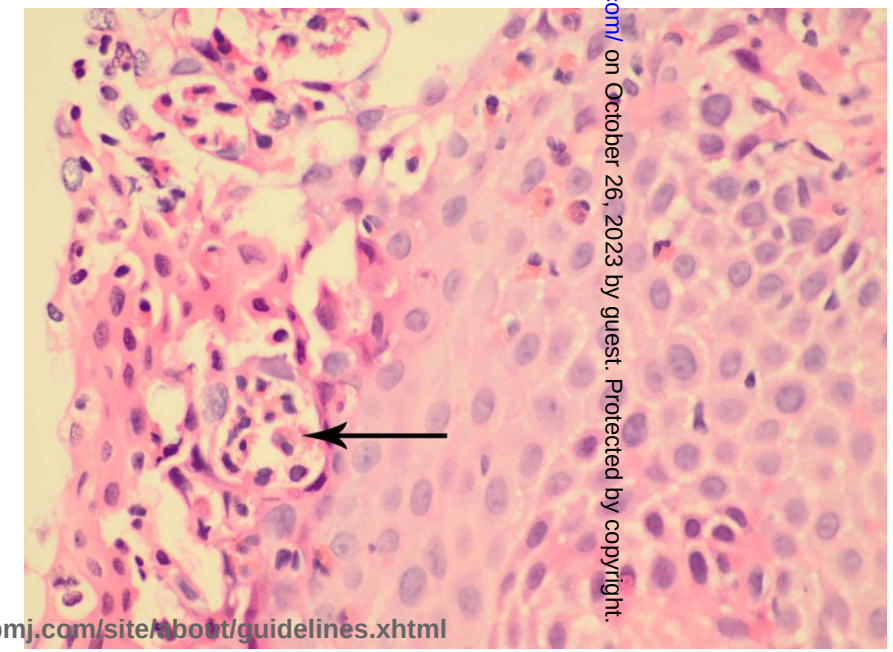

d

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any prespecified hypotheses	5
Methods			**5**
Study design	4	Present key elements of study design early in the paper	5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	5
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	NA
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*NA*
Bias	9	Describe any efforts to address potential sources of bias	NA
Study size	10	Explain how the study size was arrived at	
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	5
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	NA
		(*b*) Describe any methods used to examine subgroups and interactions	NA
		(*c*) Explain how missing data were addressed	NA
		(*d*) If applicable, explain how loss to follow-up was addressed	NA
		(*e*) Describe any sensitivity analyses	NA
Results			6-7

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	6,7
		(b) Give reasons for non-participation at each stage	NA
		(c) Consider use of a flow diagram	NA
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Table 1
		(b) Indicate number of participants with missing data for each variable of interest	Table 1
		(c) Summarise follow-up time (eg, average and total amount)	Table 3
Outcome data	15*	Report numbers of outcome events or summary measures over time	3
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	6,7
		(*b*) Report category boundaries when continuous variables were categorized	NA
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	NA
Discussion			7-12
Key results	18	Summarise key results with reference to study objectives	
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	7-12
Generalisability	21	Discuss the generalisability (external validity) of the study results	7-12
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	NA

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

Eosinophils in the oesophageal mucosa - Clinical, pathological and epidemiological relevance in children: A Cohort Study

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Eosinophils in the oesophageal mucosa - Clinical, pathological and epidemiological relevance in children: A Cohort Study

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Key words: Outcomes research, paediatric practice, gastroenterology

ABSTRACT

Objectives: Eosinophilic oesophagitis (EO) shows eosinophilic infiltration of the mucosa and can present with symptoms indistinguishable from gastro-oesophageal reflux disease (GORD). We describe the clinical, endoscopic and histopathological features of all cases of histological EO presenting during 2007- 2008 with a 2 year follow-up. The incidence of paediatric EO and the features of a subgroup with features of both GORD and EO ("overlap" syndrome-OS) are described.

Design: Biopsies with an average of 15 eosinophils/HPF were reviewed. Other histological features sought included: microabscesses, dilated intercellular spaces, basal cell hyperplasia; papillary elongation, etc. OS was suggested when there was co-existence of clinical and histological features of EO and GORD (abnormal pH study) which improved with PPI.

Setting: Tertiary care.

Participants: all cases with ≥ 15 eosinophils/HPF entered the study

Results: 24 cases of EO were identified, 13 males and 11 females. The incidence of paediatric oesophageal eosinophilia in our region was 9 per 100 000 children. 11/24 patients (46%) presented with some form of allergy, 6 with poor feeding/food aversion, 5 with dysphagia and 4 with vomiting. After follow- up, 56.5% were confirmed to have EO; 30.5% responded to treatment for GORD and were categorised as OS, 9% developed eosinophilic gastroenteritis and 4% did not have further upper gastrointestinal symptoms.

Conclusions: Accurate diagnosis of EO, especially the differentiation from GORD, requires appropriate clinico-pathological correlation. A significant proportion of patients with eosinophilia in the mucosa also have GORD ("OS"). These patients improve after treating the underlying GORD.

The study was registered as a Service Evaluation with the Trust (number SE74).

Article summary

Article focus:

1. To estimate the incidence of oesophageal eosinophilia in the paediatric population of our region
2. To describe the clinical presentation and the endoscopic appearances at presentation.
3. To appraise the natural history after 2 years follow up and to recognize the clinical features of those cases that showed an overlap with gastro oesophageal reflux

Key messages:

1. During follow-up 56.5% cases had EO confirmed; 9% improved with proton pump inhibitor treatment (overlap syndrome); 9% developed eosinophilic gastroenteritis and in 4% symptoms did not recur.

2. 3/13 (23%) patients with abnormal pH study, the failure of PPI treatment and response to oral steroids/diet placed them in the category of EO.

3. The incidence of eosinophilia in the oesophagus in our region is 9/100 000 children while that of EO is 4.5/100 000 children.

Strengths and limitations of this study:

- Defines the epidemiological features of oesophageal eosinophilia in the paediatric population in our region.
- Highlights the importance of keeping longitudinal data on these patients
- The retrospective nature prevented a uniform clinical approach.
- The small number of patients that underwent full evaluation for GORD weakens the conclusions on Overlap Syndrome.

Introduction

Gastro-oesophageal reflux (GOR) is a condition in which an abnormal reflux of gastric contents occurs into the oesophagus. It can be asymptomatic, but when it causes symptoms, it is called gastro-oesophageal reflux disease (GORD). Histologically, the mucosa of the distal oesophagus shows basal cell hyperplasia, papillary elongation and intraepithelial eosinophils (usually <15/high power field) [1].

Since Winter et al. [2] suggested that the presence of intraepithelial eosinophils in the distal oesophageal mucosa is a highly specific diagnostic criterion for GORD, several reports during the following years have identified adult and paediatric patients who failed to respond to acid blockade treatment and showed high numbers of intraepithelial eosinophils in the oesophageal mucosa. These patients presented with a variety of symptoms including poor weight gain, food refusal, dysphagia, vomiting and allergic symptoms [3-,5].

After being initially reported in 1978 in an adult patient with severe achalasia [6], Attwood et al. [7] were the first to identify eosinophilic oesophagitis (EO) as a newly recognized clinico-pathological entity in young adults, predominantly males, presenting with dysphagia in the presence of a normal barium swallow, normal endoscopy and normal oesophageal acid exposure on 24 hour pH monitoring.

EO is an emerging clinic-pathologic condition characterised by severe eosinophilia restricted to the oesophagus in patients were GORD has been excluded by normal pH monitoring and failure to respond to high dose proton pump inhibitor therapy [8,9,10]. It is a chronic interleukin (IL)-5 driven inflammatory disorder in which the aetiology seems to be linked to a combination of allergic and immunologic responses [11, 12]. The immune responses in EO are characterized by enhanced production of T helper cell (Th)-2 cytokines as a result of the interplay between genetic predisposition, environmental exposure, allergic sensitization, eosinophils, mast cells and cytokines [10, 12].

The last decade witnessed a rise in the diagnosis of this entity in both adults and children [14-16]. More recently, new clinical, endoscopic, immunologic and histological features have emerged alongside pioneer microarray genetic studies aimed to provide a more thorough understanding of the pathophysiological mechanisms involved in the development of EO [10,17,18]. The problem faced by paediatric pathologists and clinicians when first confronted to an oesophageal biopsy with intraepithelial eosinophilia is the uncertainty about what the underlying cause could be: eosinophilic oesophagitis, gastro-oesophageal reflux, allergy or eosinophilic gastroenteritis. These lead us to seek correlation between the histological features

at a first biopsy with oesophageal eosinophilia (at an average of 15 eosinophils/high power field) presenting at our institution between 2007 and 2008 and the final clinical diagnosis after a 2 -4 year follow. We also sought to define the incidence of oesophageal eosinophilia in the paediatric population of South Yorkshire, a north of England county.

MATERIAL AND METHODS

All oesophageal biopsies with an average of 15 eosinophils/ high power field (HPF) received in our department between 1st of January 2007 and 31st December 2008 were retrieved from our files and retrospectively reviewed by one of the authors as part of a service evaluation project (MC). The eosinophil count was performed on the HPF with highest concentration of intraepithelial eosinophils (ocular magnification of 10 x, lens magnification 40 x, microscopic field: 0.196 mm^2 Nikon microscope).

Histologically, EO was defined by the presence of at least 15 eosinophils/ HPF in the oesophageal mucosa in the absence of involvement of other parts of the gastrointestinal tract (eosinophil counts in the rest of the gastrointestinal tract were within the normal ranges published by DeBrosse et al. [19]). Other histological features sought in our cohort included: microabscesses (groups containing > 4 eosinophils), dilated intercellular spaces (DIS), basal cell hyperplasia (≥30% of the mucosal thickness); papillary elongation (≥70 % of the mucosal thickness), increased number of "squiggle cells" (> 6/HPF) and epithelial cell vacuolation (presence of clear vacuoles in the cytoplasm). If any of the biopsies included the lamina propria, the presence or absence of fibrosis was assessed.

