MONASH University

The Role of Food and Aeroallergens in Eosinophilic Oesophagitis
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A thesis submitted for the degree of *Doctor of Philosophy* at
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Declaration

I hereby declare that this thesis contains no material which has been accepted for the award of any other degree or diploma at any university or equivalent institution and that, to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

Student signature: Hamish Philpott　　　　**Date** ▮ 7/6/16

The undersigned hereby certify that the above declaration correctly reflects the nature and extent of the student's and co-authors' contributions to this work. In instances where I am not the responsible author I have consulted with the responsible author to agree on the respective contributions of the authors.

Main Supervisor signature:　　　　**Date** ▮

Peter Gibson

Preface

1.The serum specific IgE tests described in chapter 6 were performed by Eastern Health Department of Pathology.

2.The serum IgG tests described in Chapter 6 were performed by Healthscope Pathology (functional division).

3. The esophageal tissue was processed and haematoxylin and eosin, as well as Masson's trichrome stains were performed by Eastern Health Department of Pathology.

4. Histological assessment of oesophageal tissue leading to the determination of eosinophil counts, and the size of esophageal tissues (as discussed in chapters 4, 6 and 7) was initially performed by the Eastern Health Department of Pathology (Patrick Hosking) or by Melbourne Pathology (Prof Prithi Bhathal).

The remainder of the work was performed by myself using clinical facilities at The Alfred hospital and Eastern Health group of hospitals, as well as the Eastern Health Laboratory Box Hill (for flow cytometry) and The Department of Pharmacology (Monash University Clayton) for histopathology including immunohistochemistry.

Note regarding spelling, abbreviations, referencing and file format.

This thesis is largely based upon published works that have had to meet a range of publication formats including American or English spelling. An obvious example is the word esophagus (American) or oesophagus (English). To meet these editorial standards, abbreviations are sometimes applied, such as in the case of proton pump inhibitor responsive oesophageal eosinophilia (PPI - REE). For ease of reading in the accompanying text, I have tried to minimise abbreviations. Finally, an integrated reference list is supplied that applies to the published works and accompanying unpublished data combined into the chapters. The only exception to this principle is where there has been external correspondence (see 5.5 and 5.7) in the form of letters to the editor in relation to our published work. That is, these letters written by other authors have been left in their original form and references are not integrated in this case.

Readers of this thesis may choose to examine either the Portable Document Format (PDF) of the published works (appendices 1-8) or the word documents, the latter being integrated as chapters.

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ABSTRACTS AND PUBLICATIONS

Journal publications

1.Risk factors for eosinophilic esophagitis. Philpott H, Nandurkar S, Royce SG, Thien F, Gibson PR. Clin Exp Allergy. 2014 Aug;44(8):1012-9. doi: 10.1111/cea.12363. Review. PMID: 24990069

2.Eosinophilic esophagitis: a clinicopathological review. Philpott H, Nandurkar S, Thien F, Gibson PR, Royce SG. Pharmacol Ther. 2015 Feb; 146:12-22. doi: 10.1016/j.pharmthera.2014.09.001. Epub 2014 Sep 6. Review. PMID:25200122

3. Seasonal recurrence of food bolus obstruction in eosinophilic esophagitis. Philpott H, Nandurkar S, Thien F, Bloom S, Lin E, Goldberg R, Boyapati R, Finch A, Royce SG, Gibson PR. Intern Med J. 2015 Sep;45(9):939-43. doi: 10.1111/imj.12790. PMID: 25871330

4.Letter: seasonality in eosinophilic oesophagitis and food bolus obstruction - what about recurrent episodes? Philpott H, Nandurkar S, Royce SG, Gibson PR. Aliment Pharmacol Ther. 2015 Oct;42(7):936. doi: 10.1111/apt.13313. PMID:26331556

5.Ultrathin unsedated transnasal gastroscopy in monitoring eosinophilic esophagitis. Philpott H, Nandurkar S, Royce SG, Gibson PR.J Gastroenterol Hepatol. 2015 Oct 1. doi: 10.1111/jgh.13173. [Epub ahead of print] PMID:26426817

6.What gastroenterologists should know about testing patients with eosinophilic esophagitis for food allergies. Philpott H, Royce S, Thien F, Nandurkar S, Gibson P. Clin Gastroenterol Hepatol. 2015 May;13(5):1029-30. doi: 10.1016/j.cgh.2014.08.008. Epub 2014 Aug 12. No abstract available. PMID:25130938

7.IgG and EoE: too soon for a paradigm shift away from IgE.Philpott H, Royce S, Nandurkar S, Thien F, Gibson P.Gastroenterology. 2015 Feb;148(2):453-4. doi: 0.1053/j.gastro.2014.09.048. Epub 2014 Dec 24. No abstract available. PMID:25543056

8.Letter: an allergic phenotype in patients with eosinophilic oesophagitis and asthma. Philpott H, Gibson PR, Thien F, Nandurkar S. Aliment Pharmacol Ther. 2013 Apr;37(7):755-6. doi: 10.1111/apt.12225. No abstract available. PMID:23458535.

9. A prospective open clinical trial of a proton pump inhibitor, elimination diet and/or budesonide for eosinophilic oesophagitis. Philpott H, Nandurkar S, Royce SG, Thien F, Gibson PR. Aliment Pharmacol Ther. 2016 May;43(9):985-93. doi: 10.1111/apt.13576. Epub 2016 Mar 4. PMID:2693957

10.Reply to Editorial; Management of Eosinophilic Oesophagitis. Efficacy versus Effectiveness. Philpott H, Gibson PR. Alimentary Pharmacology and Therapeutics 2016 (in press – APT 13666).

11. Authors response; Avoiding misconceptions about elimination diet for eosinophilic oesophagitis. Philpott H, Gibson PR. Alimentary Pharmacology and Therapeutics 2016 (in press – APT 13644).

12.Allergy tests do not predict food triggers in adult patients with eosinophilic oesophagitis. A comprehensive prospective study using five modalities.Philpott H, Nandurkar S, Thien F, Royce SG, Gibson PR. Alimentary Pharmacology and Therapeutics 2016 (in press – APT – 0437-2016).

Abstracts and presentations

1. Mo1831 Trans-Nasal Gastroscopy without Sedation for Eosinophilic Esophagitis; Safe, Effective and the Ideal Method of Assessment? Hamish Philpott, Peter R. Gibson, Sanjay Nandurkar, Simon G. Royce. Gastroenterology 2014, Vol. 146, Issue 5, S-665

2. S1716 Lower esophageal preponderance of eosinophilia induced by food triggers in Diet Responsive EoE; Time and PPI dependence and mechanistic insights to antigen exposure. Hamish Philpott, Simon Royce, Sanjay Nandurkar, Peter Gibson. Am J Gastroenterol 2015 Vol 110, supplement 1.

3. Instituting Modern Management of eosinophilic esophagitis in Australia: lessons from our first 65 patients using a structured clinical algorithm. H Philpott, S Nandurkar, PR Gibson. J Gastroenterol Hepatol 2014, Vol 29 (14) 14

4. Too Much, Too Soon. Elimination diet is effective in less than 1/3 of patients with Eosinophilic Esophagitis, durability of PPIs is questionable. H Philpott, S Nandurkar, S Royce, PR Gibson. Eastern Health Research Forum (Dec 3rd 2015)

5. Ultrathin unsedated transnasal gastroscopy in monitoring eosinophilic esophagitis Philpott, S Nandurkar, S Royce, PR Gibson. Eastern Health Research Forum (Dec 3rd 2015)

6. Significant heterogeneity in the management of food bolus obstruction and divergence from Ideal management: A large multicentre study. Hamish Philpott, Sanjay Nandurkar, Steven Bloom, Ray Boyapati, Andrew Finch, Rimma Goldberg, Peter Gibson. J Gastroenterol Hepatol 2012, Vol 27 (4) S 123

CONTENTS

and is restored with treatment.

elimination diet for eosinophilic oesophagitis – authors' reply.

CHAPTER 1

1.1

Abstract

Eosinophilic oesophagitis (EoE) is a chronic inflammatory condition that afflicts children and adults, and causes oesophageal narrowing and fibrosis, with the resultant clinical sequelae of dysphagia and food bolus obstruction events (FBOE), the latter often resulting is hospitalisation, emergency endoscopic removal and rarely but catastrophically oesophageal perforation. An increasing number of patients are being diagnosed with EoE, and this is independent of a growing awareness of this recently defined condition, but rather represents a true increase in the context of a global rise (in Western first-world countries) of allergic conditions *per se*.

The mainstays of medical treatment for EoE are indefinite PPI therapy or swallowed topical corticosteroids. These therapeutic assets are highly effective but they do not ultimately target the etiologic cause of EoE. A role for aeroallergens (pollens) and dietary allergens (food) in precipitating or causing EoE has been suggested in a number of studies, albeit limited by their retrospective nature and lack of control group (in the case of aeroallergens), the predominance of paediatric literature and variable use of adjunctive treatment such as proton pump inhibitors (PPIs) (in the case of food allergens). Highly restrictive elimination diets show promise in treating EoE. Unfortunately, the role of allergy tests in directing dietary therapy remains debatable and studies are again of a retrospective nature or utilise a small range of available tests. This means that even if dietary therapy works, that patients are required to undergo many gastroscopies during their treatment course, which is both inconvenient, expensive and has the associated risk of an anaesthetic. Thus, the need both to better understand the role of food and aeroallergens (with more comprehensive prospective studies), and to offer less invasive and thereby safer endoscopic surveillance is readily apparent.

1.2

Thesis outline

This thesis is largely based upon published works that were undertaken and completed during the period of study. Thus Chapter 2 is a literature review based on two published review articles, whilst Chapters 3,4,5 and 6 are experimental chapters based on published original scientific research papers. Appropriate framing text and discussion accompanies the published works to contextualise the findings and provide a coherent narrative. Chapter 7 is as-yet unpublished original research.

Chapter 2 is a literature review.

In Chapter 3, a retrospective case-control study was used - integrating case note and laboratory record reviews, along with postcode and atmospheric pollen data, to determine the role of aeroallergens (pollens) in precipitating FBOE.

Chapters 4 and 5 are clinical studies and serve as a platform for further chapters. In particular, Chapter 4 is a prospective clinical study of the use of ultrathin unsedated transnasal gastroscopy (UTEG) in patients with EoE. This clinical instrument facilitated the safe and convenient comparison of allergy tests (Chapter 6) in determining a response to dietary therapy (compared to the gold standard of esophageal biopsy). Patients were managed according to a structured clinical algorithm (Chapter 5) that triaged patients into treatment subgroups and enabled study of putative food allergens. Furthermore, comparative data pertaining to these treatment subgroups were generated.

In Chapter 6, five different measures of potential allergic/immune sensitisation to food and aeroallergens are studied, namely skin-prick test, skin-patch test, serum-specific IgE, basophil activation test (BAT) and food-specific IgG antibodies. Dietary therapy is instigated, and in

patients responding to food exclusion, foods are introduced sequentially and biopsies taken at gastroscopy, with these results (the gold standard) being compared to the allergy tests.

Chapter 7 examines the histopathological and immunohistochemical correlates of food-antigen withdrawal and re-exposure. Region-specific and time-dependent changes in barrier integrity (desmoglein and caveolin) and disease-specific inflammatory mediators (mast cell tryptase, IgG and IgE antibodies) are considered.

Chapter 8 discusses the results of the thesis in the context of current scientific knowledge and considers future research directions.

1.3

Aims and Objectives

The broad aim of this thesis was to determine the role of food and aeroallergens in EoE. This research was undertaken on human subjects in a clinical setting. The specific aims, therefore, can be viewed as addressing issues of disease pathogenesis or of clinical management.

Questions of disease pathogenesis

1.Do aeroallergens cause EoE?

2.Do food allergens cause EoE?

3.Do measures of systemic immune activation ('allergy') detect the localised food antigen driven inflammatory infiltrate of EoE?

4.What is the nature, timing and distribution of the acute inflammatory response to food antigen exposure in patients with quiescent EoE?

5.Do patients with active EoE, or EoE treated with PPI, diet or budesonide (respectively) differ compared to healthy controls with respect to barrier integrity and inflammatory chemokine expression?

Clinically-directed aims

1.Determine if elevated levels of environmental allergens (pollens) precipitated food bolus obstruction events in patients with EoE.

2.Determine the safety, tolerability and efficacy of ultrathin unsedated transnasal gastroscopy in patients with EoE undergoing repeated gastroscopy with tissue biopsy.

3.Determine if skin prick, skin patch, serum food or aeroallergen specific IgE, serum food specific IgG antibodies and the basophil activation test, can accurately predict dietary triggers in patients with EoE.

4.Determine if dietary elimination followed by food reintroduction is a feasible treatment for patients with EoE.

CHAPTER 2

2.1.

Overview

This chapter contains two published review articles that address the pathogenesis and treatment of EoE. The first, entitled 'Risk factors for eosinophilic oesophagitis', defines the condition, details established and putative risk factors and concludes that indirect evidence only (e.g., increased numbers of patients with EoE are diagnosed in spring/summer when aeroallergens are at high atmospheric concentration) supports the role of aeroallergens in causing EoE, whilst direct evidence supports food antigens as causative (e.g. elemental diets can resolve oesophageal eosinophilia). The second, entitled 'Eosinophilic oesophagitis – a clinicopathological review', delves deeper in examining the nature of the inflammatory process and the mechanisms whereby food and/or aeroallergens may cause the condition. A further consideration, namely the nature of innate or acquired defects in barrier integrity and the mechanism of action of proton pump inhibitors is also discussed.