The clinical notes were reviewed to obtain the demographic, clinical and endoscopic features of the cohort at presentation and after a 2 year follow-up. All endoscopy procedures were performed using Olympus XP240 or XP260 scopes. "Overlap" syndrome was defined by the presence of clinical and histological features of EO together with GORD (abnormal pH study).

Our institution is the only specialist paediatric gastroenterology centre in the region. Therefore, the incidence of oesophageal eosinophilia n our region was calculated based on the population of children in our catchment area (data obtained from the United Kingdom's office of national statistics - 2009 figures) [20].

The study was registered as a Service Evaluation with the Trust (number SE74).

RESULTS

Twenty four cases fulfilled the criteria for the histological diagnosis of EO (3 other cases were excluded as the patients were diagnosed with eosinophilic gastroenteritis at presentation).

The demographic, clinical and endoscopic characteristics of our cases are shown in consecutive order in Tables 1 and 2. The cases corresponded to 13 males and 11 females. The average age was 6 years (range: 6 months-15 years). Six patients presented with poor feeding/food aversion, 5 with dysphagia and 4 with vomiting. Clinical and laboratory tests performed either before or after the index biopsy, revealed that 11/24 (46%) children had some form of allergy: 6 patients had either eczema, asthma or both (cases 1,2,9,11,15 and 18) and 4 cases improved with dairy free diet in keeping with cow milk protein allergy (cases 6,14,17,19). Another patient (case 5), although did not have clinical or histologic features of Coeliac disease, the symptoms improved after exclusion diet. Twenty two of our patients (91%) had a trial of Proton Pump Inhibitors (PPI), either before or after the biopsy results became available, without relief of their symptoms. A pH study was performed in 67% of our patients (16/24). In 7 of the 24 (29%) children (cases 4, 5, 8, 9, 10, 12, 18), EO was associated with GORD, fulfilling the criteria for the so-called “overlap syndrome”.

The endoscopic findings were described as: normal in 9 cases (38%), furrowing or trachealization in 10 cases (42%), Candida infection (white speckles) was suspected in 2 cases (8%), erythema in keeping with oesophagitis was queried in 2 cases (8%) and no description was recorded in 1 case (4%). See figure 1a.

The histologic features are shown in Table 3 and Figure 1 b-d. A total of 36 oesophageal biopsies were performed in the 24 patients of the study; although only 35/36 biopsies fulfilled the criteria of EO (this patient had another simultaneous biopsy with EO). The biopsy site was labelled as proximal in 10; middle in 2 and distal in 13. No site was recorded in 11 specimens.

The average number of eosinophils in the 35 biopsies from the 24 cases of the study was 32 (range 4-57)/HPF. When this figure was analysed per biopsy site, the corresponding average number and range of eosinophils/HPF was: 24.5 (range 4-55)/HPF in the proximal biopsies; 37.5 (range 22-55)/HPF in the middle biopsies; 38 (range 20-57)/HPF in the distal biopsies and 32 (range 16-45)/ HPF in the unknown site samples.

Other features seen in EO were various degrees of DIS; basal cell hyperplasia; papillary elongation and vacuolation of the epithelial cells. Microabscesses in the superficial mucosa were identified in 4 patients (cases 2,9,10 and 17). An interesting finding in the studied cohort was that only 10 cases had an increased number of the

so –called “squiggle” cells (more than 6 per high power field), 3 of which were later confirmed to have overlap syndrome. Most biopsies did not include lamina propria and only 8 biopsies contained a small amount of superficial lamina propria. Therefore, the presence or absence of fibrosis could not be assessed.

The clinical management and follow –up is presented in Table 4. One patient was lost from follow-up. The diagnosis of EO was confirmed in 13/23 (56.5%) cases; 7/23 (9%) patients improved with treatment for GORD and were ascribed to the “overlap syndrome”; 2/23 (9%) cases later developed eosinophilic gastroenteritis and in 1/23 (4%) case the upper gastrointestinal symptoms did not recur and the patient was later diagnosed with irritable bowel syndrome. 4/ 13 patients with EO showed no response to PPI and had a normal pH study; 6/13 patients with no response to PPI treatment improved with diet management with or without the addition of topical Budesonide (no pH study had been performed) and 3/13 patients although with abnormal pH results who did not improve with PPI treatment, responded to oral steroids and /or diet.

Our institution serves a population of 2 million, 250 000 of whom are younger than 16 years of age [20]. During the 2 year study period, 1046 patients had upper GI endoscopies with oesophageal biopsy at our hospital. The incidence of oesophageal eosinophilia in this cohort was calculated to be 2.2%; while that of EO (after further tests, treatment and 2-4 year follow-up) was 1.2%. One hundred and fifty seven (15%) of all patients referred for upper gastrointestinal endoscopy in our institution had features of oesophagitis on histology (data not shown). Therefore, the incidence of oesophageal eosinophilia among all cases with oesophagitis (24/157) was 15% while that of EO (13/157) was 8.2%. We estimate that the incidence of oesophageal eosinophilia in our region is 9/100 000 children while that of EO is 4.5/100 000 children.

DISCUSSION

The presence of eosinophilia in the gastrointestinal mucosa is seen in numerous conditions. The differential diagnosis includes IgE-mediated food allergy, eosinophilic gastroenteritis, allergic colitis, inflammatory bowel disease, hypereosinophilic syndrome, drug reactions, collagen vascular disease, parasitic infections, myeloproliferative disorders and EO [10, 17].

The hallmark of EO is the presence of > 15-20 eosinophils//HPF with preferential localization of eosinophils near the surface of the epithelium in a background of basal cell hyperplasia and papillary elongation. The number of eosinophils/HPF varies according to different investigators. While some require ≥ 20 eosinophils /HPF [8, 24,

28], others use ≥24/HPF [11] and yet now -as it is in our institution- ≥ 15 eosinophils/HPF are accepted as in keeping with EO [10,22-24],. Eosinophil microabscesses with degranulation phenomena, if present, are further supportive of this diagnosis [25,26].

In addition to the presence of eosinophils, basal cell hyperplasia and DIS have also been reported to be a frequent finding in EO [17, 25, 27-29]. The mechanism of DIS is through loss or rearrangement of intercellular glycoconjugates that "seal" the intercellular spaces, impairing sodium transport and causing water accumulation in the intercellular space [30, 31]. It is possible that eosinophilic infiltration causes mucosa cell damage and increased permeability that render the oesophageal mucosa susceptible to injury by gastric acid [25]. The presence of lamina propria fibrosis has also been described as a feature of EO and can be related to the occurrence of oesophageal stenosis [24,32].

The symptoms of EO are often difficult to distinguish from those of GORD thus posing a management dilemma [21]. These include vomiting, regurgitation, nausea, epigastric pain, heartburn, food aversion, dysphagia and failure to thrive [8,11,17,33, 34], all of which were present in our cases (see Table 1). Interestingly, the most common presenting features in our cohort were symptoms related to allergy (11/24). Dysphagia, which was present in only 5 of our cases, has been reported as the most common feature of EO in patients, both adults and children [15,16, 27,35].

We calculated the incidence of oesophageal eosinophilia among all cases of oesophagitis in our region to be 15%; although the incidence of EO is only 8.2%. This figure is higher than the 6.8% reported by Fox et al [35] and lower than the incidence documented by Lim et al [33]. The incidence of oesophageal eosinophilia in our region is 9/100 000 children, while the incidence of EO is half of this amount. This figure is less than the 2-4 per 10 000 children cited by Noel et al [14] and Rothenberg [17]. However, Straumann et al [14] reported an average annual incidence of 1.438 cases per 100 000 population throughout a 16 year observation period (range 0-6). This wide range of figures probably reflects the different population studied, differences in the diagnostic thresholds or under-recognition of the condition.

As previously seen in both adults and children [9, 14, 15], we have also noticed a marked increase in the number of cases of EO during the last few years (data not shown). As a matter of fact, a few years ago paediatric EO was not offered as a diagnosis at all. It is likely that the raising incidence of EO could be due to increased recognition by both gastroenterologists and pathologists, and increasing number of

endoscopy procedures performed in patients with upper gastrointestinal tract symptoms.

Classically, EO shows a male to female ratio of 3 to 1 [13, 15, 22, 27]. Our cohort failed to show a male predominance when EO and OS were analysed together (11 males : 9 females). However, this became apparent when only cases of EO were analysed (9 males : 4 females). .

The endoscopic appearance of EO is puzzling. Endoscopy is described as showing a ring-like oesophagus ("trachealization"), longitudinal linear furrows, friability or multiple small white papules suggestive of Candida [19,21,27, 28]. Interestingly, a study that addressed the correlation between endoscopic and histologic features demonstrated a striking accumulation of eosinophils in those biopsies taken from "white" fungal –looking areas [11]. In a paediatric series, white specks were described in approximately 30% of the cases and have been demonstrated to have a specificity of 95% [27,33] However, histologically severe EO can be associated to normal-looking mucosa at endoscopy [34]. Indeed, 9 of our cases had normal endoscopy.

Eosinophils are specialised cells that contain granule proteins, cytokines, platelet activating factors and leukotrienes. Their main role is traditionally thought to be combating parasitic infections, although they can be stimulated by a variety of other triggers such as tissue injury, allergens and viruses [17]. Their cytoplasmic granules contain a major basic protein, eosinophil cationic protein, peroxidase and a neurotoxin, that has been linked to the presence of dysphagia in many patients [36]. Some authors postulate that EO is a IL-5 driven inflammatory disorder of the oesophagus in which the aetiology could be linked to a combination of allergic and immunologic responses [11,12]. Allergic disorders are noted to be more common in patients with EO than in those with GORD, and the majority of patients show food and aeroallergen hypersensitivity identified by skin prick tests, food specific radioallergosorbent testing (RAST) or both [10,12,17, 37-39]. Eleven (46%) of our cases of oesophageal eosinophilia and 5/13 (38.5%) with EO had an associated allergic condition including asthma, eczema and cow milk protein allergy. One additional patient, although did not have clinical or histologic features of Coeliac disease, improved with a gluten-free diet. Coeliac disease and EO has been reported in 6 patients [40]. However, the eosinophilic infiltration in the oesophagus did not improve with gluten free diet in these cases [40]. The relevance of these findings suggests the need to refer patients with EO for food allergy evaluation, a practice more commonly seen in paediatric than adult gastroenterology practice [41].

Eotaxins are a group of chemokines that are relatively specific for eosinophils and have a key role in the modulation of eosinophil accumulation in the gastrointestinal tract [17]. All eotaxins act on a selective transmembrane eotaxin CCR3 receptor primarily expressed on eosinophils. The same eotaxin CCR3 receptor is also expressed in gastrointestinal mast cells. Using genetic microarray expression profile analysis, Blanchard et al [18] demonstrated an approximately 50 fold overexpression of the gene of eotaxin-3 in the oesophageal mucosa of patients with EO compared to controls, suggesting a role of eotaxin in the pathogenesis of EO. The level of eotaxin-3 mRNA and protein strongly correlated with the number of eosinophils in the oesophageal mucosa. They also showed that mast cell gene expression is highly increased in EO. This correlates with the description of increased number of mast cells and mast cell degranulation in oesophageal biopsies of patients with EO [17,18, 23,25].

pH studies are generally considered useful to distinguish patients with EO from those with GORD [10,12,33]. However, and although our results are limited due to the retrospective nature of the study and the small number of cases, these suggest that there is overlap in the clinical and histological features of EO and GORD. Both ends of the spectrum are not so difficult to recognize but sometimes differentiating between these 2 conditions can be challenging [23]. Moreover, the possibility of an "overlap" group showing features of both conditions has been demonstrated [25, 42, 43]. The identification of those patients with overlap syndrome has therapeutic implications, as the addition of acid blockade and prokinetic agents can aid in healing by reducing exposure to acid which adds a further insult to the mucosa. Thirty percent (7/23) of our cases with oesophageal eosinophilia had evidence of GORD that improved with PPI treatment ("overlap syndrome"). This figure is approximately three-quarters of the 40% reported by Remedios et al. in adults with EO [42]. A recent report demonstrated resolution of oesophageal eosinophilia in three children with clinical symptoms as well as endoscopic features of EO following a course of proton pump inhibitor therapy [43], indicating that a large number of eosinophils can be seen in patients with GORD. The underlying proposed mechanism is that either EO causes a dysfunction of the lower oesophageal sphincter or an allergy type reaction of the oesophageal mucosa to reflux contents [16,28,42]. This would explain why as many as 94% of children with EO exhibit reflux symptoms refractory to proton pump inhibitor therapy [43]. In line with this proposal, 3/13 cases that were clinically categorised as EO after 2 years follow –up demonstrated abnormal pH results but their symptoms did not improve with PPI treatment although disappeared or markedly improved with oral steroids and /or diet.

Rothenberg [17] indicated that the presence of 7 to 20 to 24 eosinophils/HPF likely represents a combination of GORD and food allergy, while more than 20 to 24 eosinophils/HPF is characteristic of EO. In our study, the number of intraepithelial eosinophils in the "overlap" group was between 16 to 57/HPF. Results from a recent histomorphological and immunohistological investigation performed in adult patients with EO concluded that the differential diagnosis of EO and GORD cannot be based on counts of eosinophils alone, and that the presence and intensity of secondary changes such as basal cell hyperplasia, DIS and vacuolization of keratinocytes would be helpful to better delineate these two conditions [25]. If EO is suspected, endoscopy with biopsy and histology is critical to achieve the correct clinicopathologic diagnosis.

In summary, we have presented the clinical, endoscopic histological and epidemiological features of oesophageal eosinophilia in our area. A significant proportion of patients had either EO or EO associated with GORD ("overlap syndrome"). Further studies need to be done in order to delineate the interaction between genetic factors, allergens and eosinophils. This would help to plan interventionist measures that could remedy the perceived worldwide increasing incidence of EO. The fact that after 2-4 years of treatment and follow-up, approximately 2/3 patients were diagnosed as classical EO and 1/3 as the so- called OS (GORD + OE) highlights the importance of keeping longitudinal data on these patients. As a consequence of the study, we have now introduced a register of patients with features of EO, aiming to gather long term follow- up data which could assist in the identification of further histological and/or clinical characteristics that would allow better management of the disease.

Competing interest: None

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Word count: 3,227

Authors Contribution:

Dr Marta Cohen designed the study, acquired the histological data, analysed the histology and was the main author in the task of writing and approving the final

version of the study.

Prithviraj Rao: designed and acquired the clinical and endoscopic data; critically reviewed and improved the article and approved the final version to be published.

Mike Thomson: designed and acquired the clinical and endoscopic data; critically reviewed and improved the article and approved the final version to be published.

Dr Mudher Al Adnani: analysed the histology, contributed to writing the initial draft, critically reviewed the article and approved the final version to be published.

Data Sharing:

Technical appendix and dataset available from the corresponding author at Marta.Cohen@sch.nhs.uk

Consent was not specifically obtained as this was a retrospective study, but the presented data are anonymised and risk of identification is low.

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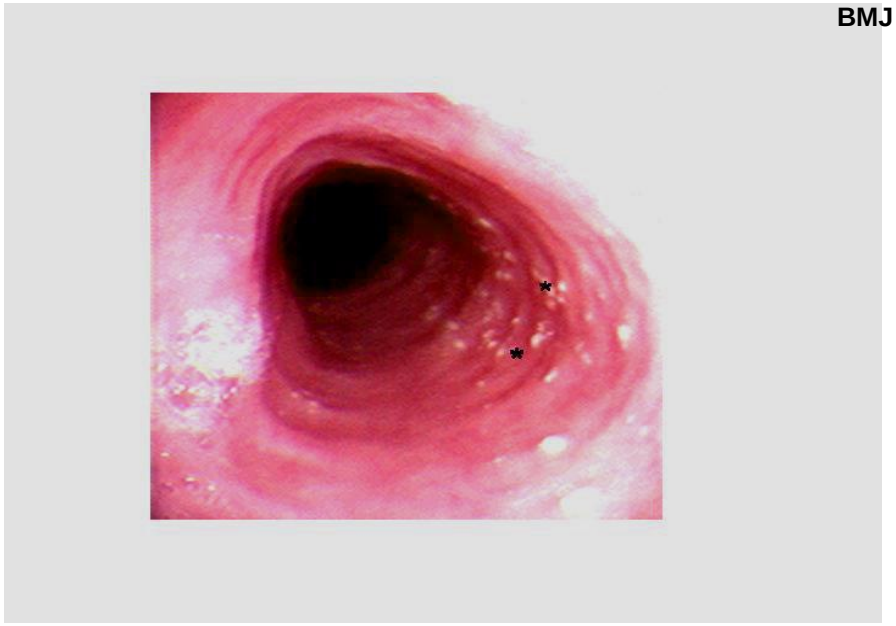
Figures 1

a: Endoscopic appearance of eosinophilic oesophagitis showing “trachealization” of the oesophagus and white speckles (*); b: Biopsy from the middle oesophagus depicting 53 eosinophils/high power field. These were located toward the surface of the mucosa (H&E x 40. Case 2); c: Dilatation of the intercellular spaces (curved arrow), papillary elongation and basal cell hyperplasia were frequent changes present in biopsies with eosinophilic oesophagitis (H&E x 40. Case 4); d: Microabscesses containing more than 4 eosinophils present near the surface of the mucosa (arrow) (H&E x 40. case 10).



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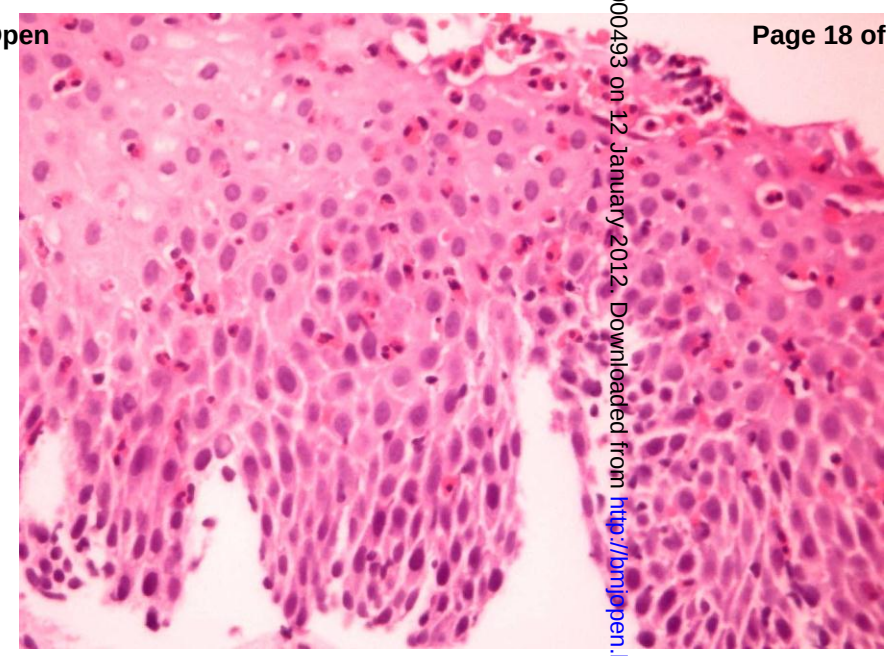