2.2

Risk factors for eosinophilic esophagitis. Philpott H, Nandurkar S, Royce SG, Thien F, Gibson PR. Clin Exp Allergy. 2014 Aug;44(8):1012-9. doi: 10.1111/cea.12363. Review. PMID: 24990069

Summary

Eosinophilic esophagitis (EoE) is a chronic antigen driven disease, whereby food and/or aeroallergens result in inflammation and luminal narrowing, and the clinical symptoms of dysphagia and food bolus obstruction events (FBOE). Established risk factors are male gender, Caucasian race and atopy. Increased risk amongst family members, and a single nucleotide polymorphism (SNP) in a gene coding thymic stromal lymphopoietin (TSLP) on the pseudoautosomal region of the X and Y chromosomes supports a genetic predisposition. Environmental factors including the timing and nature of food and aeroallergen exposure to the developing immune system may be important, whilst esophageal barrier function integrity and the influence of microbiota are worthy of future research.

Introduction

Eosinophilic oesophagitis is conceptualised as a chronic antigen-driven disease, whereby food and/or aeroallergens stimulate an eosinophil-rich infiltrate in the oesophagus that produces the clinical syndrome of dysphagia, feeding difficulties (in young children) and food bolus obstruction[1]. EoE was first recognised as a distinct clinical entity only recently in 1993[2]. The epidemiology, and the basic scientific and clinicopathological data are hence somewhat limited, and, to date, the most clearly defined risk factors for EoE are gender (male predominance), race (mainly a disease of white Caucasians) and atopy (elevated serum IgE to common aeroallergens and other allergic conditions, asthma, seasonal rhinitis and atopic dermatitis). Other putative risk factors include alterations in barrier function (e.g. from gastro-oesophageal reflux disease (GERD), variation in the nature and timing of oral antigen exposure (e.g. secondary to infant feeding practices, proton pump inhibitor use and commercial food processing) and variation in the nature and timing of aeroallergen exposure (seasonal, geographical and secondary to migration and factors relating to fibrous remodelling, e.g. ACE gene polymorphisms and TGF-β polymorphisms).

The reasons for this gender and racial difference are not known, but could include genetic factors transferred on the sex chromosomes, mitochondrial DNA or possibly the pathoplastic effects of sex hormones on inflammation and fibrosis[3-6]. A history of EoE in a first-degree relative has been reported in 23–37% of cases (adult vs. paediatric), supporting a genetic basis, although a more modest familial trend was demonstrated recently (about 3%) [7, 8]. Genetic studies have been useful in correlating single nucleotide polymorphisms (SNPs) with a propensity to develop atopic conditions, including EoE. Notably, thymic stromal lymphopoietin (TSLP), eotaxin 3 and filaggrin SNPs appear to predispose to EoE, and a SNP for TGF-β predicts response to corticosteroids[9-11].

This review examines the basic scientific, epidemiological and clinical studies relating to the pathogenesis of EoE and attempts to highlight unexplored risk factors with a view to future research and therapeutic innovation (see Table 1).

Epidemiology – A Western disease on the increase?

The history of EoE is short. Prior to 1990s, only a few case reports of oesophageal eosinophilia had been reported, with Attwood, Levine and Saul, and Vitellas being amongst the first to suggest the existence of a distinct clinicopathological entity[2, 12, 13]. What is *not* known is if EoE is a disease of modern life, or if the apparent increase in the last 20 years is a result of awareness by clinicians and researchers armed with modern endoscopic equipment able to make the diagnosis with the mandatory oesophageal biopsy. For example, some patients who were once assumed to have gastroesophageal reflux disease (GERD) with stricture formation may now be called EoE. To date, several large uncontrolled retrospective studies from North America, Western Europe and Australia demonstrate an increasing incidence and/or prevalence of EoE[14-16]. Interestingly, other atopic conditions that are better characterised such as atopic dermatitis and food allergy *per se* have increased amongst children in the USA in the last 14 years[17].

Theories relating to the apparent increase in EoE are broad. They are mostly borrowed from research on other atopic conditions and relate to recent changes in living conditions and medical treatments, such as exposure to bacterial pathogens, moulds and animal antigens (i.e. the hygiene hypothesis), or a decrease of *Helicobacter pylori* infection in Western populations [18, 19]. Furthermore, use of PPI, a decrease in consumption of fresh fruit and vegetables and in increase in processed foods have also been suggested as causes for the apparent rise in allergic conditions including EoE[20, 21]. Whilst hypotheses abound, good quality prospective data collection in relation to EoE is needed before conclusions can be reasonably made.

Age, gender and genetics

EoE is a disease of both children and adults. The majority of cases diagnosed in childhood are between 5 and 10 years of age, although cases in very young children are seen[22, 23]. In adults, the mean age of diagnosis is in the late 30s, with almost all cases diagnosed before the age of 50[15, 24]. It is notable that amongst both children and adults, EoE mainly afflicts males with a male to female ratio of approximately 3:1 in most series[1, 25].

The distinction between adult and paediatric EoE may relate to the increased recognition of the condition, rather than two rigidly distinct entities. In other words, in the past, paediatric cases may have been missed and are now being diagnosed in adulthood. A recent prospective study suggests this is the case, demonstrating that EoE in more than 70% of paediatric cases remains active on transition to young adulthood over a period of 5 years' observation[26]. The authors suggest that both paediatric- and adult-onset conditions exist (as is the case with asthma), but that a significant number of current paediatric-onset cases will progress to adulthood in what is increasingly viewed as a chronic condition.

The male predominance of EoE is unique when compared to other apparently related conditions, such as asthma, atopic dermatitis and seasonal rhinitis, that share key similarities such as the eosinophilic infiltrate and atopy defined as an elevated IgE to common food or environmental allergens[17, 27, 28]. Classical food allergy (characterised by anaphylaxis or angioedema within minutes to hours of food ingestion) demonstrates a male predominance in early life that disappears or even slightly favours females in adulthood according to some studies[17]. Asthma again shows a male predominance in childhood that disappears later in favour of females[28]. Finally, atopic dermatitis favours females in both children and adults[27].

Thymic stromal lymphopoietin (TSLP) is a cytokine produced by epithelial cells that is central to the pathogenesis of EoE, as demonstrated by animal models and recent human studies[6, 9, 29]. Intriguingly, the gene coding for the TSLP receptor (TSLP-R, that is, cytokine receptor-like factor-2 or CRLF2) is found on a pseudo-autosomal region of the X and Y chromosomes (Xp22.3 and Yp 11.3), and statistical analysis has demonstrated that a single nucleotide polymorphism of this region predisposes male patients to develop EoE[6]. Furthermore, a SNP in the region coding TSLP itself (5q 22.1) predisposes to EoE[6]. Thus, TSLP-related inflammatory pathways may in part contribute to the gender predominance.

Other factors related to gender predominance can be considered, but remain unstudied. First, mitochondrial DNA is inherited from the mother, and it is notable that mitochondrial dysfunction has been attributed to result from allergen exposure in an animal airways model[3]. Furthermore, a maternal history of atopy may predispose to asthma to a greater extent than a paternal one, and a SNP in a region of mitochondrial DNA has been linked to elevated IgE levels and atopy[3]. Secondly, relaxin is a hormone present in large amounts in pregnancy, in small amounts in non-pregnant females of reproductive age and possibly also in males[30]. It may have the potential to decrease fibrosis in a range of organs, as demonstrated by animal models[31]. It is acknowledged that most patients with EoE never become pregnant (many paediatric patients and a male predominance in adulthood). A study of relaxin in human bronchial biopsies suggests an association with the remodelling process in asthma, whilst a greater expression of relaxin receptors in female compared with male cruciate ligament tissue has been cited as an explanation for greater tissue laxity in females[32]. Further research may allow the therapeutic use of relaxin in the future.

SNPs unrelated to gender have been implicated as risk factors for EoE[33]. Eotaxin-3, a cytokine expressed by epithelium, plays a central role in eosinophil recruitment to the oesophagus, and

a SNP (+2496 GG on chromosome 7) correlates with EoE development[34]. A SNP coding for filaggrin, an epithelial structural protein, has also been implicated in EoE, whilst a SNP coding for TGF correlates with response to inhaled corticosteroids (see below). Gene expression studies have also detailed a 'genetic signature or thumbprint' of EoE, useful in differentiating this condition from the more common GERD, although arguably such research may document the response to inflammation as opposed to causative sequences[10].

Table 1. Risk factors for EoE

ESTABLISHED RISK FACTORS	
Risk Factor	**Proposed Mechanism/s**
Male gender	TSLP on sex chromosomes[29], relaxin*[35]
Caucasian	Non X linked SNP's e.g. for Filaggrin, Eotaxin 3[36]
Atopy	IgE mediated inflammatory infiltration[37]
PUTATIVE RISK FACTORS	
Impaired barrier function (may due to GORD, be genetic, e.g. Filaggrin or due to e.g. altered microbiota)	Increased antigen exposure to esophageal mucosa[20]
Aeroallergens in spring/summer	Exposure of air passages leading to an inflammatory reaction and trafficking of eosinophils to the esophagus[33]
Impaired tolerance to food antigens*	Immune reaction, may be increased in infants not exposed to a wide range of foods, in those born by C section[38]
Commercially prepared foods*	Agglutinated proteins incite immune reaction[21]
Proton pump inhibitor use	Gastric pH is higher, and hence proteins are not denatured and greater antigen exposure may result[39]
Migration as an adult*	Novel antigens incite immune reaction
Increased fibrotic remodelling	Decreased relaxin expression, SNP's for TGF B, SNP's for ACE[32, 40]
Living in a temperate or arid climate	Low vitamin D levels and/or higher aeroallergen exposures[41]

*denotes a hypothesis generated from other related disease processes or anecdotal observation.

The timing and nature of food and aeroallergen exposures

The dominant theory pertaining to the likely pathogenesis of EoE is that food antigens are causative[42].The use of an elemental diet can eliminate eosinophilic infiltration in the oesophagus in up to 90% of children and 75% of adults, and the less restrictive six-food elimination diet is successful in approximately 65% of adults and children [1].Gonsalves et al. not only demonstrated endoscopic and histological recurrence of the condition following successful treatment with the six-food elimination diet, but also histological and in many cases observable endoscopic recurrence after reintroduction of the putative foods[24].

EoE can be viewed as a form of food allergy with distinct features, lacking the acute 'allergic' features (anaphylaxis or angioedema characteristic of classical food allergy or oral food allergy syndrome) but sharing the atopic profile of the suffers (that is, elevation in serum IgE to aero and/or food allergens, and frequent co-morbid atopic conditions such as asthma or rhinitis)[43].As food allergy may be considered a defect of immune tolerance, and antigen exposure is a factor in the development of tolerance, the timing and magnitude of antigen exposure in shaping the immune system (e.g., the type of extent of food and aeroallergen exposure) may be important in disease pathogenesis.

Much ongoing research is dedicated to factors influencing sensitisation and tolerance to food antigens with reference to classical IgE-mediated food allergy[17]. This may be of relevance to EoE, particularly given the observed increase in incidence over time (see below). For several decades, allergy-prevention guidelines have stressed late introduction of food antigens to infants. However, food allergy amongst children has increased during this period. Animal models have since suggested that both oral and cutaneous exposure to antigens in early life may be integral to achieving immune tolerance [17]. As a result, guidelines issued in the last few years have advocated cautious exposure to a wide range of foods from 4 months of age, whilst

maintaining breastfeeding as the primary source of nutrition. Breastfeeding during the first 6 months of life may decrease the risk of food allergies, although the mechanisms have not be delineated[44]. Also, the use of acid-suppressing medication may increase food allergen sensitisation, possibly by decreasing protein degradation of food antigens and increasing intestinal exposure to intact antigens[45].

It has also been proposed that aeroallergens may cause or contribute to the pathogenesis of EoE. The supportive data are limited to uncontrolled observational studies and an animal model. Case reports detail sudden symptomatic worsening following seasonal aeroallergen exposure, and sublingual immunotherapy has been hypothesised to both cause (precede the diagnosis) and cure (disappearance of EoE following the treatment of rhinitis) the condition[46-48]. Mishra et al. showed that ovalbumin-sensitised mice developed oesophageal eosinophilia in response to airway but not gastrointestinal rechallenge[49]. Almansa et al. and Moawad et al. both demonstrated a seasonal peak of EoE diagnosed at gastroscopy[33, 50]. The assertion of these studies is that patients with EoE present in spring/summer when aeroallergens are at their peak atmospheric concentration. Notable weaknesses of both studies are the lack of a control group and the fact that the case definition included all-comers (i.e. both newly diagnosed and past cases). Both were also retrospective and, hence, susceptible to recall bias.

In an attempt to address some of the methodological issues related to the study of potential seasonality in presentation of EoE, our group performed a retrospective study of all food bolus obstruction episodes occurring across five metropolitan hospitals over a period of 10 years. As food bolus obstruction events (FBOEs) are one of the key clinical features of EoE, and as FBOEs are caused by EoE or GERD in similar proportion, a control group as such (i.e., GERD) is hence included. In this study of 1135 individuals, cases of GERD and EoE were both evenly distributed across the year. Thus, amongst the 85 patients who were diagnosed with EoE, the

FBOEs were evenly distributed throughout the year. Again, the notable weaknesses are the retrospective nature of data collection. Also, disappointingly, few patients (5–45%) underwent biopsies at gastroscopy[51].