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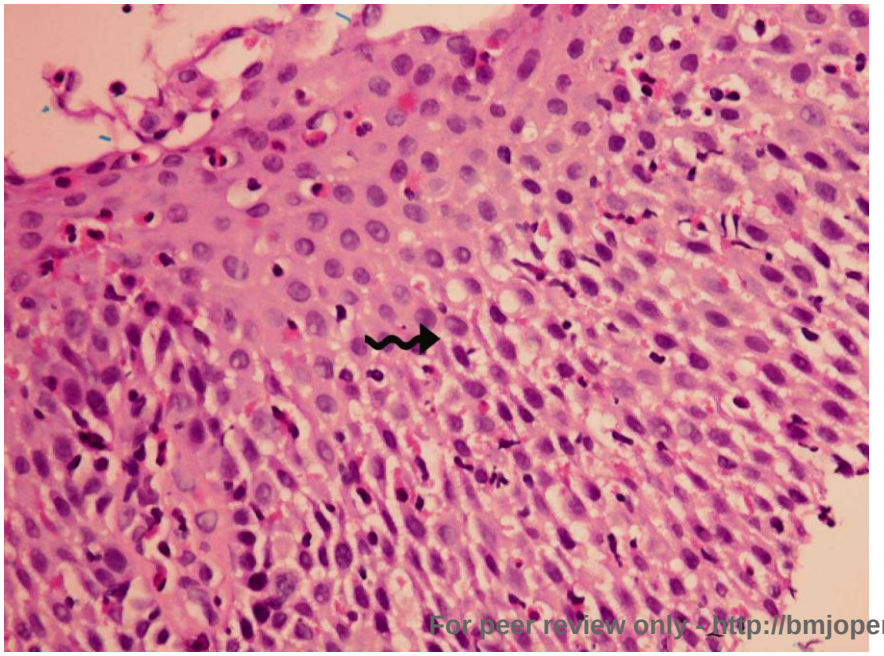

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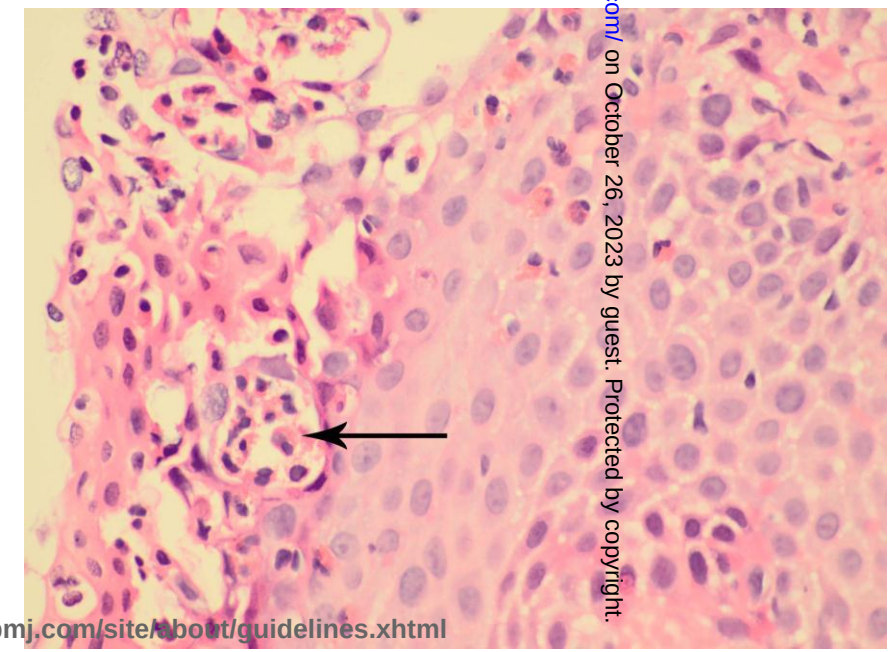

d

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any prespecified hypotheses	5
Methods			**5**
Study design	4	Present key elements of study design early in the paper	5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	5
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	NA
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*NA*
Bias	9	Describe any efforts to address potential sources of bias	NA
Study size	10	Explain how the study size was arrived at	
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	5
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	NA
		(*b*) Describe any methods used to examine subgroups and interactions	NA
		(*c*) Explain how missing data were addressed	NA
		(*d*) If applicable, explain how loss to follow-up was addressed	NA
		(*e*) Describe any sensitivity analyses	NA
Results			6-7

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	6,7
		(b) Give reasons for non-participation at each stage	NA
		(c) Consider use of a flow diagram	NA
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Table 1
		(b) Indicate number of participants with missing data for each variable of interest	Table 1
		(c) Summarise follow-up time (eg, average and total amount)	Table 3
Outcome data	15*	Report numbers of outcome events or summary measures over time	3
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	6,7
		(*b*) Report category boundaries when continuous variables were categorized	NA
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	NA
Discussion			7-12
Key results	18	Summarise key results with reference to study objectives	
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	7-12
Generalisability	21	Discuss the generalisability (external validity) of the study results	7-12
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	NA

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

Eosinophils in the oesophageal mucosa - Clinical, pathological and epidemiological relevance in children: A Cohort Study

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Eosinophils in the oesophageal mucosa: Clinical, pathological and epidemiological relevance in children. A cohort study.

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Key words: Outcomes research, paediatric practice, gastroenterology

ABSTRACT

Objectives: Eosinophilic oesophagitis (EO) shows eosinophilic infiltration of the mucosa and can present with symptoms indistinguishable from gastro-oesophageal reflux disease (GORD). We describe the clinical, endoscopic and histopathological features of all cases of histological EO presenting during 2007- 2008 with a 2 year follow-up. The incidence of paediatric EO and the features of a subgroup with features of both GORD and EO ("overlap" syndrome-OS) are described.

Design: Biopsies with an average of 15 eosinophils/HPF were reviewed in the cohort. Other histological features sought included: microabscesses, dilated intercellular spaces, basal cell hyperplasia; papillary elongation, etc. OS was suggested when there was co-existence of clinical and histological features of EO and GORD (abnormal pH study) which improved with PPI.

Setting: Tertiary care.

Participants: all cases with ≥ 15 eosinophils/HPF entered the study

Results: 24 cases of EO were identified, 13 males and 11 females. The incidence of paediatric oesophageal eosinophilia in our region was 9 per 100 000 children. 11/24 patients (46%) presented with some form of allergy, 6 with poor feeding/food aversion, 5 with dysphagia and 4 with vomiting. After follow- up, 56.5% were confirmed to have EO; 30.5% responded to treatment for GORD and were categorised as OS, 9% developed eosinophilic gastroenteritis and 4% did not have further upper gastrointestinal symptoms.

Conclusions: Accurate diagnosis of EO, especially the differentiation from GORD, requires appropriate clinico-pathological correlation. A significant proportion of patients with eosinophilia in the mucosa also have GORD ("OS"). These patients improve after treating the underlying GORD.

The study was registered as a Service Evaluation with the Trust (number SE74).

Article summary

Article focus:

1. To estimate the incidence of oesophageal eosinophilia in the paediatric population of our region
2. To describe the clinical presentation and the endoscopic appearances at presentation.
3. To appraise the natural history after 2 years follow up and to recognize the clinical features of those cases that showed an overlap with gastro oesophageal reflux

Key messages:

1. During follow-up 56.5% cases had EO confirmed; 9% improved with proton pump inhibitor treatment (overlap syndrome); 9% developed eosinophilic gastroenteritis and in 4% symptoms did not recur.

2. 3/13 (23%) patients with abnormal pH study, the failure of PPI treatment and response to oral steroids/diet placed them in the category of EO.

3. The incidence of eosinophilia in the oesophagus in our region is 9/100 000 children while that of EO is 4.5/100 000 children.

Strengths and limitations of this study:

- Defines the epidemiological features of oesophageal eosinophilia in the paediatric population in our region.
- Highlights the importance of keeping longitudinal data on these patients
- The retrospective nature prevented a uniform clinical approach.
- The small number of patients that underwent full evaluation for GORD weakens the conclusions on Overlap Syndrome.

Introduction

Gastro-oesophageal reflux (GOR) is a condition in which an abnormal reflux of gastric contents occurs into the oesophagus. It can be asymptomatic, but when it causes symptoms, it is called gastro-oesophageal reflux disease (GORD). Histologically, the mucosa of the distal oesophagus shows basal cell hyperplasia, papillary elongation and intraepithelial eosinophils (usually <15/high power field) [1].

Since Winter et al. [2] suggested that the presence of intraepithelial eosinophils in the distal oesophageal mucosa is a highly specific diagnostic criterion for GORD, several reports during the following years have identified adult and paediatric patients who failed to respond to acid blockade treatment and showed high numbers of intraepithelial eosinophils in the oesophageal mucosa. These patients presented with a variety of symptoms including poor weight gain, food refusal, dysphagia, vomiting and allergic symptoms [3-,5].

After being initially reported in 1978 in an adult patient with severe achalasia [6], Attwood et al. [7] were the first to identify eosinophilic oesophagitis (EO) as a newly recognized clinico-pathological entity in young adults, predominantly males, presenting with dysphagia in the presence of a normal barium swallow, normal endoscopy and normal oesophageal acid exposure on 24 hour pH monitoring.

EO is an emerging clinic-pathologic condition characterised by severe eosinophilia restricted to the oesophagus in patients in whom GORD has been excluded by normal pH monitoring and failure to respond to high dose proton pump inhibitor therapy [8,9,10]. It is a chronic interleukin (IL)-5 driven inflammatory disorder in which the aetiology seems to be linked to a combination of allergic and immunologic responses [11, 12]. The immune responses in EO are characterized by enhanced production of T helper cell (Th)-2 cytokines as a result of the interplay between genetic predisposition, environmental exposure, allergic sensitization, eosinophils, mast cells and cytokines [10, 12].

The last decade witnessed a rise in the diagnosis of this entity in both adults and children [14-16]. More recently, new clinical, endoscopic, immunologic and histological features have emerged alongside pioneer microarray genetic studies aimed to provide a more thorough understanding of the pathophysiological mechanisms involved in the development of EO [10,17,18]. The problem faced by paediatric pathologists and clinicians when first confronted with an oesophageal biopsy showing intraepithelial eosinophilia is the uncertainty about what the underlying cause could be: eosinophilic oesophagitis, gastro-oesophageal reflux, allergy or eosinophilic gastroenteritis. These lead us to seek a correlation between

the histological features at a first biopsy with oesophageal eosinophilia (with an average of at least 15 eosinophils/high power field) presenting at our institution between 2007 and 2008 and the final clinical diagnosis after a 2 -4 year follow. We also sought to define the incidence of oesophageal eosinophilia in the paediatric population of South Yorkshire, a north of England county.

MATERIAL AND METHODS

All oesophageal biopsies with an average of at least 15 eosinophils/ high power field (HPF) received in our department between 1st of January 2007 and 31st December 2008 were retrieved from our files and retrospectively reviewed by one of the authors as part of a service evaluation project (MC). The eosinophil count was performed on the HPF with highest concentration of intraepithelial eosinophils (ocular magnification of 10 x, lens magnification 40 x, microscopic field: 0.196 mm^2 Nikon microscope).

Histologically, EO was defined by the presence of at least 15 eosinophils/ HPF in the oesophageal mucosa in the absence of involvement of other parts of the gastrointestinal tract (eosinophil counts in the rest of the gastrointestinal tract were within the normal ranges published by DeBrosse et al. [19]). Other histological features sought in our cohort included: microabscesses (groups containing > 4 eosinophils), dilated intercellular spaces (DIS), basal cell hyperplasia (≥30% of the mucosal thickness); papillary elongation (≥70 % of the mucosal thickness), increased number of “squiggle cells” (> 6/HPF) and epithelial cell vacuolation (presence of clear vacuoles in the cytoplasm). If any of the biopsies included the lamina propria, the presence or absence of fibrosis was assessed.

The clinical notes were reviewed to obtain the demographic, clinical and endoscopic features of the cohort at presentation and after a 2 year follow-up. All endoscopy procedures were performed using Olympus XP240 or XP260 scopes. “Overlap” syndrome was defined by the presence of clinical and histological features of EO together with GORD (abnormal pH study).

Our institution is the only specialist paediatric gastroenterology centre in the region. Therefore, the incidence of oesophageal eosinophilia in our region was calculated based on the population of children in our catchment area (data obtained from the United Kingdom’s office of national statistics - 2009 figures) [20].

The study was registered as a Service Evaluation with the Trust (number SE74).

RESULTS

24 cases fulfilled the criteria for the histological diagnosis of EO (3 other cases were excluded as the patients were diagnosed with eosinophilic gastroenteritis at presentation).

The demographic, clinical and endoscopic characteristics of our cases are shown in consecutive order in Tables 1 and 2. There were 13 males and 11 females. The average age was 6 years (range: 6 months-15 years). 6 patients presented with poor feeding/food aversion, 5 with dysphagia and 4 with vomiting. Clinical and laboratory tests performed either before or after the index biopsy, revealed that 11/24 (46%) children had some form of allergy: 6 patients had either eczema, asthma or both (cases 1,2,9,11,15 and 18) and 4 cases improved with dairy free diet in keeping with cow milk protein allergy (cases 6,14,17,19). Another patient (case 5), although did not have clinical or histologic features of Coeliac disease, the symptoms improved after exclusion diet. 22 of our patients (91%) had a trial of Proton Pump Inhibitors (PPI), either before or after the biopsy results became available, without relief of their symptoms. A pH study was performed in 67% of our patients (16/24). In 7 of the 24 (29%) children (cases 4, 5, 8, 9, 10, 12, 18), EO was associated with GORD, fulfilling the criteria for the so-called “overlap syndrome”.

The endoscopic findings were described as: normal in 9 cases (38%), furrowing or trachealization in 10 cases (42%), Candida infection (white speckles) was suspected in 2 cases (8%), erythema in keeping with oesophagitis was queried in 2 cases (8%) and no description was recorded in 1 case (4%). See figure 1a.

The histologic features are shown in Table 3 and Figure 1 b-d. A total of 36 oesophageal biopsies were performed in the 24 patients of the study; although only 35/36 biopsies fulfilled the criteria of EO (this patient had another simultaneous biopsy with EO). The biopsy site was labelled as proximal in 10; middle in 2 and distal in 13. No site was recorded in 11 specimens.

The average number of eosinophils in the 35 biopsies from the 24 cases of the study was 32 (range 4-57)/HPF. When this figure was analysed per biopsy site, the corresponding average number and range of eosinophils/HPF was: 24.5 (range 4-55)/HPF in the proximal biopsies; 37.5 (range 22-55)/HPF in the middle biopsies; 38 (range 20-57)/HPF in the distal biopsies and 32 (range 16-45)/ HPF in the unknown site samples.

Other features seen in EO were various degrees of DIS; basal cell hyperplasia; papillary elongation and vacuolation of the epithelial cells. Microabscesses in the superficial mucosa were identified in 4 patients (cases 2,9,10 and 17). An interesting

finding in the studied cohort was that only 10 cases had an increased number of the so –called "squiggle" cells (more than 6 per high power field), 3 of which were later confirmed to have overlap syndrome. Most biopsies did not include lamina propria and only 8 biopsies contained a small amount of superficial lamina propria. Therefore, the presence or absence of fibrosis could not be assessed.

The clinical management and follow –up is presented in Table 4. One patient was lost from follow-up. The diagnosis of EO was confirmed in 13/23 (56.5%) cases; 7/23 (9%) patients improved with treatment for GORD and were ascribed to the "overlap syndrome"; 2/23 (9%) cases later developed eosinophilic gastroenteritis and in 1/23 (4%) case the upper gastrointestinal symptoms did not recur and the patient was later diagnosed with irritable bowel syndrome. 4/ 13 patients with EO showed no response to PPI and had a normal pH study; 6/13 patients with no response to PPI treatment improved with diet management with or without the addition of topical Budesonide (no pH study had been performed) and 3/13 patients although with abnormal pH results who did not improve with PPI treatment, responded to oral steroids and /or diet.

Our institution serves a population of 2 million, 250 000 of whom are younger than 16 years of age [20]. During the 2 year study period, 1046 patients had upper GI endoscopies with oesophageal biopsy at our hospital. The incidence of oesophageal eosinophilia in this cohort was calculated to be 2.2%; while that of EO (after further tests, treatment and 2-4 year follow-up) was 1.2%. 157 (15%) of all patients referred for upper gastrointestinal endoscopy in our institution had features of oesophagitis on histology (data not shown). Therefore, the incidence of oesophageal eosinophilia among all cases with oesophagitis (24/157) was 15% while that of EO (13/157) was 8.2%. We estimate that the incidence of oesophageal eosinophilia in our region is 9/100 000 children while that of EO is 4.5/100 000 children.

DISCUSSION

The presence of mere "*eosinophilia*" in the gastrointestinal mucosa is seen in numerous conditions. The differential diagnosis includes IgE-mediated food allergy, eosinophilic gastroenteritis, allergic colitis, inflammatory bowel disease, hypereosinophilic syndrome, drug reactions, collagen vascular disease, parasitic infections, myeloproliferative disorders and EO [10, 17].

The concept of EO is more complex than the simple presence of eosinophils in the mucosa. In EO the occurrence of > 15-20 eosinophils//HPF is *restricted* to the oesophagus and these have a preferential localization near the surface of the epithelium in a background of basal cell hyperplasia and papillary elongation. The

number of eosinophils/HPF varies according to different investigators. While some require ≥ 20 eosinophils /HPF [8, 9,10 24, 28], others use ≥24/HPF [11] and yet now -as it is in our institution- ≥ 15 eosinophils/HPF are accepted as in keeping with EO [10,22-24],. Eosinophil microabscesses with degranulation phenomena, if present, are further supportive of this diagnosis [25,26]. Many studies also indicate that in EO GORD needs to be excluded by normal pH monitoring and failure to respond to high dose proton pump inhibitor therapy [8,9,10].

In addition to the presence of eosinophils, basal cell hyperplasia and DIS have also been reported to be a frequent finding in EO [17, 25, 27-29]. The mechanism of DIS is through loss or rearrangement of intercellular glycoconjugates that "seal" the intercellular spaces, impairing sodium transport and causing water accumulation in the intercellular space [30, 31]. It is possible that eosinophilic infiltration causes mucosa cell damage and increased permeability that render the oesophageal mucosa susceptible to injury by gastric acid [25]. The presence of lamina propria fibrosis has also been described as a feature of EO and can be related to the occurrence of oesophageal stenosis [24,32].

The symptoms of EO are often difficult to distinguish from those of GORD thus posing a management dilemma [21]. These include vomiting, regurgitation, nausea, epigastric pain, heartburn, food aversion, dysphagia and failure to thrive [8,11,17,33, 34], all of which were present in our cases (see Table 1). Interestingly, the most common presenting features in our cohort were symptoms related to allergy (11/24). Dysphagia, which was present in only 5 of our cases, has been reported as the most common feature of EO in patients, both adults and children [15,16, 27,35].

We calculated the incidence of oesophageal eosinophilia among all cases of oesophagitis in our region to be 15%; although the incidence of EO is only 8.2%. This figure is higher than the 6.8% reported by Fox et al [35] and lower than the incidence documented by Lim et al [33]. The incidence of oesophageal eosinophilia in our region is 9/100 000 children, while the incidence of EO is half of this amount. This figure is less than the 2-4 per 10 000 children cited by Noel et al [14] and Rothenberg [17]. However, Straumann et al [14] reported an average annual incidence of 1.438 cases per 100 000 population throughout a 16 year observation period (range 0-6). This wide range of figures probably reflects the different population studied, differences in the diagnostic thresholds or under-recognition of the condition.