The role of aeroallergens in the pathogenesis of EoE may foreseeably be as a cofactor in some or many patients, with food antigens playing a more dominant role. The ability of an elemental diet to induce complete histological remission in a majority supports this assertion. As many patients with EoE have coexistent atopic disease (especially seasonal rhinitis), it is also possible that seasonal worsening of that disease may contribute to EoE by facilitating secondary trafficking of eosinophils to the oesophagus. This has been demonstrated both in the research setting, where exposure of the bronchioles to aeroallergens at the time of bronchoscopy can induce nasal mucosal eosinophilia, and clinically in relation to the control of asthma and rhinitis[52, 53]. The 'common airway' hypothesis asserts that control of rhinitis improves asthma therapy.

Geographical disparity in the prevalence of EoE has been suggested by a retrospective epidemiological study examining climatic regions in North America[54]. Arid/temperate zones manifested higher rates of diagnosis of EoE on endoscopic biopsy. Tropical regions manifested the fewer relative diagnoses of EoE, using total cases undergoing oesophageal biopsy as the reference[54]. The reason for a geographical disparity is not known, but could include variations in atmospheric pollen counts, which are higher in low humidity zones, and/or regions where putative tree or grass pollens are abundant. Alternatively, the role of serum vitamin D levels has been proposed as a protective factor in tropical environments in atopic conditions per se, using adrenaline auto-injectors or hospital admissions for anaphylaxis as a surrogate marker[55]. It is notable that conflicting data concerning the geographical influence on atopy exist; a recent

Australian study demonstrated that distance away from the equator and, therefore, sunlight actually predisposes to atopy[56]. Much remains to be clarified.

The effects of migration on the development of EoE have not been explored. Migration between countries and climate zones has been proposed as a factor influencing the development and severity of other atopic conditions, particularly rhinitis. Asian immigrants to Melbourne, Australia were observed to develop asthma more commonly than Australian-born non-Asian and Australian-born Asian populations[57]. The length of stay positively correlated with the likelihood of symptom development. The new occurrence of rhinitis amongst children who had migrated to Italy also correlated with the length of time living in the new region. We have observed in our own patient group of adult patients a disproportionately large number who have migrated from overseas in young adulthood. The scientific validity of this observation cannot be asserted; however, it is conceivable that exposure to novel (to the migrant) region-specific aero or food allergens may precipitate the disease. Further study seems warranted.

Barrier function and microbiota

Impairments in the structural integrity of the oesophageal mucosal barrier may predispose to and/or perpetuate EoE. Research focussed on EoE directly is lacking, but much attention and importance have been placed on variations in epithelial barrier function in patients with atopic dermatitis. Filaggrin is a structural protein of critical importance in the development of dermatitis. In health, filaggrin is found in the stratum corneum and binds keratin and intermediate filaments[11]. The gene for pro-filaggrin, which is subsequently phosphorylated to filaggrin, is located on chromosome 1. Specific mutations within this locus have been described to cause or greatly increase the risk of ichthyosis vulgaris and, of great relevance to EoE, atopic dermatitis[11]. As human skin is composed of stratified squamous keratinising epithelium, the importance of filaggrin in keratin attachment is apparent. However, the squamous epithelium

of the oesophagus does not undergo keratinisation to a significant extent. Yet, both of these conditions, along with asthma, correlate with an SNP coding for filaggrin (R501X and 2282 del4), albeit to a lesser extent in the case of asthma and EoE[58, 59]. Blanchard demonstrated that whilst SNPs for filaggrin correlated with the development of EoE (independent of the risk of atopic dermatitis), that filaggrin expression measured by mRNA analysis was decreased in all patients, and was influenced by interleukin-13 production. Hence, it remains to be seen if the association between filaggrin and EoE is more than a confounding factor, simply demonstrating the coexistence of EoE with atopic dermatitis, or occurring as a secondary response to eosinophilic inflammation, or if instead there is a hitherto unknown role for filaggrin in the oesophagus that contributes to the pathogenesis of EoE. The evidence thus far suggests that filaggrin is not of central importance in EoE as immunohistochemical staining of the oesophagus has failed to reveal filaggrin expression[11].

Proteins aside from filaggrin, such as occludins, claudins and cadherins, may be important in EoE. Patients with atopic dermatitis have demonstrable decreases in the production of tight junction proteins, claudin-1 and claudin-23[60]. Furthermore, single nucleotide polymorphisms in claudin-1 have been demonstrated in patients with AD, implying that decreased protein expression results in disease, rather than being a result of the dermatitis [60]. A deficiency of the cadherin desmoglein 1 may result in impaired barrier integrity in patients with EoE, and the expression of desmoglein 1 is influenced by interleukin 13, that is, in turn commonly elevated in this patient group[61]. Patients with connective tissue disease have been noted to have an eightfold increase in prevalence of EoE, the cause unknown, but conceivably could relate to barrier function or may relate to allergic inflammation (see TGF-β). It is interesting to note that disruption of tight junction proteins is thought to contribute to the increase in size of intracellular spaces in patients with GERD and that treating patients with EoE using proton pump inhibitors improves the histology in many patients and is considered a first-line therapy[62,

[63]. It could be hypothesised that GERD may cause or precipitate EoE[39]. Abnormalities in tight junction protein and hence barrier function warrant further study.

Microbiota of the oesophagus and possible effects on barrier function and immune tolerance may be important in the pathogenesis of EoE. Limited studies in patients with GERD, Barrett's oesophagus and oesophageal adenocarcinoma have reported a decrease in the microbial diversity in these diseases, with a propensity to be colonised with *Clostridium consisus* in one study[64, 65]. It is not known if the difference in microbial diversity represents a causal role for some bacteria, or rather a secondary change to altered local conditions. Little is known about the microbial profile in patients with EoE.

Microbiota of the large intestine (and presumably the oesophagus) is established in the days and weeks following delivery of the infant and may influence the development of food allergy. Children born via caesarean section and those administered probiotics in the first 6 months of life may have a lower rate of food allergy[66]. Bacterial colonisation of the skin is important in atopic dermatitis, with different populations in those with the condition compared with healthy controls and demonstrable improvement with antibiotics in some patients[67]. Defensins are proteins that a secreted at the mucosal surface and play a role in maintaining microbial homoeostasis, being considered part of the innate immune system. It has been noted that patients with atopic dermatitis and, more recently, those with EoE have a decreased expression of defensins (using techniques *in vitro*)[68]. Further, studies *in vivo* appear warranted.

Are all cases of EoE the same? – Fibrosis and stricture formation

A key question in managing any chronic illness is prognostic evaluation, counselling and (potentially) appropriate management selection. A spectrum of clinical, endoscopic and

histological pictures emerges across patients with the condition, including those with recurrent, frequent food bolus obstruction, those with severe oesophageal narrowing limiting scope passage and those with variable eosinophil counts or lamina propria thickness at biopsy[15, 69]. To date, neither the endoscopic appearance nor the eosinophil counts have been predictive of symptom severity or response to therapy[40, 70]. Notably, the diagnostic validity of endoscopic visualisation alone (in the absence of biopsy) in diagnosis is poor. Furthermore, the absolute eosinophil count does not correlate with symptom severity as measured by questionnaire [1, 71].Schoepfer in a retrospective study of 200 patients demonstrated increased stricture formation in those patients whose diagnosis was delayed; for example, 17% had strictures that were diagnosed within 2 years of the onset of symptoms, compared with > 60% when the diagnosis was delayed > 14 years[72]. Hence, it is apparent that diagnosis (and presumably treatment) alters the natural history of the condition. Moreover, a mandate to treat patients not only to alleviate symptoms but also to avoid future pathological oesophageal narrowing is present. Current first-line treatments for EoE include swallowed corticosteroids (dry powder or gel) as well as dietary therapy. Short-term prospective studies have shown that both may result in a complete or near complete disappearance of eosinophils and reduction in epithelial as well as lamina propria thickness[1, 24, 73]. It is logical to assume that maintenance of these therapies will alleviate symptoms and minimise stricture formation long term, although data are not yet forthcoming.

The search for prognostic variables that will predict severe progression with stricture formation or response to treatment is a worthy research goal. Aceves et al. demonstrated that oesophageal remodelling in EoE was reversed with topical steroids in some patients and that a SNP coding for TGF-β (19q13) predicted treatment response in this group[9, 74]. It has also been observed that TGF-β receptor (9q 22) mutations occur in the childhood-onset Loeys Dietz syndrome (characterised by high IgE levels, multiple atopic conditions and eosinophilia including the gastrointestinal tract) suggesting that mutations in this receptor may strongly predispose to

human atopic conditions, and demonstrating the complexity of the relationship between allergic inflammation and mediators thought to promote fibrosis[75]. Furthermore, Abonia et al. demonstrated TGF-β expression by mast cells and eosinophils again highlighting this complexity[71]. It is conceivable that such an approach correlating genetic alterations in terms of key pathological processes of inflammation and fibrosis and response to therapy may result in improved understanding and therapeutic advances. Polymorphisms in the angiotensin converting enzyme (ACE) gene (17q 22.3 – more than a dozen mutations noted), already implicated in cardiovascular disease and pulmonary fibrosis, are one such example[76]. Already, human trials on the angiotensin 2 receptor antagonist (losartan) in the treatment of EoE have commenced.

Conclusion

EoE is disease that presents with dysphagia and/or food bolus obstruction events. Gender (male), race (Caucasian) and atopy confer increased risk. The elemental diet and to a lesser extent the six-food elimination diet result in complete remission in many, implying that food antigens are causative. The timing and nature of food antigen exposure may be important in inducing or reversing immune tolerance and may explain the apparent increasing incidence of the condition. Aeroallergens may play a role. Oesophageal barrier function, microbiota and the clarification of factors influencing fibrous remodelling appear important areas for future study and understanding of this newly recognised condition.

2.3

Abstract

Eosinophilic esophagitis (EoE) is considered to be a chronic antigen driven disease whereby food and/or aeroallergens induce a chronic inflammatory infiltrate in the esophagus, resulting in pathological hyperplasia of the epithelia and muscular layers, and fibrosis of the lamina propria (referred to collectively as remodelling) and the symptoms of dysphagia and food impaction. EoE shares features with other atopic conditions asthma and atopic dermatitis, such as a TH2 cytokine milieu and a mixed inflammatory infiltrate of eosinophils, mast cells and lymphocytes. Relatively distinct features include the strong male predominance amongst adult patients, and the expression of the eosinophil chemokine eotaxin 3. Current first line treatments such as strict dietary modification and corticosteroids fail many patients. Looking forward, clarification of distinct genotype/phenotype associations, determining the reversibility of remodelling following treatment, and the development of new pharmacotherapies that target fibrotic pathways (as opposed to eosinophilic inflammation per se) or specifically improve barrier integrity appear relevant.

Key Words

Eosinophil

Esophagitis

Dysphagia

Allergy

Remodelling

Diet

Abbreviations

EoE – eosinophilic esophagitis

TSLP – thymic stromal lymphopoietin

Interleukin – IL (e.g. IL – 3)

Atopic dermatitis (AD)

Vascular cell adhesion protein 1 (VCAM -1)

Antigen presenting cell (APC)

Major basic protein (MBP)

Transforming growth factor beta (TGF – β)

Intracellular adhesion molecule (ICAM)

Interferon (IFN)

Vascular endothelial growth factor (VEGF)

Epithelial mesenchymal transition (EMT)

Introduction

Eosinophilic esophagitis (EoE) presents in adults as dysphagia and food impaction. The pathophysiological correlates of these symptoms are thought to comprise (1) acute narrowing of the esophageal lumen by inflammation and oedema, (2) fixed narrowing and limited distensibility of the lumen by remodelling and (3) dynamic and variable narrowing caused by muscular contraction or spasm[77-79]. The relative contribution of these three pathological processes to the clinical syndrome is not known, although the focus of research and treatment relates to remodelling.

Eosinophilic esophagitis (EoE) is considered to be a chronic antigen driven disease, whereby food and/or aeroallergens induce an eosinophilic infiltration in the esophagus [43]. Remodelling refers to the structural changes caused by acute and chronic inflammation, namely epithelial hyperplasia, fibrosis of the lamina propria and muscular hypertrophy (smooth and longitudinal) of the esophagus resulting from an inflammatory infiltrate typical of a TH2-mediated milieu[80]. The mechanism of injury has been demonstrated using animal models and in vivo human studies (before and after disease modifying treatments) and may be conceptualised to involve cells (e.g., eosinophils, mast cells, epithelial cells and fibroblasts) cytokines (e.g., interleukins IL-4,5 and 13, and the chemokine Eotaxin 3) and adhesion molecules (e.g., integrins and vascular cell adhesion protein 1 (VCAM-1))[81, 82].The precise sequence of events and the dominant cellular signalling or adhesional molecules involved are not yet fully elucidated. Importantly, however, treatments such as topical corticosteroids and dietary modification may at least partially reverse the pathological changes in a significant number of patients, with resultant improvements in swallowing reported in some studies[74, 83]. Other atopic conditions, such as asthma and atopic dermatitis (AD) manifest tissue remodelling, again typified by cellular infiltration and fibrosis, and the significant body of research in these fields provides

valuable potential clues to the pathogenesis of EoE, and may direct future research[58, 84]. The role of epithelial barrier function, epithelial defence, and repair and bacterial colonisation (both crucial in the pathogenesis of AD) are neglected areas of research. The genetic and racial predilection of EoE as a disease predominantly of white male Caucasians also deserves careful consideration [85].