As previously seen in both adults and children [9, 14, 15], we have also noticed a marked increase in the number of cases of EO during the last few years (data not shown). As a matter of fact, a few years ago paediatric EO was not offered as a

diagnosis at all. It is likely that the rising incidence of EO could be due to increased recognition by both gastroenterologists and pathologists, and increasing number of endoscopy procedures performed in patients with upper gastrointestinal tract symptoms.

Classically, EO shows a male to female ratio of 3 to 1 [13, 15, 22, 27]. Our cohort failed to show a male predominance when EO and OS were analysed together (11 males : 9 females). However, this became apparent when only cases of EO were analysed (9 males : 4 females). .

The endoscopic appearance of EO is puzzling. Endoscopy is described as showing a ring-like oesophagus ("trachealization"), longitudinal linear furrows, friability or multiple small white papules suggestive of Candida [19,21,27, 28]. Interestingly, a study that addressed the correlation between endoscopic and histologic features demonstrated a striking accumulation of eosinophils in those biopsies taken from "white" fungal –looking areas [11]. In a paediatric series, white specks were described in approximately 30% of the cases and have been demonstrated to have a specificity of 95% [27,33] However, histologically severe EO can be associated with normal-looking mucosa at endoscopy [34]. Indeed, 9 of our cases had normal endoscopy.

Eosinophils are specialised cells that contain granule proteins, cytokines, platelet activating factors and leukotrienes. Their main role is traditionally thought to be combating parasitic infections, although they can be stimulated by a variety of other triggers such as tissue injury, allergens and viruses [17]. Their cytoplasmic granules contain a major basic protein, eosinophil cationic protein, peroxidase and a neurotoxin, that has been linked to the presence of dysphagia in many patients [36]. Some authors postulate that EO is an IL-5 driven inflammatory disorder of the oesophagus in which the aetiology could be linked to a combination of allergic and immunologic responses [11,12]. Allergic disorders are noted to be more common in patients with EO than in those with GORD, and the majority of patients show food and aeroallergen hypersensitivity identified by skin prick tests, food specific radioallergosorbent testing (RAST) or both [10,12,17, 37-39]. Eleven (46%) of our cases of oesophageal eosinophilia and 5/13 (38.5%) with EO had an associated allergic condition including asthma, eczema and cow milk protein allergy. One additional patient, although not showing clinical or histologic features of Coeliac disease, improved with a gluten-free diet. Coeliac disease and EO has been reported in 6 patients [40]. However, the eosinophilic infiltration in the oesophagus did not improve with gluten free diet in these cases [40]. The relevance of these

findings suggests the need to refer patients with EO for food allergy evaluation, a practice more commonly seen in paediatric than adult gastroenterology practice [41].

Eotaxins are a group of chemokines that are relatively specific for eosinophils and have a key role in the modulation of eosinophil accumulation in the gastrointestinal tract [17]. All eotaxins act on a selective transmembrane eotaxin CCR3 receptor primarily expressed on eosinophils. The same eotaxin CCR3 receptor is also expressed in gastrointestinal mast cells. Using genetic microarray expression profile analysis, Blanchard et al [18] demonstrated an approximately 50 fold overexpression of the gene of eotaxin-3 in the oesophageal mucosa of patients with EO compared to controls, suggesting a role of eotaxin in the pathogenesis of EO. The level of eotaxin-3 mRNA and protein strongly correlated with the number of eosinophils in the oesophageal mucosa. They also showed that mast cell gene expression is highly increased in EO. This correlates with the description of increased number of mast cells and mast cell degranulation in oesophageal biopsies of patients with EO [17,18, 23,25].

pH studies are generally considered useful to distinguish patients with EO from those with GORD [10,12,33]. However, and although our results are limited due to the retrospective nature of the study and the small number of cases, our findings -in agreement with those obtained by other authors [25,42,43]- suggest that there is overlap in the clinical and histological features of EO and GORD. Both ends of the spectrum are not so difficult to recognize but sometimes differentiating between these 2 conditions can be challenging [23]. Moreover, the possibility of an “overlap” group showing features of both conditions has also been demonstrated in previous studies [25, 42, 43]. The identification of those patients with overlap syndrome has therapeutic implications, as the addition of acid blockade and prokinetic agents can aid in healing by reducing exposure to acid which adds a further insult to the mucosa. Thirty percent (7/23) of our cases with oesophageal eosinophilia had evidence of GORD that improved with PPI treatment (“overlap syndrome”). This figure is approximately three-quarters of the 40% reported by Remedios et al. in adults with EO [42]. A recent report demonstrated resolution of oesophageal eosinophilia in three children with clinical symptoms as well as endoscopic features of EO following a course of proton pump inhibitor therapy [43], indicating that a large number of eosinophils can be seen in patients with GORD. The underlying proposed mechanism is that either EO causes a dysfunction of the lower oesophageal sphincter or an allergy type reaction of the oesophageal mucosa to reflux contents [16,28,42]. This would explain why as many as 94% of children with EO exhibit reflux symptoms refractory to proton pump inhibitor therapy [43]. In line with this

proposal, 3/13 cases that were clinically categorised as EO after 2 years follow –up demonstrated abnormal pH results but their symptoms did not improve with PPI treatment although disappeared or markedly improved with oral steroids and /or diet.

Rothenberg [17] indicated that the presence of 7 to 20 to 24 eosinophils/HPF likely represents a combination of GORD and food allergy, while more than 20 to 24 eosinophils/HPF is characteristic of EO. In our study, the number of intraepithelial eosinophils in the "overlap" group was between 16 to 57/HPF. Results from a recent histomorphological and immunohistological investigation performed in adult patients with EO concluded that the differential diagnosis of EO and GORD cannot be based on counts of eosinophils alone, and that the presence and intensity of secondary changes such as basal cell hyperplasia, DIS and vacuolization of keratinocytes would be helpful to better delineate these two conditions [25]. If EO is suspected, endoscopy with biopsy and histology is critical to achieve the correct clinicopathologic diagnosis.

In summary, we have presented the clinical, endoscopic histological and epidemiological features of oesophageal eosinophilia in our area. A significant proportion of patients had either EO or EO associated with GORD ("overlap syndrome"). Further studies need to be done in order to delineate the interaction between genetic factors, allergens and eosinophils. This would help to plan interventionist measures that could remedy the perceived worldwide increasing incidence of EO. The fact that after 2-4 years of treatment and follow-up, approximately 2/3 patients were diagnosed as classical EO and 1/3 as the so- called OS (GORD + OE) highlights the importance of keeping longitudinal data on these patients. As a consequence of the study, we have now introduced a register of patients with features of EO, aiming to gather long term follow- up data which could assist in the identification of further histological and/or clinical characteristics that would allow better management of the disease.

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Contributorship statement:

Dr Marta Cohen designed the study, acquired the histological data, analysed the histology and was the main author in the task of writing and approving the final version of the study.

Dr Prithviraj Rao: designed and acquired the clinical and endoscopic data; critically reviewed and improved the article and approved the final version to be published.

Mike Thomson: designed and acquired the clinical and endoscopic data; critically reviewed and improved the article and approved the final version to be published.

Dr Mudher Al Adnani: analysed the histology, contributed to writing the initial draft, critically reviewed the article and approved the final version to be published.

Data Sharing:

Technical appendix and dataset available from the corresponding author at Marta.Cohen@sch.nhs.uk

Consent was not specifically obtained as this was a retrospective study, but the presented data are anonymised and risk of identification is low.

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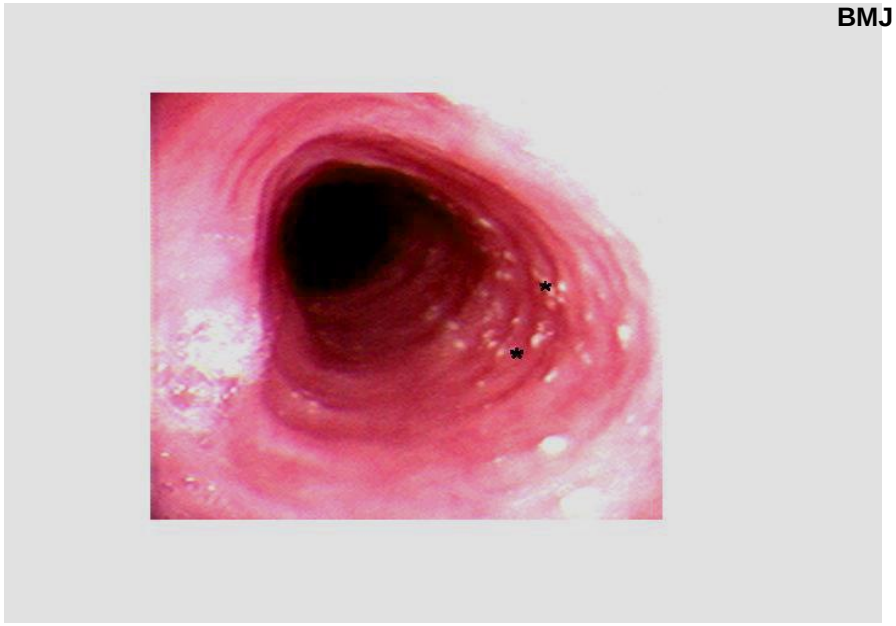
Figures 1

a: Endoscopic appearance of eosinophilic oesophagitis showing “trachealization” of the oesophagus and white speckles (*); b: Biopsy from the middle oesophagus depicting 53 eosinophils/high power field. These were located toward the surface of the mucosa (H&E x 40. Case 2); c: Dilatation of the intercellular spaces (curved arrow), papillary elongation and basal cell hyperplasia were frequent changes present in biopsies with eosinophilic oesophagitis (H&E x 40. Case 4); d: Microabscesses containing more than 4 eosinophils present near the surface of the mucosa (arrow) (H&E x 40. case 10).



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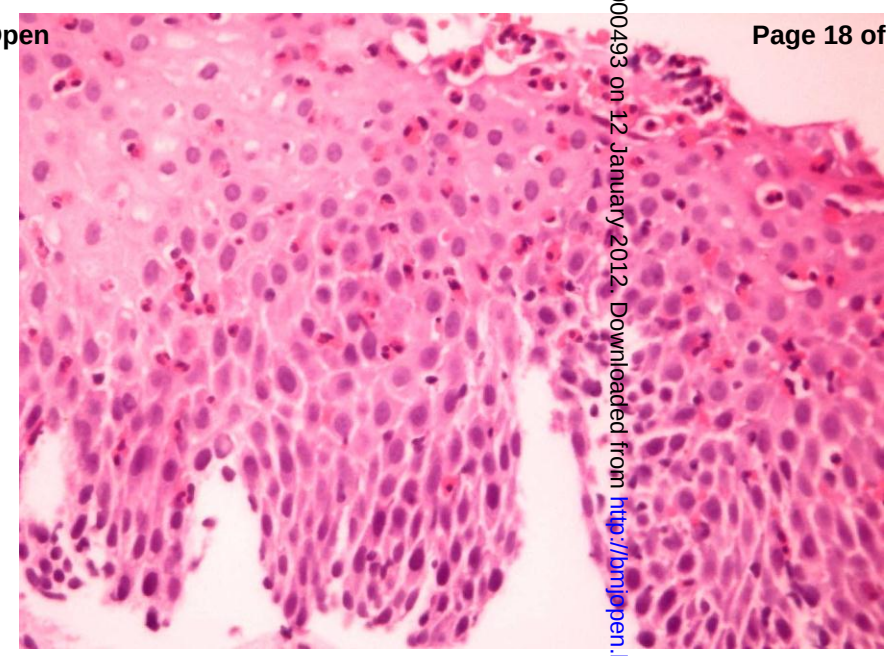

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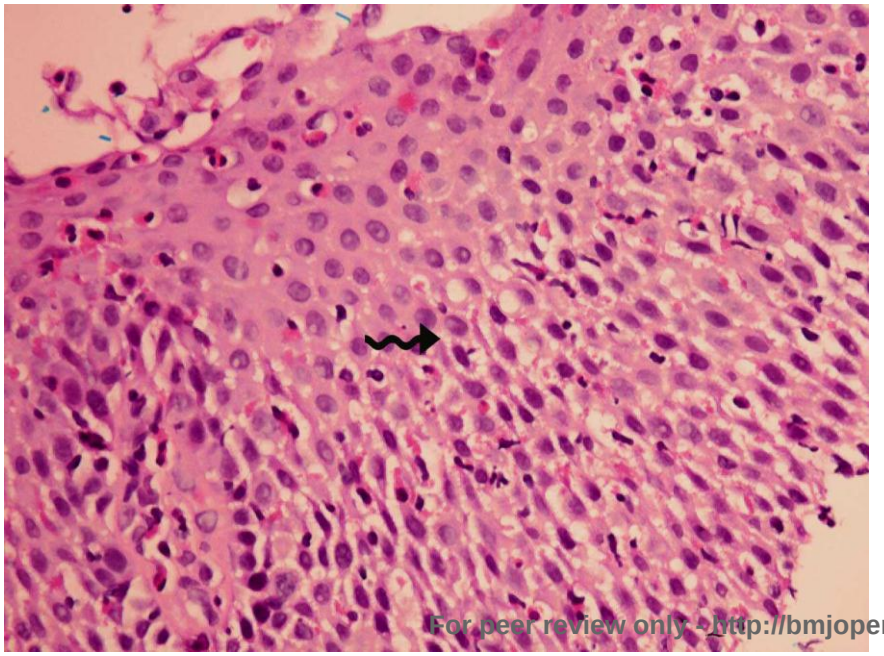

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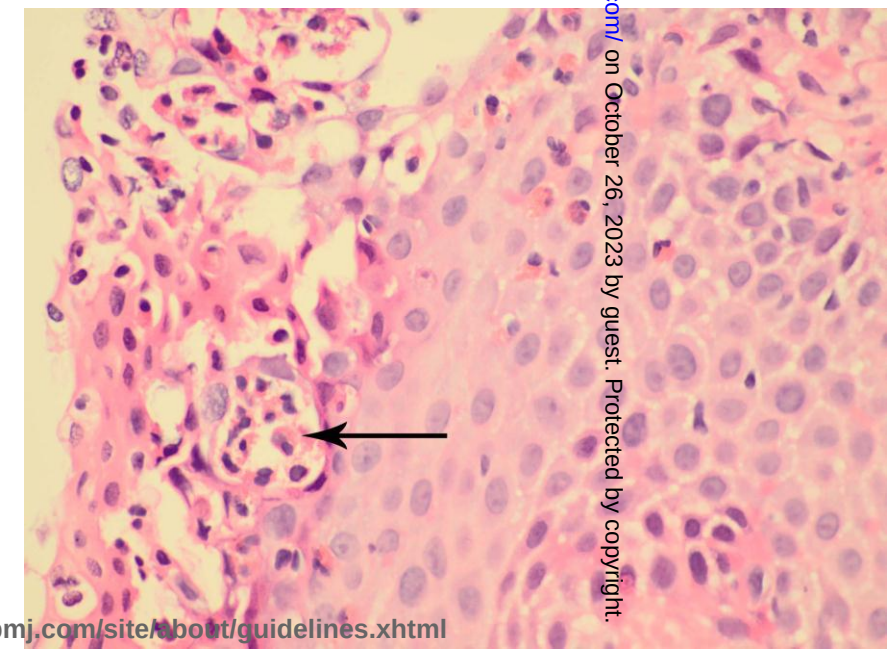

d

Table 1: Demographic features, clinical history and endoscopic characteristics of the cohort children with oesophageal eosinophilia on histology. Pre and post-biopsy diagnosis and initial clinical management of the cohort

Case	Age	Sex	History	Endoscopy	Clinical Dx pre-biopsy result	Clinical diagnosis (pre-biopsy)	PPI trial (pre or post biopsy)	pH study	Clinical diagnosis post biopsy
1	23 m	M	Allergy. Eczema. Asthma. Feeding problems	Corrugated. Furrowing. Trachealization	EO	EO	Y (post)	Normal	EO
2	44 m	M	Dysphagia. Intractable asthma	Corrugated. Furrowing	EO	EO	Y (post)	Normal	EO
3	35 m	F	Reflux. Food aversion	Furrowing. Ridging	GORD	GORD	Y (pre)	Normal	EO
4	51 m	F	Failure to thrive. Feeding problems. Developmental delay. Epilepsy	Normal	GORD	GORD	Y (pre)	ND	EO + GORD (OS)
5	9 y	F	Poor weight gain. Epigastric pain	Normal	GORD	GORD	Y (pre)	Normal	EO
6	19 m	M	No weight gain. Poor appetite	Normal	?GORD or non - ulcer dyspepsia	?GORD or non- ulcer dyspepsia	Y (post)	ND	EO
7	39 m	F	Feeding problems. Russell Silver syndrome. Food aversion.	White speckles. ?Candida oesophagitis	?GORD	?GORD	Y (pre)	ARI of 13%	EO + GORD (OS)
8	15 y	M	Epigastric pain	Severe oesophagitis	?GORD	?GORD	Y (pre)	ArRI of 8.8%	EO + GORD (OS)
9	6 m	F	Vomiting and mucosy diarrhoea. Asthma.	No information available	GORD	GORD	Y (pre)	ARI of 19.6%	EO + GORD (OS)
10	15 y	M	Dysphagia. GORD	Normal	?GORD	?GORD	Y (pre)	Normal	EO + GORD
11	23 m	M	Allergy. Eczema. Asthma. Feeding problems	Furrowing. Trachealization	?EO	?EO	Y (post)	ND	EO
12	15 m	F	?GORD	Normal	?GORD	?GORD	Y (post)	Normal	EO
13	13 y	M	Heartburn. Difficulty swallowing solids/liquids. Family history of peptic ulcer.	Furrowing	?GORD	?GORD	Y (pre)	AR Iof 12.2%	EO + GORD (OS)
14	16 m	F	? Cow milk protein intolerance	Normal	?CMPA	?CMPA	Y (post)	Normal	EO
15	12 y	M	Asthma. Eczema. Food bolus obstruction.	Furrowing	Achalasia of the cardia. ?EO	Achalasia of the cardia? EO	Y (pre)	ND	EO

Case	Age	Sex	History	Endoscopy	Clinical Dx pre-biopsy result	Clinical diagnosis (pre-biopsy)	PPI trial (pre or post biopsy)	pH study	Clinical diagnosis post biopsy
16	11 y	M	Food bolus obstruction	Normal. Pre-pyloric Ulcer	Gastritis	Gastritis	Y (pre)	ND	EO
17	12 m	F	?CMPA	?Candida esophagitis	?CMPA	?CMPA	Y (post)	ARI of 16.8%	EO + GORD (OS)
18	14 y	F	Asthma. Eczema.	Furrowing	?EO	?EO	Y (post)	ARI of 10.7%	EO + GORD (OS)
19	6 m	M	Failure to thrive. Diarrhoea. ?Protein loosing enteropathy	Normal	Likely CMPA	Likely CMPA	ND	ND	EO
20	42 m	F	Abdominal pain. ?Coeliac disease. Low IgA. Poor weight gain.	Normal	Iron deficiency aneamia ?Coeliac disease	Iron deficiency aneamia ?Coeliac disease	ND	ND	EO
21	14 y	M	Diarrhoea	Furrowing	?EO	?EO	Y (pre)	ND	EO
22	10 y	F	Heartburn. Reflux. Vomiting.	Trachealization	?GORD	?GORD	Y (pre)	173 reflux episodes/ 24 hours (normal is < 75)	EO + GORD (OS)
23	16 m	M	Vomiting	Esophagitis	?GORD	?GORD	Y (pre)	Normal	EO
24	11 m	M	Vomiting. Failure to thrive. Medullobla stoma in remission	Corrugated and furrowed esophagus. Trachealization	?EO. ?GORD	?EO. ?GORD	Y (pre)	ND	EO