The natural history of EoE in the era of disease-modifying treatments (elimination diets and corticosteroids), and the clinicopathological correlations between remodelling, acute inflammation and esophageal dysmotility remain to be determined[1, 24]. The clinical and research tools that determine symptoms (such as dysphagia scores) and esophageal function (manometry) and structure (endoscopic biopsy) have limitations. Improvements in technical utilisation (such as measuring esophageal distensibility instead of contractility and obtaining deeper endoscopic specimens including the lamina propria and muscularis) hold considerable promise[79, 86]. Clarifying the relationship between mucosal inflammation, esophageal motility, esophageal distensibility and the symptoms of dysphagia and food bolus obstruction will enable useful treatment end-points to be determined and potentially drive therapeutic innovation.
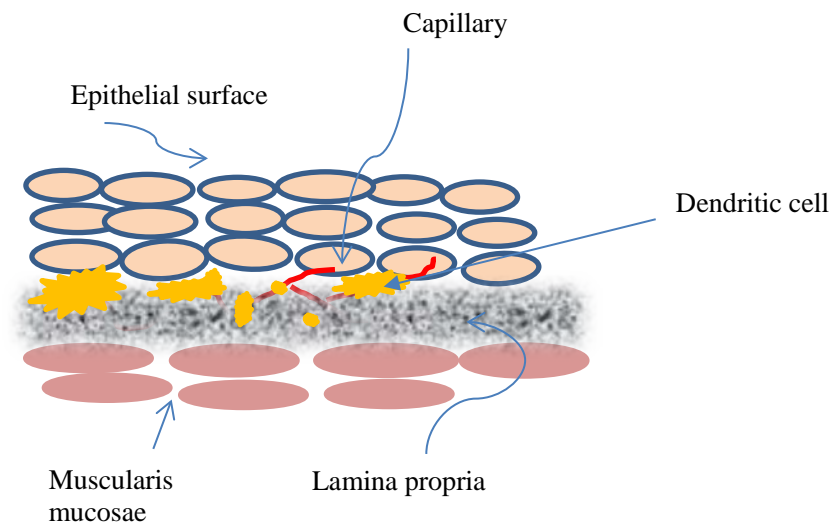
Figure 1. Normal esophagus

Esophageal barrier function is maintained by an orderly arrangement of epithelial cells maintained by gap junction proteins. The muscularis is striated (upper 1/3 of the oesophagus) and smooth (lower 2/3) of the oesophagus. Antigen presentation may occur by dendritic cells or possibly epithelial cells

Epithelial hyperplasia

Disruption of barrier integrity

Lamina propria fibrosis and thickening

Muscular hypertrophy and hyperplasia

Eosinophilic infiltration
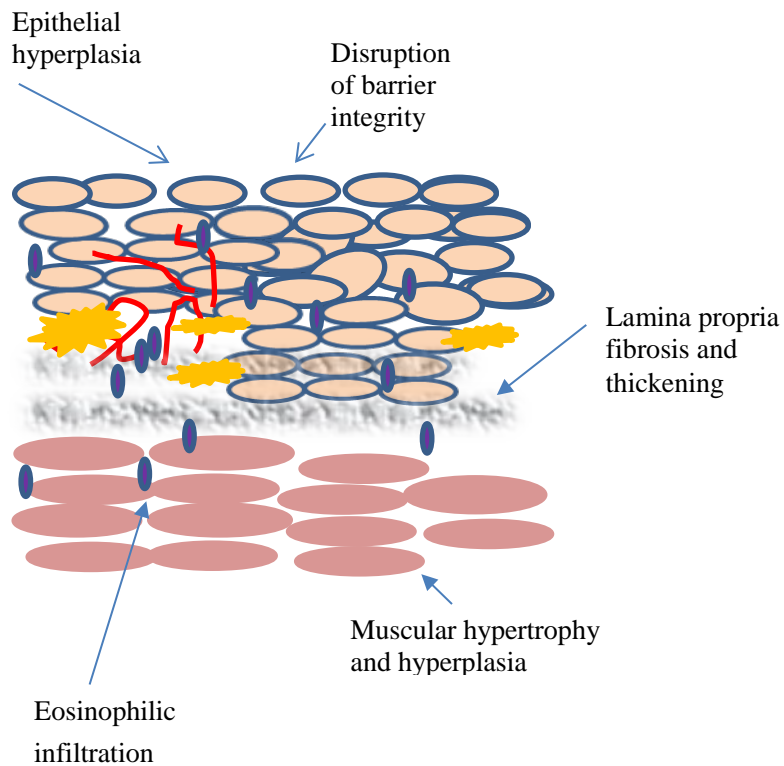
Figure 2. Eosinophilic esophagitis

Epithelial barrier integrity is disrupted, allowing greater contact between antigens and dendritic cells. The epithelial layer is thickened and disorderly, and the inflammatory infiltrate rich in eosinophils extends throughout all layers, and may contribute to dysmotility. Angiogenesis is present; the esophagus is friable and bleeds easily at endoscopy. The lamina propria is thickened and fibrotic. The clinical sequelae of the pathological changes are dysphagia and food bolus obstruction due to luminal narrowing, limited distensibility and disturbance of peristalsis.

1. Antigen presentation

EoE is viewed as an antigen-driven disease. The striking success of dietary therapy (up to 65% of patients improve on a 6 food elimination diet and 95% improve on an elemental diet) suggests that direct contact with the esophageal mucosa leads to antigen presentation and a localised inflammatory infiltration[24, 83].It is also possible that the exposure of the small bowel, which is rich in lymphoid follicles and immunologically active, may lead to immune activation and subsequent migration of the eosinophils to the esophagus. This is the implication of a recent study, that demonstrated increased intestinal permeability in patients with EoE, that was reversible with treatment (using diet or corticosteroids)[87]. Another hypothesis, suggested by the observation that there is a seasonal peak of patients presenting with clinical symptoms of EoE (correlating with high aeroallergen levels in the atmosphere) is that distant contact with the respiratory epithelium of the nose or airways leads to trafficking of eosinophils to the esophagus [33]. This hypothesis is supported directly by a murine model, in which antigenic exposure of the nasal and not the esophageal mucosa leads to esophageal infiltration with eosinphils in ovalbumin sensitised mice, and indirectly by human studies of asthma and rhinitis, where stimulation of the nasal or distal airway mucosa leads to an infiltration in the opposing mucosal surface respectively[49, 88].

If it is assumed that direct exposure of the esophageal epithelial surface to ingested food leads to the eosinophilic infiltration, the question remains as to how the inflammatory cascade then proceeds. The esophageal mucosa is stratified squamous and partially-keratinising in type, whereby up to 30 layers of epithelium separate the luminal contents and, therefore, potential food antigens from the lamina propria, where mast cells and (transiently) eosinophils may reside. There is only secretion of mucus from submucosal glands in the lower esophagus. This contrasts with airway epithelium, where a layer of ciliated epithelial cells are interposed with

goblet cells that secrete mucus and thereby potentially trap antigen. It is hence apparent that, in health, the physical interaction of the food antigen with inflammatory cells such as mast cells residing in the lamina propria will be limited. Several alternative mechanisms may facilitate the interaction of antigen with inflammatory cells, including the potential of the esophageal epithelium and/or the eosinophils themselves to function as antigen presenting cell (APCs)[43, 89]. It is interesting to note that the same physical barrier - multiple layers, lack of mucus to trap antigen - exists in the skin, yet the exposure to aeroallergens such as dust mite is thought to play a role in the pathogenesis of atopic dermatitis (AD Dendritic cells, lying within the epithelial layers present antigen in the skin of patients with AD, potentially explaining the immune activation in spite of the barrier function mentioned[90]. Dendritic cells are present in the lamina propria of the normal esophagus, are found in increased numbers in patients with Barretts esophagus and esophageal adenocarcinoma but are not found in increased number in patients with EoE, suggesting a role for non-professional APCs such as epithelial cells[82]. Abnormalities in the barrier function of the skin have been proposed as factors in the pathogenesis of AD, both predisposing and perpetuating the condition by enabling increased antigen exposure[90]. The barrier function of the esophagus is discussed below.

2. Inflammatory cell infiltration

Eosinophils

Eosinophils define EoE, in name, diagnosis (>15 eosinophils per high power field following endoscopic biopsy is required to confirm the condition) and response to treatment [91]. Furthermore, eosinophils are key players in the process of remodelling. The normal esophagus does not contain eosinophils, although a non-specific eosinophilic inflammatory reaction may occur, for example, in patients with gastroesophageal reflux disease (GORD) and viral esophagitis[91]. Eosinophils are derived from myeloid precursors in the bone marrow and mature in response to IL-5, subsequently circulating in the blood for up to 20 hours and residing in the tissues for between 2-14 days. Eosinophils remain increased in number in the esophagus of patients with EoE (who are untreated), but absolute density or numbers may fluctuate over time [38].

Cytokines and chemokines drive the migration of eosinophils into the esophagus. Cytokines such as IL – 5, IL-9 and IL – 13 and the chemokines, eotaxin 1, 2, and 3, are of central importance, as determined by elevated circulating levels and mRNA expression of esophageal tissue in human patients with EoE, and as demonstrated in murine knockout models (see below)[92-94]. Studies *in vitro* of human lung and endobronchiolar tissues suggest a role for vascular adhesion mediated by VCAM-1 and P-selectin on the endothelial surface along with P-selectin glycoprotein and very late protein-4 on eosinophils in facilitating eosinophil attachment and migration into tissues, a process governed by integrins [95].

Once in the esophagus, eosinophils may reside in the intraepithelial spaces where they can form microabscesses when clustered, in the lamina propria and, in some cases, muscular layers although the prevalence of the last two locations is difficult to determine given the limited

sampling capability of standard endoscopic biopsies[96, 97]. Eosinophils release a range of mediators stored in secondary granules. These include major basic protein (MBP), eosinophil peroxidase, eosinophil cationic protein and eosinophil-derived neurotoxin the release of which causes local tissue damage and esophageal dysmotility, and may secondarily activate mast cells[98-100]. MBP comprises a major component by volume of the secondary granules and putatively plays a significant role in causing fibrotic remodelling of the esophagus[80, 101]. MBP may act on esophageal epithelial cells, leading to production of fibroblast growth factor-9 (FGF-9) that in turn promotes epithelial hyperplasia [102]. Transforming growth factor-β (TGF-β) is also produced by eosinophils. This growth factor promotes activation of quiescent fibroblasts to myofibroblasts and, in turn, the production of fibrotic tissue in the lamina propria[71]. TGF-β also may contribute to smooth muscle contraction, hyperplasia and hypertrophy.

Eosinophils are both attracted and activated by cytokines and interleukins secreted by other cell types. They are capable of cytokine secretion themselves, which influences the inflammatory process, as demonstrated by studies *in vitro* of human cells[103]. Recently, the importance of IL-9 production by eosinophils in patients with EoE and of the ability of this cytokine to attract mast cells were demonstrated [104]. Further clarification of the role of eosinophils in EoE appears warranted. Eosinophils have, for instance been demonstrated in vivo to have an immunoregulatory role, eosinophil granule proteins decreasing the proliferation of lymphocytes (using donor eosinophils from healthy controls) and have also been shown to influence T cells, skewing development toward a Th 2 –like profile[105, 106].

It is apparent that eosinophils contribute to the key pathological processes of tissue remodelling, namely epithelial hyperplasia, subepithelial fibrosis, and muscular hypertrophy

and hyperplasia. Eosinophil density may be decreased by administering corticosteroids or instituting dietary therapy, and this reduction has been found in some studies to correlate with a reduction in remodelling or a return to a more histologically normal esophagus [74, 83]. Determining if the eosinophil count following endoscopic biopsy is a reliable marker of successful treatment and correlates closely with the reversal of pathological remodelling and symptom resolution need clarification.

Mast cells

Mast cells (MC) are found in small numbers in the normal esophagus, residing only in the lamina propria[91]. In EoE, increased density of MCs are found, both in the connective tissues and also within the intraepithelial and muscular layers[77]. These cells are derived from CD34^{+} progenitors in the bone marrow, but mature in the tissues and do not circulate in the blood stream[80].

Mast cells are classically associated with the type 1 hypersensitivity reaction, whereby an antigen comes into contact with specific IgE-bound to mast cells leading to activation, degranulation and release of a range of mediators such as histamine, eicosanoids and cytokines[107]. Mast cells bearing IgE have been demonstrated in the esophagus of patients with EoE in a human study, as well as animal models of disease [93, 108]. The evidence that mast cells are important in disease pathogenesis is furthered by the correlation between successful treatment with corticosteroid therapy, dietary therapy and mast cell density[93, 108] Some have suggested that 'mastocytic esophagitis' would be a better term for EoE, given the relatively greater specificity of histological techniques such as mast cell tryptase in determining the diagnosis of EoE, as compared to eosinophil density, which is non- specific [71]. Mast cells may modulate remodelling via the production of TGF-β, which in turn governs connective tissue production and also possibly smooth muscle contractility [74]. Importantly, mast cells, but not

eosinophils, may cause smooth muscle spasm[74]. In models of asthma, mast cells may release TGF-β and, in turn, increase the expression of adhesion molecules, intercellular adhesion molecule (1CAM) and VCAM[109]. The role of mast cells in inflammation, remodelling and esophageal dysmotility is an area worthy of further research.

B-lymphocytes and IgE antibodies

B cells, identified by staining for CD-20 (human B cell lymphocyte restricted differentiation antigen), are found in the esophageal mucosa of patients with EoE. IgE is also demonstrable, suggesting, along with the observation that skin prick tests and serum specific IgE to food and/or aeroallergens are frequently positive, that class switching to IgE antibody production by B-cells occurs in response to TH2 cytokines[108]. Furthermore, a growing body of literature suggests that the removal of the putative food allergens by, for example, elemental or six-food elimination diets, reverses the eosinophilic infiltration and remodelling in these patients[24, 83, 110].

Whilst EoE is considered an antigen-driven disease, the significance of IgE antibodies in the pathogenesis is debatable. The variably reported positive and negative predictive values of skin prick and patch tests, and antigen-specific IgE antibodies in determining a response to food elimination diets, and the heterogeneity of the responses to food or environmental allergens despite a florid infiltrate of eosinophils, hampers a definitive assertion to this regard[24, 83, 111]. Binding and removing IgE from the circulation with the monoclonal antibody, omalizumab, may be greatly decrease IgE levels has had limited success in EoE [112, 113]. The failure of this medication to deliver universally positive results also supports the suggestion that a TH2 mediated cytokine production, rather that B cell antibody production is of greater importance in the pathogenesis of EoE.

T Lymphocytes

Patients with EoE have an increased density of T cells (CD8^{+}, CD4+ and CD3^{+}) compared to the normal esophagus[98]. The assertion that EoE is a TH-2 mediated disease is upheld by the finding that the cytokines, IL-4, IL-5 and IL-13, are produced in greater quantities by monocytes following antigen exposures in the peripheral blood of patients with EoE compared to healthy controls, and that mRNA for these cytokines is overexpressed in esophageal biopsies. Furthermore, the mRNA levels decrease following corticosteroid therapy[94, 114]. It is interesting to note that TH-1 cytokines, TNF-β and IFN-α are also increased following antigen exposure of monocytes and are found in increased quantities in esophageal mucosal biopsies, and that skin patch tests (generally considered a measure of cellular or delayed hypersensitivity) are often positive to common food and aeroallergens[111, 114].

The cytokines IL-4, IL-5, IL-13 and possibly IL-9 are produced by TH-2 and TH-9 cells, and drive the eosinophilic and mastocytic infiltrate characteristic of EoE[104]. It has been proposed that the expression of eotaxin-3 by the esophageal epithelium, VEGF by the endothelium and integrins by the interstitium attracts these cells to the esophagus, whereby activation and degranulation occurs, modulating local tissue damage by MBP, histamine and other mediators[80, 101]. Furthermore, growth factors such as TFG-β and FGF-9 (fibroblast growth factor 9) are released by eosinophils and mast cells that activate quiescent fibroblasts to myofibroblasts, and drive hyperplasia of epithelium and smooth muscle that complete the cycle of remodelling[115]. It can hence be appreciated that T helper cells are central to the pathogenesis of EoE and the resultant esophageal remodelling. Accordingly, murine T helper deficient mice do not develop EoE[98].

Given the role of T helper cells in EoE, some focus of research has been directed at T-regulatory cells that express immunomodulatory cytokines such as IL-2 and IL-10). A small study of paediatric patients with EoE or GORD revealed an increase number of T regulatory cells compared with those of control patients, but no difference between those with different forms of esophageal inflammation[115]. Murine models have suggested a reduction of regulatory T cells, and a reduction in the esophageal eosinophilia with the infusion of T regulatory cells. Further research appears warranted in humans [116].

Basophils and the TSLP basophil axis (see also gender differences)

An exciting recent hypothesis, supported by a murine model and a single human study is that basophils and the epithelial cytokine thymic stromal lymphopoietin (TSLP) are integral to the development of EoE, and that disease development can occur in the absence of IgE and IL-5. Noti et al, in a landmark paper demonstrated that mice that were sensitised to ovalbumin and then re-exposed would develop changes representative of EoE, whereas mice that were treated in addition with antibodies to basophils and TSLP respectively did not develop esophageal eosinophilia[29]. Remarkably, mice did develop disease even when IgE antibodies were administered, strongly supporting a non- IgE mediated, TSLP/basophil mediated pathogenesis. A further human study demonstrated increased expression of TSLP (immunohistochemistry of esophageal biopsies) and cells resembling activated basophils in these biopsy specimens (flow cytometry). This alternative disease model would appear to offer great promise in understanding and potentially treating at least a subset of patients with EoE in the future, with monoclonal antibodies against TSLP representing one possible option[117].

3. Remodelling

Epithelial Cells

The esophagus is lined by squamous partially keratinised epithelium. Patients with EoE develop epithelial hyperplasia, possibly in response to MBP and TGF-β produced by eosinophils. A complex positive feedback loop has been proposed to explain the recruitment and maintenance of eosinophilic and mastocytic infiltration and epithelial hyperplasia [71]. Eotaxin 3 is produced by esophageal epithelium in response to IL-13, which in turn attracts eosinophils expressing the CCR-3 (eotaxin 3) receptor, promoting the remodelling described. Finally, it has been proposed that mast cells may produce IL – 9 in turn causing increased IL-13 production by TH-2 cells, thus completing the loop (see figure)[71]. Eotaxin 3 appears to be of central importance in the pathogenesis of EoE, with histological and mRNA studies of biopsy specimens demonstrating a specificity of this chemokine in patients with EoE compared to those with GERD, and some (but not all) studies demonstrating a correlation between disease activity, successful treatment and Eotaxin 3 levels[80, 118]. Furthermore, a single nucleotide polymorphism in the gene encoding eotaxin 3 has been demonstrated in some patients with EoE [119, 120].

As well as participating in the process of inflammation and remodelling characteristic of EoE, it is possible that inherited or acquired defects in esophageal epithelial barrier function may contribute to the development and/or perpetuation of EoE. Filaggrin is a structural protein of critical importance in the development of dermatitis. In health, filaggrin is found in the stratum corneum and binds keratin and intermediate filaments[11]. The gene for profilaggrin, which is subsequently phosphorylated to filaggrin, is located on chromosome 1. Specific mutations within this locus have been described to cause or greatly increase the risk of icthyosis vulgaris and, of great relevance to EoE, atopic dermatitis[11]. Since human skin is composed of stratified

squamous keratinising epithelium, the importance of filaggrin in keratin attachment is apparent. However, the squamous epithelium of the esophagus doesn't undergo keratinisation to a significant extent. Yet both of these conditions, along with asthma correlate with the filaggrin genetic loci albeit to a lesser extent in the case of asthma and EoE [90]. It remains to be seen if the association between filaggrin and EoE is more than a confounding factor, simply demonstrating the co-existence of EoE with atopic dermatitis, or if instead there is a hitherto unknown role for filaggrin in the esophagus that contributes to the pathogenesis of EoE. The evidence thus far suggests that filaggrin is not of central importance in EoE, as immunohistochemical staining of the esophagus failed to reveal filaggrin expression [11].

Proteins aside from filaggrin, such as occludins and claudins, may be important in EoE. Patients with atopic dermatitis have demonstrable decreases in the production of tight junction proteins, claudin-1 and claudin-23[121]. Furthermore, single nucleotide polymorphisms in claudin-1 have been demonstrated in patients with AD, implying that decreased protein expression results in disease, rather than being a result of the dermatitis[121]. It is interesting to note that disruption of tight junction proteins is thought to contribute to the increase in size of intracellular spaces in patients with GERD, and that treating patients with EoE using proton pump inhibitors improves the histology in many patients, and is considered a first line therapy[63, 122]. Abnormalities in tight junction protein and hence barrier function may precipitate and perpetuate EoE and warrant further study.

Esophageal muscle

The esophageal muscle layer is predominantly striated in the cervical esophagus (the upper third), a mixture of striated and smooth muscle in the middle third and smooth muscle alone in

the lower third. The muscle layers themselves are oriented in a circular (inner) and longitudinal (outer) fashion[123].

The inflammatory infiltrate of EoE that involves the muscular layer includes both mast cells and eosinophils, the former predominating in one study [74]. The availability of muscle tissue for study has hampered research as standard gastroscopy forceps will sample muscle tissue in <20% [83]. It is possible that both structural alterations (myocyte hypertrophy and hyperplasia along with inflammatory infiltration) and dynamic changes (muscular contraction) contribute to the clinical syndrome of dysphagia and food bolus obstruction. Mediators released from mast cells, including histamine, have the ability to cause muscle contraction and hyperplasia according to animal studies and one study of humans, and a correlation between the number of mast cells and the expression of TGF-β in the esophageal muscle layer have been demonstrated, suggesting a role for mast cells in driving remodelling[74]. Future studies aiming for systematic sampling of the muscular layer across a range of patients with variable disease severity seem necessary. It is yet to be determined, for example, whether the refractory dysphagia typical of some patients with EoE represents ongoing inflammation in the muscle layer or muscular dysmotility, or simply subepithelial fibrosis.

Epithelial mesenchymal transition

Epithelial mesenchymal transition (EMT) refers to the process whereby epithelial cells may lose their typical histological and immunohistochemical appearance, and functional properties to instead acquire the structure and function of mesenchymal cells, such as motility (instead of adherent tight junctions) and depolarised cytoskeletal arrangements (vimentin instead of cytokeratin in epithelial cells)[124]. Myofibroblasts, the quintessential mesenchymal cells characteristic of the remodelling process in asthma, can both synthesise extracellular matrix such as collagen and express alpha-smooth muscle actin (αSMA), possessing contractile

properties relevant to airway narrowing[125]. Furthermore myofibroblasts may differentiate to smooth muscle cells and contribute to the muscle thickening typical of chronic asthma [125].

The same process of EMT, and the resultant fibrosis and smooth muscle hyperplasia observed in asthma may occur in EoE [124]. Histological as well as sonographic endoscopic assessment demonstrate thickening of the lamina propria and esophageal muscular layer[126]. Immunohistochemical and mRNA of tissue in studies of patients with EoE pre and post treatment with corticosteroids demonstrate reversible EMT as defined by expression of mRNA or cell surface protein of cytokeratin or vimentin [124]. These changes correlated with a reduction in eosinophil number and immunohistochemical stains for TGF-β [124]. It is hence apparent that the EMT of EoE is an important step in the remodelling of EoE, and that this area warrants further study in line with asthma research[127].

Production of extracellular matrix (ECM) – the process of subepithelial fibrosis

Subepithelial fibrosis is characterised by an increase in the thickness and density of collagen bundles, and an increase in fibroblast density[80]. Several studies have employed a three-point scoring system to denote the severity of fibrosis according to these three indices[83, 86]. The production of the ECM by myofibroblasts that have been activated by TGF-β secreted by a range of cells (e.g., eosinophils, mast cells, epithelial cells) leads to SMAD-dependent signalling, upregulating fibrogenic genes such as collagen, alpha- SMA and periostin[118]. Indeed, mRNA studies have found that patients with EoE express SMAD-2 and 3 and periostin at high levels in the esophageal tissues compared to patients with GERD[95]. Subepithelial fibrosis is dynamic and does respond in some patients who receive either dietary restriction (the six-food elimination diet or the elemental diet) or corticosteroid therapy. The reversal of the fibrous remodelling appears to correlate with the disappearance of eosinophils [128]. Determining which

patients will respond to therapy remains a research question worthy of consideration. Periostin is traditionally viewed as a cell adhesion molecule regulating extracellular matrix deposition. However, considerable research focus on its role in mediating a range of biological processes involved in the fibrous remodelling of EoE, such as binding and facilitating cross-linking of collagen, potentially functioning to increase eosinophil adhesion to integrins and inducing epithelilal mesenchymal transition, is ongoing[36]. Intriguingly, the upregulation of periositin is only partially reversed by corticosteroid administration, suggesting a causative role for this protein in EoE, and reinforcing the potential importance in disease pathogenesis[36].

Angiogenesis

Angiogenesis, the formation and development of new blood vessels, is a feature of eosinophilic esophagitis. The angiogenic factor vascular endothelial growth factor alpha (VEGF-A), angiogenin and IL-8 have been implicated in promoting this pathological process, and it is notable that eosinophil-depleted mice have decreased angiogenesis [129, 130]. The abnormal vascularisation of the diseased esophagus typical of EoE may contribute to the friability and propensity to bleed at the time of endoscopy, and circulating inflammatory cells including eosinophils would have ready access to the site due to the angiogenesis, potentially perpetuating the disease process. A decrease in VEGF has been demonstrated following treatment with corticosteroids and dietary therapy respectively[83, 86]. It is interesting that Siglec-F, sialic acid immunoglobulin-like lectin, a protein that is highly expressed on eosinophils, may facilitate eosinophil adhesion and contribute to angiogenesis according to a murine model. Anti-Siglec-F was shown in a mouse model to reverse remodelling, eosinophilia and angiogenesis, raising the possibility of the use of this agent as a novel therapy in EoE[130].