Dx: Diagnosis; m: Months; M: Male; EO: Eosinophilic oesophagitis; F: Female; GORD: Gastro-oesophageal reflux disease; y: Year; CMPA: Cow milk protein allergy.
PPI: Proton Pump Inhibitor ; EO: Eosinophilic oesophagitis; Y: Yes ; GORD : Gastro-oesophageal reflux disease ; OS : Overlap syndrome ; CMPA: Cow milk protein allergy ; N : No. ND : Not done. ARI : abnormal reflux indez

Case	Clinical Dx pre-biopsy	E/HPF (Biopsy site)	DIS	Basal cell hyperplasia	Cell vacuolisation	Squiggle cells	Papillary elongation
1	EO	5/hpf (P) 33/hpf (D)	+	+	+	Not increased	+
2	EO	55/hpf (P,M,D) *	+++	+++	+++	Not increased	+++
3	GORD	16/hpf (un)	+++	+++	++	Increased	++
4	GORD	50/hpf (un)	+++	+++	++	Not increased	+++
5	GORD	40/hpf (D)	No	+	No	Not increased	No
6	?GORD or non- ulcer dyspepsia	22/hpf (un)	+++	++	++	Increased	No
7	?GORD	46/hpf (un)	+++	+++	+++	Not increased	+++
8	?GORD	30/hpf (P, D)	+++	+++	+++	Increased	+++
9	GORD	0/hpf (P) 24/hpf (D) *	+++	+	+	Not increased	+
10	?GORD	40/hpf(D) *	+++	+++	+++	Increased	+++
11	?EO	30/hpf(P) 50/hpf(D)	++	++	++	Not increased	++
12	?GORD	35/hpf(D)	++	++	++	Not increased	++
13	?GORD	30/hpf(P) 20/hpf(M) 20/hpf(D)	++	++	++	Mild increase	++
14	?CMPA	4/hpf(P)	0	0	0	Not increased	0
		35/hpf(D)	+++	+++	+++	Not increased	+++
15	Achalasia cardia ?EO	25/hpf (P) 34/hpf(D)	++	++	++	Not increased Increased	+++
16	Gastritis	40/hpf(un)	+++	+++	+++	Not increased	++
17	?CMPA	24/hpf(un) *	++	++	++	Not increased	++
18	?EO	26/hpf(P) 43/hpx(D)	+++	+++	+++	Increased Not increased	+++
19	Failure to thrive	21/hpf(un)	+	+	+	Not increased	0
20	Iron deficiency aneamia ?coeliac disease	25/hpf(un)	+	0	0	Not increased	0
21	?EO	15/hpf(un)	+	+++	+	increased	+++
22	?GORD	16/hpf(P)	0	+	0	Increased	++
		57/hpf(D)	+++	+++	+++	Increased	+++
23	?GORD	45/hpf (un)	+	+	+	Not increased	0
24	?EO ?GORD	46/hpf	+++	++	++	Increased	+

Table 2: Initial clinical diagnosis (pre - biopsy) and histologic features present in the cohort group.

Dx: Diagnosis; E/HPF: Eosinophils/ high power field (40x); DIS: Dilatation of intercellular space; EO: Eosinophilic oesophagitis;
P: Proximal; M: Middle; D: Distal; un: Unknown; *: Microabscesses; +: Mild; ++: Moderate; +++: Marked;
GORD: Gastro-oesophageal reflux disease; CMPA: Cow milk protein allergy.

Table 3: Clinical diagnosis, treatment and follow up of up to 4 years.

Case	Pre-treatment Clinical Diagnosis (after biopsy)	Treatment & Follow-up (2009-2011)	Final clinical diagnosis
1	EO	Initial histological response to Anti-IL 5 for up to 6 months but then rebound EO warranting treatment with 6 weeks of exclusive Elemental feed with good clinical and histological response. Symptoms have now rebound and currently on introduction of dietary protein. Feed aversive behaviour. Atopy.	EO
2	EO	Initial clinical and histological response to egg, wheat, banana and nut free diet. However, with worsening asthma his symptoms have re-surfaced i.e. vomiting and low appetite with some clinical response to Leukotriene receptor antagonist.	EO
3	EO	Exclusion diet. Gradual improvement of symptoms and at discharged from care at 2 years.	EO
4	EO+ GORD (OS)	Clinical improvement in symptoms with long term PPI and Domperidone at 2 year follow up. Was gradually being weaned off PPI at 2 years follow up.	GORD (OS)
5	EO	Short term P.P.I and gradually weaned off by 1 year of age.	GORD (OS)
6	EO	Some clinical response to dairy free diet but then went onto develop Feed aversive behaviour and slow transit constipation on follow up at 2 yrs. Parents not keen on re-assessment scope as dietary restriction very difficult due to behavioural difficulties.	EO
7	EO + GORD (OS)	Had a gastrostomy. With improvement in nutrition and weight gain, significant improvement in asthma and gastrointestinal symptoms and discharged from care at 2 years follow up.	EO
8	EO+ GORD (OS)	PPI treatment for 6 months with resolution of symptoms.	GORD (OS)
9	EO+GORD (OS)	Initial clinical response to 6 week elemental feed regime and gradual re-introduction of proteins in diet over 3 months. Kept on PPI for 1 year and weaned off by 2nd year. No gastrointestinal or respiratory symptoms at 2 year follow up.	GORD (OS)
10	EO+ GORD (OS)	Stayed on PPI with partial control of symptoms and care then transferred to	GORD (OS)

		adult gastroenterologist.	
11	EO	Given a trial of PPI with no improvement in symptoms of vomiting, feed aversion and eczema. Then was found to have multiple food allergies on RAST testing. At 6 months of age, put on 6 protein free diet (milk, soya, egg, nuts, fish, wheat) with complete resolution of symptoms by 9 months. On re-introduction was symptomatic with milk, soya, eggs and wheat but asymptomatic with other nuts and fish . Discharged from gastro follow up at 14 months.	EO
12	EO	EO identified at the time of gastrostomy insertion along with significant acid reflux . Trial of PPI had some improvement but no resolution. Later Neocate and dairy/Soya free diet with both clinical and histological resolution at 9 months post diagnosis . She then went into adoption and care was transferred.	GORD (OS)
13	EO + GORD (OS)	Persistence of symptoms despite of topical steroids. At 2-year follow up, was commenced on oral steroids and had a clinical response to his dysphagia.	EO
14	EO	Subsequently developed eosinophilic gastroenteritis. Excellent response to dairy free diet on follow up.	Eosinophilic gastroenteritis
15	EO	Initial poor response to topical Budesonide. Subsequently symptomatic improvement on 6 protein elimination diet.	EO
16	EO	Partial response to PPI but had PEG inserted during the same procedure. After 6 weeks trial of PPI, went onto Neocate (elemental feed) for 6 weeks . At 18 months dairy and soya re-introduced in diet with tolerance.	EO
17	EO + GORD (OS)	Lost to follow –up.	
18	EO + GORD (OS)	Partial response to PPI alone and then subsequently put on dairy free diet with clinical improvement in symptoms. Gradually weaned off PPI and at 2 years back onto dairy with no recurrence of symptoms.	GORD (OS)
19	EO	Subsequently developed eosinophilic gastroenteritis. Good response to dairy, soya and wheat free diet.	Eosinophilic gastroenteritis
20	EO	Persistently positive Coeliac serology. Re-scoped after 18 months on a gluten containing diet with no evidence of Coeliac disease but persistence of EO. Symptoms of pain did not improve on dairy or wheat free diet and was put on topical Budesonide with some positive response.	EO

21	EO	No upper gastrointestinal l symptoms after histological diagnosis. Only had intermittent diarrhoea which was subsequently diagnosed as Irritable bowel syndrome and responded well to Mebeverine and Loperamide.	Irritable bowel syndrome
22	EO + GORD (OS)	Initial lack of response to PPI and anti-histamine but at 2 year follow -up responded to dairy free diet.	EO
23	EO	Improvement on dairy and soya free diet. Symptoms appear to be re-surfacing on reintroduction of soya. Due to have a repeat endoscopy.	EO
24	EO	No improvement in symptoms on dairy free diet and some response to Topical Budesonide gel. Lost to follow-up	EO

PPI: Proton Pump Inhibitor ; EO: Eosinophilic oesophagitis; IL-5: Interleukin-5; GORD : Gastro-oesophageal reflux disease ; OS : Overlap syndrome ; RAST test: Radioallergosorbent test.

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any prespecified hypotheses	5
Methods			**5**
Study design	4	Present key elements of study design early in the paper	5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	5
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	NA
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*NA*
Bias	9	Describe any efforts to address potential sources of bias	NA
Study size	10	Explain how the study size was arrived at	
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	5
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	NA
		(*b*) Describe any methods used to examine subgroups and interactions	NA
		(*c*) Explain how missing data were addressed	NA
		(*d*) If applicable, explain how loss to follow-up was addressed	NA
		(*e*) Describe any sensitivity analyses	NA
Results			6-7

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	6,7
		(b) Give reasons for non-participation at each stage	NA
		(c) Consider use of a flow diagram	NA
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Table 1
		(b) Indicate number of participants with missing data for each variable of interest	Table 1
		(c) Summarise follow-up time (eg, average and total amount)	Table 3
Outcome data	15*	Report numbers of outcome events or summary measures over time	3
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	6,7
		(*b*) Report category boundaries when continuous variables were categorized	NA
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	NA
Discussion			7-12
Key results	18	Summarise key results with reference to study objectives	
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	7-12
Generalisability	21	Discuss the generalisability (external validity) of the study results	7-12
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	NA

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.