Microbiota

Microbiota of the esophagus and possible effects on barrier function may be important in the pathogenesis of EoE. Limited studies in patients with GERD, Barrett's esophagus and esophageal adenocarcinoma [131] have reported a decrease in the microbial diversity in these diseases, with a propensity to be colonised with *Clostridium consisus* in one study [132]. It is not known if the difference in microbial diversity represents a causal role for some bacteria, or rather a secondary change to altered local condition. Little is known about the microbial profile in patients with EoE. This is arguably an important area given the ability of skin infection to modulate the severity of atopic dermatitis and for antibiotics to improve the condition. Defensins are proteins that a secreted at the mucosal surface and play a role in maintaining microbial homeostasis, being considered part of the innate immune system. It has been noted that patients with atopic dermatitis and, more recently, those with EoE have a decreased expression of defensins (using techniques *in vitro*) [133]. Further, studies *in vivo* appear warranted.

Gender differences

There is a male predominance of EoE with a male: female ratio of 3 or 4:1, but this remains unexplained. This differs from asthma where a bimodal gender distribution pattern occurs where asthma is more common in young male children, but adult females are more likely to suffer from asthma[134, 135]. Interestingly sensitisation to common food and environmental allergens as determined by skin prick or serum-specific IgE tests) show no sex difference[134]. It could be hypothesised that a further factor may then modulate the response to allergen exposure.

TSLP is a protein product mainly of epithelial cells that closely resembles IL-7 in structure and function, and promotes a characteristic TH-2-mediated milieu via the activation of

dendritic cells in AD and asthma[136]. Single nucleotide polymorphisms in the gene coding for TSLP at 5q22 are associated with predisposition to asthma, AD and EoE[136]. Intriguingly, the gene coding for the TSLP receptor is located at Xp22.3/Yp11.3, and polymorphisms at both sex linked loci may predispose to EoE in male but not female patients[137]. Recently, the 'TSLP-basophil axis' was studied and found to play a central role in eosinophilic inflammation of the esophagus in both mouse and human models, whereby disease did not develop in the absence of TLSP, and single nucleotide polymorphisms associated with gain of function correlated with increased disease activity[29]. Further clarification concerning the role of TSLP is clearly warranted.

Nitrous oxide-mediated relaxation of smooth muscle in the bladder is reduced in the presence of testosterone in a rodent model. Effects of TGFβ in causing cardiac fibrosis and mediating adverse outcomes following myocardial infarction in males are attributed to testosterone[5, 138]. Relaxin is a hormone present in large amounts in pregnancy, small amounts in non-pregnant females of reproductive age and also in males. It may have the potential to decrease fibrosis in a range of organs, as demonstrated by animal models[139, 140]. A study of relaxin in human bronchial biopsies suggests an association with the remodelling process in asthma, whilst a greater expression of relaxin receptors in female compared with male cruciate ligament tissue has been cited as an explanation for greater tissue laxity in female[84, 141]. Further research may allow the therapeutic use of relaxin in the future.

4. Alterations in esophageal function (biomechanics)

From a theoretical standpoint, it is apparent that the dysphagia and the food bolus obstruction events that occur in EoE may be caused by fixed narrowing (remodelling), acute narrowing (inflammation and oedema) and esophageal dysmotility or spasm, alone or in combination. As fixed narrowing is demonstrable as focal strictures at endoscopy or barium swallow, the emphasis in research has been on remodelling. Routine esophageal manometry has not uniformly demonstrated abnormalities in patients with EoE, although high-amplitude abnormal distal contractions have been reported in some patients and findings consistent with nutcracker esophagus found in a subset of patients with a high eosinophil count[79, 142, 143]. Manometry findings in another study were similar in patients with EoE to those with GORD, both having limited peristaltic activity, perhaps demonstrating the importance of a control group[144]. Research methodologies utilising the barostat or planimetry, which assess esophageal distensibility, however, have defined abnormalities in a significant percentage of patients, with decreased distensibility and pan-esophageal pressurisation characteristic[144, 145]. Further research, particularly delineating the change in distensibility with treatment, and correlation with traditional measures of treatment success such as patient reports of dysphagia and eosinophil count at gastroscopy are needed.

Acute or subacute infiltration by inflammatory cells may also explain the dysphagia and food bolus obstruction events. Significant improvement in these features has been demonstrated within six weeks of the commencement of dietary modification, inhaled corticosteroids and even oral corticosteroid therapy. It remains to be determined if complete symptom resolution can be achieved using these treatments and many patients notably do not respond. The presence of eosinophils and mast cells may contribute to the esophageal dysfunction via the production of products of degranulation such as tryptase, major basic protein and eosinophil derived

neurotoxin (see below), as well as causing inflammation and oedema [99]. Correlations between eosinophil and mast cell densities and dysphagia have been noted, and future definition of this potential pathophysiological phenomenon appears important in establishing valid treatment goals[24].

Figure 3. Suggested clinical management algorithm for patients with EoE
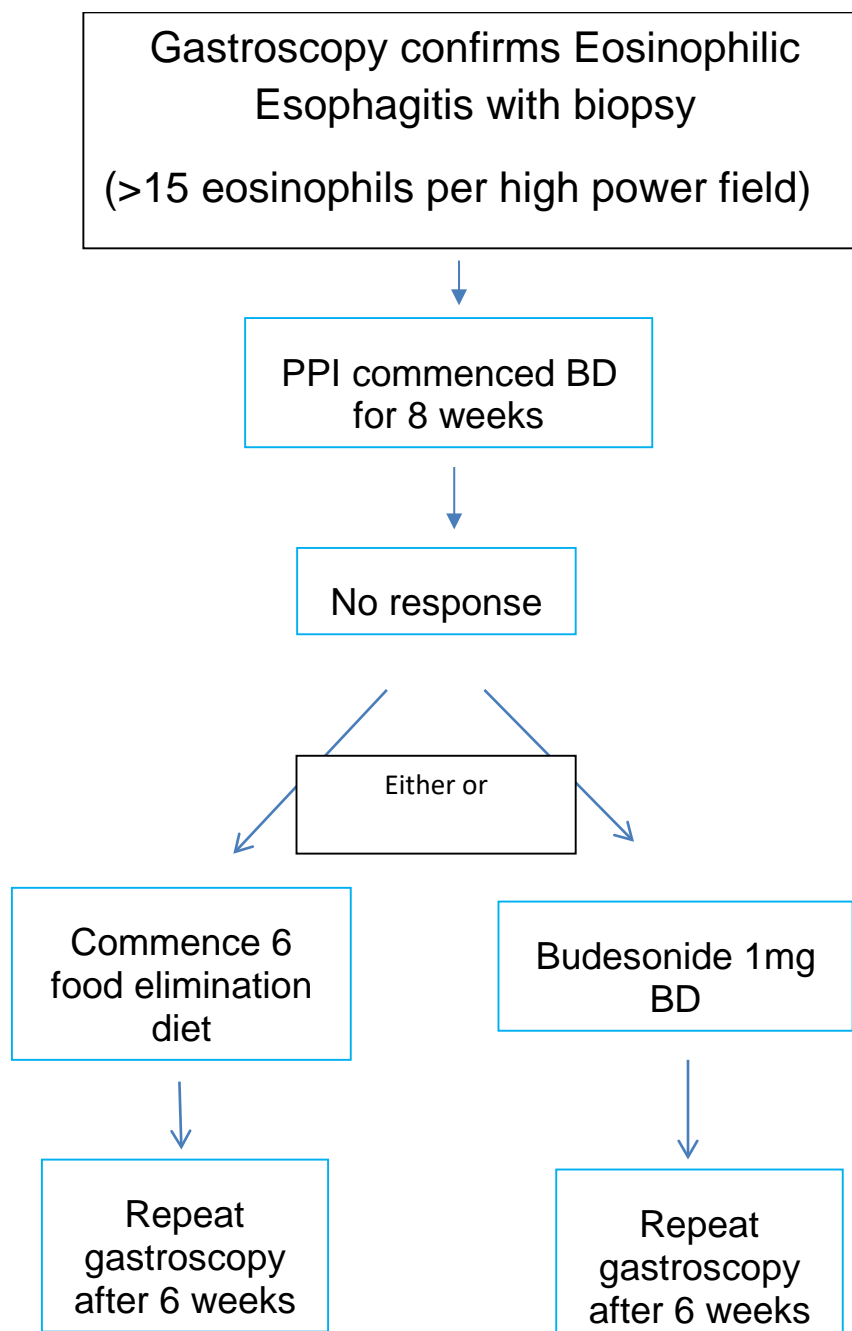
```
┌─────────────────────────────────────────────┐
│   Gastroscopy confirms Eosinophilic          │
│       Esophagitis with biopsy                │
│                                              │
│   (>15 eosinophils per high power field)     │
└─────────────────────────────────────────────┘
                     │
                     ▼
            ┌──────────────────┐
            │ PPI commenced BD │
            │    for 8 weeks   │
            └──────────────────┘
                     │
                     ▼
            ┌──────────────────┐
            │   No response    │
            └──────────────────┘
                   ╱    ╲
                  ╱      ╲
         ┌────────────────┐
         │    Either or   │
         └────────────────┘
        ╱                    ╲
       ▼                      ▼
┌─────────────────┐    ┌─────────────────┐
│   Commence 6    │    │ Budesonide 1mg  │
│ food elimination│    │       BD        │
│      diet       │    │                 │
└─────────────────┘    └─────────────────┘
         │                      │
         ▼                      ▼
┌─────────────────┐    ┌─────────────────┐
│     Repeat      │    │     Repeat      │
│   gastroscopy   │    │   gastroscopy   │
│  after 6 weeks  │    │  after 6 weeks  │
└─────────────────┘    └─────────────────┘
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Treatment

There are compelling reasons to advocate aggressive early treatment of EoE, and there are significant shortcomings in the current treatment options. It is now apparent that EoE is a chronic fibro - stenosing disease that likely benefits from prompt recognition and therapy[69, 72]. It is possible that broadening the treatment goals to include measurable changes and return of normal esophageal distensibility may guide treatment type, duration and potentially define a need for additional agents to address not just eosinophilic inflammation (the current sole focus of treatment) but also lamina propria and muscular thickening and disturbances in physiological peristalsis. It should be noted that it is not clearly established that controlling the eosinophilic inflammation will correct all of the pathological changes and return the esophagus to normal structure and function, although some studies are suggestive (admittedly hampered by the lack of lamina propria and muscularis sampling)

Existing guidelines advocate the use of dietary therapy such as the six-food elimination diet or corticosteroids as first line in the management of EoE, and suggest esophageal dilatation at endoscopy as an alternative in refractory cases[42]. Unfortunately, many patients do not respond or have an inadequate response to either or both therapies, as determined by symptoms and/or subsequent endoscopy and biopsy. The response to a six-food elimination diet in adult and paediatric patients approaches 65%, albeit in a controlled trial situation, whilst swallowed corticosteroids as dry powder or gel solutions result in histological improvement in 50% to 90% of patient[24, 146]. Dietary therapy is restrictive and hence potentially cumbersome. Neither strategy is curative. Patient's rapidly relapse following cessation of treatment. Basic scientific research supports this – that is after treatment with corticosteroids eosinophils do not revert to a normal phenotype, rather the capacity of eosinophils to adhere to epithelium is diminished[147]. Corticosteroids may also cause oral candidiasis and a hoarse voice in up to 25% of patients'

long term, whilst suppression of the hypothalamopituitary axis and reduction of bone mineral density are potential issues [148].

Immunotherapy (subcutaneous or oral/sublingual) could be used as treatment for EoE, and one case series supports this approach[48]. However the fact that immunotherapy for seasonal rhinitis has potentially caused EoE suggests great caution should be exercised, and further research occur before such therapy can be advocated[46]. Furthermore, immunotherapy for classical food allergy (anaphylactic) is not an established treatment, and the efficacy of the elimination or elemental diets in EoE suggests that food antigens are the likely trigger, again raising questions about the rationale of this approach.

The mast cell stabiliser (sodium cromoglycate) and the leukotriene receptor antagonist (monteleukast) have both been used to treat EoE but without success. Monteleukast does not result in a reduction in esophageal eosinophil count, nor does it sustain histological remission in those treated with corticosteroids[149]. Sodium cromoglycate has been used in a number of paediatric patients and found to be ineffective[85]. Extrapolating from other gastrointestinal diseases characterised by chronic inflammation (e.g. Crohns disease and ulcerative colitis) the thiopurines have been used successfully in a case series of 3 patients, with relapse of the condition following the cessation of this medication supporting a treatment effect[150]. Nonetheless, the potential side effects of this medication class (increased risk of non – melanomatous skin cancer, increased risk of lymphoma, immunosuppression) make this an unattractive option, given that EoE is not in itself associated with a decreased life expectancy.

Figure 4. Components of the 6 food elimination diet

1. Wheat
2. Egg
3. Milk
4. Soy
5. Nuts
6. Seafood

Esophageal dilatation at endoscopy is an effective treatment in some patients, providing immediate relief of dysphagia. Initial safety concerns relating to the potential to cause esophageal perforation have been moderated by the publication of a retrospective cohort of 207 adult patients, in which there were no reported perforations although significant chest discomfort and odynophagia occurred in 45%[151]. In this same study, swallowing was normalised in 50% of patients. Other smaller and prospective studies have suggested clinical remission of dysphagia in up to 90% of patients, which is durable in 50% of these patients at 24 months [152].

It is hence apparent that current treatments have variable and suboptimal success rates, and may be poorly tolerated. The need for alternative management approaches is evident. Biological agents have been trialled in EoE, although to date the efficacy has been disappointing and the cost remains prohibitive. Monoclonal antibodies to IL-5 (mepolizumab and reslizumab), have been trialled, with reslizumab demonstrating a dose related ability to decrease esophageal intraepithelial eosinophil counts in comparison to placebo in a large study of paediatric patients[153]. Unfortunately, even at maximal dose, reductions in the eosinophil count were modest (the study expressing the change in eosinophil count as a percentage, rather than the ability to decrease the eosinophil count to less than 5 per high power field as is

customary). Furthermore, all groups, including placebo demonstrated an improvement in symptoms, and the difference between groups was not significant. Mepolizumab was used in a small placebo controlled trial of adult patients, and like reslizumab achieved a significant reduction in eosinophils, but not symptoms with between 4 and 13 weeks of follow up[25].

 The CRTH2 receptor antagonist OCOO459 has been trialled in a group of 26 adult patients with EoE. After 26 weeks of therapy, there was a modest decrease in the eosinophil count, but with the mean eosinophil count at the conclusion of therapy in this group of patients with severe EoE decreasing only to 75 per hpf, the utility must be questioned, despite an improvement in the physician rated disease activity index[154]. Omalizumab, a monoclonal anti – IgE antibody has been trialled, and has not proven successful according to a randomised controlled trial, as well as case reports[113]. Neither histology nor clinical symptoms were improved. Similarly, infliximab (an anti-TNF agent) was studied in a single series of adults patients with EoE, and was   not   effective[155].

Borrowing from established treatments of other related conditions (asthma and atopic dermatitis) may potentially be extended to EoE, with likely benefit. The calcineurin inhibitors (tacrolimus and the related pimecrolimus) have been used topically in atopic dermatitis, as well as orally in refractory cases (including with cyclosporine)[156]. It is foreseeable that a gel suspension of pimecrolimus or tacrolimus could be used (these agents are fortunately free of the side effect of gum hyperplasia that occurs with cyclosporine). Methotrexate is another option, case reports indicating potential efficacy in atopic dermatitis[157]. Disturbances in the esophageal microbiota have not been characterised in EoE, but potential abnormalities could be treated with antibiotics, once again an established treatment in AD[158].

TSLP may activate basophils and drive a Th2 like mediated disease milieu independent of IgE antibodies (see below). A trial of a monoclonal antibody against TSLP in allergic airways disease in cynomolgus monkeys was successful[159]. This is particularly pertinent to EoE, given the SNPs coding for TSLP and TSLP receptor that correlate with EoE in particular[137]. Siglecs are cell surface proteins expressed on eosinophils, basophils and mast cells. Siglec F, and the related Siglec 8 in humans govern eosinophil apoptosis, and hence the observation that Siglec F may decrease eosinophilic inflammation in murine airways may lead to human trials in the future[160]. Finally, sphingosine kinase is an enzyme implicated in IgE mediated mast cell responses. Inhibition of sphingosine kinase -1 decreases airway hyper-responsiveness and allergic inflammation in murine inflammatory airways disease models[161]. Again EoE, as a mast cell rich inflammatory disease, may foreseeably be helped by this approach and research appears warranted.

All of the pharmacological treatments discussed work by reducing the (mainly) eosinophilic infiltrate. Thinking more broadly, agents that may modulate barrier membrane integrity, and thus minimise antigen exposure, or those that address the end result of eosinophilic infiltration, namely subepithelial fibrosis can be considered. Barrier integrity is a considerable focus of treatment for atopic dermatitis, where factors that denude the epithelium of the protective moisture retaining lipid surface layer, such as harsh soaps, are minimised, and moisturisers and emollients are used extensively. Furthermore, using antibiotics may improve barrier integrity[90]. One existing treatment for eosinophilic esophagitis, namely PPIs, may act by improving barrier function of the esophagus[162]. Patients with coeliac disease have impairments in intestinal permeability and barrier function, and the agent larazotide, that promotes tight junction assembly has been shown to return epithelial integrity to a more normal phenotype [163]. As antigen presentation may occur in the duodenum as opposed to the esophagus in EoE, targeting

intestinal barrier function may have a role in reducing antigen presentation. It remains to be seen if this agent will have a similar effect in the duodenum of patients with EoE.

Treatments that address fibrosis of the lamina propria are needed. Borrowing from research in cardiovascular medicine and hepatology, angiotensin 2 receptor blockers inhibit TGF-B production, that is significantly expressed in EoE (see above). For example, ACE inhibitors and AT 2 blockers have been demonstrated to influence myocardial remodelling positively post myocardial infarction[76]. A trial has been commenced examining the role of losartan in paediatric EoE patients. Relaxin has been used and demonstrated positive effect in murine asthma models of bronchiolar remodelling[139]. As expert clinical commentators suggest that the fibrosis and hence dysphagia induced by EoE may persist long after the resolution of inflammation, and currently only responds to dilatation at endoscopy, further therapeutic agents are needed.

Conclusion

EoE is a chronic antigen-driven disease characterised clinically by dysphagia and recurrent food bolus obstruction events. Despite a great deal of recent research interest, many questions remain. Apparently related conditions such as asthma and atopic dermatitis provide clues as to the likely mechanisms and promising research and therapeutic targets for the future. It is likely that both inherited (gender and individual genes) as well as acquired factors (e.g. dietary allergens, microbiota) are significant. Clarifying the relationship between the cessation of inflammation, and potential improvements in symptoms and reversal of remodelling will enable treatment end-points to be established and guide therapeutic advances.

2.4

Summary – Solving the riddle of food and aeroallergens?

EoE is a condition characterised and diagnosed by an eosinophilic oesophageal infiltrate and patients present with dysphagia and FBOE. The weight of evidence favours food antigens as the major cause, however the significance of aeroallergens and the feasibility of food antigen restriction are key areas of debate and research interest. The finding that PPIs resolve oesophageal eosinophilia in a large number of patients apparently independent of their ability to decrease oesophageal acid exposure has led to a new subcategory of patients; those with PPI –REE. Defining subtypes of patients with oesophageal eosinophilia, the mechanism of PPI effect and the interplay with food antigens may provide insights into disease pathogenesis.

A significant impediment to both research and clinical management has been the need for invasive, risky and expensive histological sampling with gastroscopy. Gaps in knowledge include the atopic profile of patients with EoE outside of the Northern Hemisphere, potential food and aeroallergen cross-sensitisation and the ability of allergy tests to guide dietary therapy. Future research must be directed both to refine diagnostic methods and ultimately further mechanistic insights with a view to therapeutic innovation.

CHAPTER 3

3.1

Overview

This chapter consists principally of an original published research paper. Prior to the publication of the manuscript, evidence supporting or refuting the role of aeroallergens in eosinophilic oesophagitis was either based on animal models or on observational and uncontrolled human studies, for example those showing that eosinophilic oesophagitis was diagnosed more frequently during the pollen season. Furthermore, studies were limited to the Northern Hemisphere where the aeroallergens of significance vary from our Australian cohort. The background literature and its limitations, and the reasons for choosing the study design are addressed within the manuscript itself.

3.2

Philpott, H. L., Nandurkar, S., Thien, F., Bloom, S., Lin, E., Goldberg, R., Boyapati, R., Finch, A., Royce, S. G. and Gibson, P. R. (2015), Seasonal recurrence of food bolus obstruction in eosinophilic esophagitis. Internal Medicine Journal, 45: 939–943. doi: 10.1111/imj.12790

Background

Eosinophilic esophagitis (EoE) is a newly recognised condition that is apparently increasing in prevalence, and the aetiology is poorly understood. The role of aeroallergens in EoE is controversial, given the success of dietary therapy. Massive aeroallergen exposure leading to food bolus obstruction events (FBOE) has been described, and the diagnosis of EoE by esophageal biopsy noted to be more common in the pollen season according to previous case series.

Aim

To determine if a seasonal variation and a geographical variation occurred in EoE presenting as FBOE in adults, and to track the prevalence of FBOE and EoE over time.

Method

A retrospective case–control study analysis was performed from January 2002 to January 2012 to identify all FBOE in adults presenting to five tertiary hospitals in Melbourne, Australia. Endoscopy, histopathological reports, case notes and blood tests were examined, and postcodes recorded. Records of pollen counts were obtained. Cases were defined according to oesophageal biopsy and grouped based on month of diagnosis. All other causes of FBOE served as controls.

Results

One thousand, one hundred and thirty-two FBOE were identified. Biopsies were only performed in 278 of these cases, and 85 patients were found to have EoE after biopsy. Patients with EoE were younger (mean age 38 years, range 18–72) compared with those with alternative diagnosis (mean age 64.4 range 22–92), more likely to be male (M : F = 4:1 compared with 1.68:1) and had a higher eosinophil count in venous blood. Overall no seasonality was demonstrated in FBOE secondary to any diagnosis, although the six cases of recurrent FBOE secondary to EoE mainly occurred in the grass pollen season in subsequent years. FBOE cases were evenly distributed throughout metropolitan Melbourne irrespective of population density. EoE as a percentage of FBOE increased over time.

Conclusion

Seasonal aeroallergens may be important for a subgroup of patients with EoE presenting as recurrent FBOE. Esophageal biopsies are performed in a minority of patients, representing a significant departure from ideal management and contributing to recurrent unnecessary FBOE. EoE is an increasingly important cause of FBOE.

Introduction

Eosinophilic esophagitis (EoE) has short history, being formally defined in 1993, and may be increasing in prevalence[149, 164]. EoE is a chronic antigen-driven disease manifesting clinically as dysphagia and FBOE, and pathophysiologically is characterised by luminal narrowing and limited distensibility secondary to a mixed eosinophil-rich inflammatory infiltrate, epithelial hyperplasia, lamina propria fibrosis and muscular dysmotility[135]. Esophageal eosinophilia is a non-specific finding and may occur, for example, in gastro-esophageal reflux disease (GERD). The significance of proton-pump inhibitor (PPI)-responsive esophageal eosinophilia is debated[165]. The role of food allergens is well established; an elemental diet is effective in >90%, the six-food elimination diet effective in >65%, and culprit food allergens are identifiable on rechallenge and re-biopsy at endoscopy [24] [166]. Aeroallergens as a contributor to disease pathogenesis are, in contrast, supported only by case studies and case series involving adults, showing increased diagnoses during the pollen season, a finding refuted by a recent paediatric study [33, 47, 50, 167]. A large cross-sectional study of pathology records also supports a role for aeroallergens; more cases of esophageal eosinophilia were found in climatic zones and regions where pollen counts are known to be high, such as temperate as opposed to tropical locations and areas that are less densely populated and hence possibly more vegetated[41]. Thus, in the absence of a control group, and the inability to distinguish esophageal eosinophilia related to GERD or at least responsive to PPIs from a key indicator event of disease activity, the validity of these studies must be questioned.

FBOE can be secondary to EoE in up to 50% of cases, while other causes include GERD, benign strictures and esophageal malignancy [168]. The suggestion that FBOE may be used as a surrogate marker of EoE disease activity is supported by case reports describing these events following massive aeroallergen exposure. Furthermore, apparently new cases of EoE in adults are more commonly documented in the pollen season, and often present for diagnosis with

FBOE [33].It is acknowledged that food bolus impaction may result from chronic rather than acute pathological changes in the esophagus (such as lamina propria fibrosis), and that factors unrelated to disease activity (such as the consistency of food ingested) may also play a role. Nonetheless, it is our hypothesis that the inhalation or ingestion of aeroallergens may lead to increased disease activity with resultant luminal narrowing and may in turn cause food bolus obstruction. With this in mind, and considering the limitations of previous studies relating to aeroallergens including the lack of a control group, heterogeneity of clinical presentation, unknown significance of esophageal eosinophilia, the current study aimed to determine if a seasonal and geographical pattern exists in patients with EoE presenting with FBOE and to examine the seasonality of representation of FBOE. Additional aims were to determine if this presentation is becoming more common using all other cases as a control and to define the quality of diagnostic processes in patients presenting with FBOE.

Methods

A retrospective review of the computer databases of five large tertiary hospitals in Melbourne, Australia, was undertaken to identify patients with FBOE, using the International Classification of Diseases (ICD) 9 code CM 935.1. The databases were searched between February 2002 and February 2012. Case files (electronic or hard copy) were retrieved and analysed. Included were patients aged 18 years and over who underwent an upper gastrointestinal endoscopy and had biopsies of the esophagus performed during the admission. Excluded were those who did not receive an endoscopy and biopsy and those with oropharyngeal or tracheal obstruction miscoded as an esophageal event. Cases were defined as having >15 eosinophils per high power field (area $0.19 \, mm^2$) anywhere in the esophagus at endoscopy as determined by the relevant histopathology report. Controls were those with an esophageal biopsy and any diagnosis other than EoE that explained the FBOE. Patient age, gender, date of birth, postcode, clinical

diagnosis, co-morbidities, medications, endoscopic diagnosis, histopathology report, eosinophil count and specific IgE to common food or aeroallergens in the serum (where available) was recorded. The postcodes were characterised according to the regional population density with reference to data from the Australian Bureau of Statistics. A higher frequency of cases per head of population from postcodes with lower population density was anticipated to define a geographical effect. The date of presentation with FBOE was recorded and compared with Melbourne pollen counts recorded by the Melbourne University Department of Botany. As the predominant aeroallergen in Victoria is Rye grass, seasonality was expected to be defined as increased numbers of EoE presenting as FBOE in the months September through to January when high levels of Rye grass pollen are present (although a small amount of annual variation does occur and could be tracked with the pollen count data). The study was approved by local hospital ethics committees.

Microsoft access database and statistical software were used to collate and analyse the data. Continuous data were expressed as means, and categorical data as percentages. Actual and expected frequencies between groups (EoE vs other) were compared using the Chi-squared test. Continuous data were compared with the Student's *t*-test. A probability value ≤0.05 was considered statistically significant.

Results

A total of 1132 patients was admitted with FBOE to the five hospitals over a 10-year period. As shown in Figure 1, 854 were excluded due to the presence of oropharyngeal pathology ($n = 57$) or the absence of esophageal biopsy ($n = 797$). Thus, 278 patients met the inclusion criteria, of whom 85 were diagnosed with EoE. The diagnoses in the other 193 patients are shown in Table 1, the most common alternative being GERD. Comparison of the characteristics of the

two groups of patients showed that those with EoE were younger and more likely to be male (Table 1).

Figure 1. Identification of food bolus obstruction events (FBOE) subgroups.

Table 1. Characteristics of the patients presenting with foreign body obstruction of the esophagus and having oesophageal biopsies performed			
	Eosinophilic esophagitis $n = 85$	**Other diagnosis**[a] $n = 193$	*P*-value
1. [a] Gastro-esophageal reflux disease (104), neoplasia (19), candidiasis (12), non-food foreign body (13), Barrett's esophagus (13), Schatzki ring (13), motility disorder (3), normal esophagus (17).			
Mean age (range) (years)	38 (18–72)	64 (22–92)	0.001
Proportion male (male : female ratio)	81% (4:1)	63% (1.68:1)	0.002
Mean peripheral blood eosinophil count (reference range $0–0.2 \times 10^9$/L)	0.237 (0.0–1.0) (available on 42)	0.06 (0.0 to 0.4) (available on 84)	0.235
Proportion living in area of low population density	22%	19%	0.6394

Dividing the data into two periods, 2002–2006 and 2007–2012, the number of cases of FBOE seen at the hospitals had increased over time, as shown in Table 2. The proportion having esophageal biopsies had not significantly increased, but the proportion who were diagnosed with EoE had increased by 56% (*P* = 0.029).

Table 2. Patients presenting with foreign body obstruction of the esophagus, the frequency of esophageal biopsy and diagnosis of eosinophilic esophagitis according to the time period		
	2002–2006	**2007–2012**
1. *P = 0.029; Chi-squared = 5.2819.		
Total cases of foreign body obstruction	571	707
Biopsies performed (percentage of cases)	110 (19%)	168 (24%)
Eosinophilic esophagitis		
● Number of diagnoses	25	60
● EoE as percentage of biopsies	23%*	36%*

Seasonality was not demonstrated in cases of EoE, nor the control group with an even number of cases occurring across the seasons (Fig. 2). Furthermore, equal proportions of patients residing in low population density regions of Melbourne occurred in both groups.

Figure 2. Number of cases presenting with foreign body obstruction of the esophagus according to the diagnosis and season. No significant differences were observed for the proportion of those with eosinophilic esophagitis (EoE) (Chi-squared test). (■), EoE; (■), non-EoE
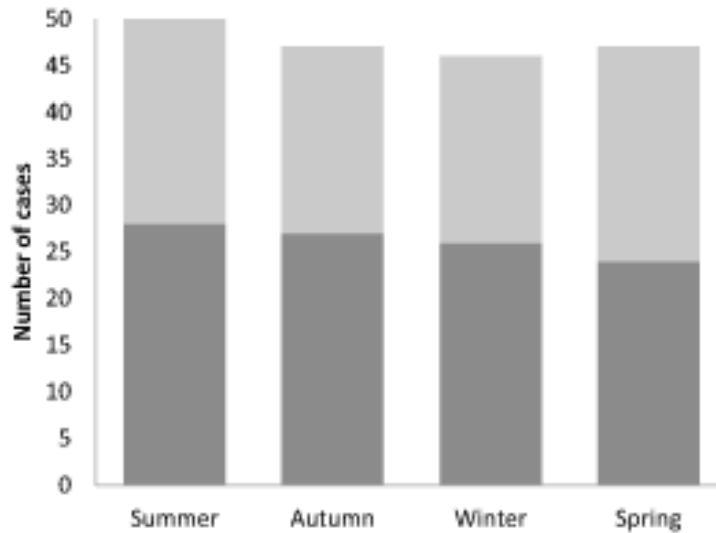
	Summer	Autumn	Winter	Spring
*Total	50	47	46	47
*EoE	22	20	20	23

Recurrent FBOE in subsequent years were identified in six of those with EoE and 19 with alternative diagnosis (Table 3). Recurrent FBOE within the same calendar year (usually within months) were noted in some patients, mainly those with a diagnosis of esophageal neoplasia and are not shown in the table. The demographics of those with recurrent FBOE did not differ from those with single episodes. It is notable that the pattern of recurrence did differ between groups – that is when the recurrence occurred in subsequent years those with EoE were more likely to represent in the same season and even calendar month, and usually in the grass pollen season.

	Eosinophilic esophagitis	Other diagnosis	P-value
Table 3. Characteristics of the patients having recurrent foreign body obstruction of the esophagus†			
1. †Only recurrences of FBOE that occurred in subsequent years are recorded. ‡Two patients had more than one recurrence, again in different months of the year and were incorporated in this data. NS, not significant.			
Number	6	19‡	
Biopsy performed at first foreign body obstructive event	1/6 (16%)	4/19 (21%)	NS
Mean age (range) (years)	39.1 (21–54)	62 (range 22–90)	NS
Proportion male	83%	73%	NS
Peripheral blood eosinophil count	0.4 (range 0.2–0.7; 95% CI) (4 available)	0.1 (range 0.5-0.2) (95% CI) (11 available)	NS
Recurrence same month in subsequent years	67%	10%	0.005
Occurrence in peak pollen season for rye grass (1 October to 1 January)			

†Only recurrences of FBOE that occurred in subsequent years are recorded. ‡Two patients had more than one recurrence, again in different months of the year and were incorporated in this data. NS, not significant.

Discussion

A primary aim of the current study was to find evidence for the importance or otherwise of aeroallergens in the pathogenesis of EoE. Previous studies lacked controls and had heterogeneity of presentations. The use of FBOE as a measure of seasonal variation was novel as it provided a ready control subgroup with non-EoE causes. The results clearly show that the presentation of EoE with FBOE is equally distributed throughout the year, as were non-EoE causes. Since FBOE is one of the most common presenting features of EoE, we contend that this indicates that factors unrelated to seasonal variation in pollen count are most important for the *majority* of patients. However, the other novel feature of the present study was the examination of recurrent FBOE in terms of seasonality, which has not been previously addressed despite the description of the phenomenon for recurrent FBOE in patients with hiatus hernia and in those with EoE[169, 170].The observation that recurrent FBOE demonstrates a seasonal trend that correlates with the appearance of the major allergenic pollen, ryegrass, suggests that, for a *subgroup* of patients with EoE, aeroallergens are important and that seasonal variations in disease activity could be expected. It is proposed that these dichotomous observations may assist in further characterisation of the relevance of food versus aeroallergens in driving disease activity. It could be inferred from the success of dietary therapy in the majority of patients that food allergens are important for most, but that aeroallergens are important for some. Future prospective longitudinal studies monitoring patients maintained on food allergen exclusion diets throughout the calendar year are indicated to further refine this hypothesis.

The characteristics of the patient population in our study are typical of those reported in previous literature relating to EoE and FBOE *per se*. Patients with EoE were more likely to be male and were younger that those with alternative diagnoses[37]. The causes of FBOE were also similar to previous studies. The apparent increase in EoE as proportion of total FBOE also

supports previous research[164] Patients with EoE were no more likely to reside in low density regions where exposure to aeroallergens is greater, although it is acknowledged that some of the population may be mobile, thus minimising the effect of local variations in pollen count. Also, the referral base of the hospitals involved may not fairly reflect the overall prevalence of the condition in the state of Victoria.

A supplementary aim for the study was to examine the current clinical practice around the diagnosis of patients presenting with FBOE. Critical to the diagnosis of EoE is the taking of esophageal biopsies for histopathological assessment. As the diagnosis of EoE has longer term implications and specific therapies are efficacious in preventing further problems, it would seem good clinical practice to pursue the reason why patients are presenting with an obstructed esophagus. Despite this, biopsies were on average taken in only one in four cases presenting with FBOE. While biopsy at the time of clearing the esophagus of the foreign body is often regarded as not of priority, the fact that few are coming back from repeat endoscopy in this case series and a diagnosis is not being reached in 75% is not acceptable, given the efficacy of dietary and topical therapies. Furthermore, among patients with recurrent FBOE, an esophageal biopsy was performed in less than 20% on the first presentation, suggesting that expensive and potentially risky hospital admission with endoscopic intervention could have been avoided.

Several limitations are present in the study design and are acknowledged. The hypothesis the FBOE occurs when EoE is most active, when an inflammatory infiltrate is florid, underlies the design of our study. It is plausible that several other factors could contribute, including the more chronic pathological change of subepithelial fibrosis, as well as external factors such as the intake of dense foods such as steak or chicken that conceivably may be ingested according to the season (e.g. barbeques) and in the setting where chewing may be suboptimal. Nonetheless, the use of a control group (all other causes of FBOE) should mitigate this issue.

The retrospective nature of data acquisition relies heavily on correct coding, and the number of identified cases may have been underestimated. One potential modifying factor, medication use, particularly of PPI and corticosteroids, was not reliably recorded and hence not analysed. Finally, but unavoidably, the fact that few patients underwent histological assessment of the esophagus greatly reduced the sample size and reliability of the observations.

Conclusion

The present study has shown that adult patients with EoE presenting with FBOE do so evenly throughout the year, suggesting that for most patients non seasonal factors are more important. Of importance, however, was the pattern of seasonal recurrence in patients with EoE corresponding with the grass pollen exposure. Hence, the results have suggested that aeroallergens may be of great relevance to some patients with EoE. Of perhaps more alarming significance was the relative rarity of the practice of biopsying the esophagus at endoscopy, representing a significant departure from ideal management, and contributing to recurrent FBOE. There is a need for prioritisation of performing biopsies at the index endoscopy.

3.3

Discussion

Our manuscript suggests that aeroallergens have a very limited role in patients with eosinophilic oesophagitis presenting with food bolus obstruction events, based on the failure to demonstrate seasonality in the occurrence of this phenomenon. It is acknowledged that the failure to obtain biopsies of the esophagus in 75% of individuals is a significant methodological weakness that limits the strength of the conclusions. However, the fact that recurrent episodes may be more common in a small subset of patients indeed raises the possibility that in a minority aeroallergens *are* of importance. Since the publication of our paper, another group overseas performed a very similar study, and the results differed in that seasonality was demonstrated. However, the validity of these findings was compromised by a lack of available data pertaining to pollen counts and thus the potential that the wrong months were analysed, as well as an inability to provide data on recurrent episodes (which as we found were relatively common) and the fact that few biopsies were performed demonstrating non-specific oesophagitis (which is at odds to previous research). Our concerns about this manuscript were communicated to the authors by means of a 'letter to the editor' (attached – see 3.4). The authors agreed that these aspects were important limitations worthy of future enquiry (attached – see 3.5).

3.4

Philpott, H., Nandurkar, S., Royce, S. G. and Gibson, P. R. (2015), Letter: seasonality in eosinophilic oesophagitis and food bolus obstruction – what about recurrent episodes? Alimentary Pharmacology & Therapeutics, 42: 936. doi: 10.1111/apt.13313

Sirs;

We read the paper by Sengupta *et al*. with interest, and have the following observations and queries stemming from our own similar study[171].

Firstly, the rate of previous oesophageal food bolus impaction was recorded as high (45%) among those with eosinophilic esophagitis (EoE), yet data regarding repeated episodes are not forthcoming, despite a 10-year analysis. We encourage explanation and if possible provision of such data, given our findings that suggest a subgroup of patients with EoE (and not other aetiologies) have seasonal oesophageal food bolus impaction[51].

Secondly, oesophageal food bolus impactions are more common in spring/summer, a finding that the authors attribute potentially to elevated pollen counts. Our pollen season (Australia) for the pathogenic species (rye grass) is regularly between 9 and 11 weeks duration. Have the authors considered their region-specific pollen counts in this time-dependent manner in relation to oesophageal food bolus impaction?

Thirdly, the predominance of EoE as the established cause of oesophageal food bolus impaction is notable, while few cases of erosive reflux (four in total) are diagnosed. Is it the supposition that erosive reflux is a rare cause of oesophageal food bolus impaction, or rather that few biopsies are taken for cases other than suspected EoE?

Finally, the documentation of adverse events associated with the management of oesophageal food bolus impaction is admirable. The high rate of complications including aspiration coupled with the majority of procedures being performed using conscious sedation is of note. We suggest there is thus a case for mandatory general anaesthesia (hence protecting the airway).

3.5

Sirs;

We appreciate the commentary by Philpott *et al*. regarding our study.[1, 2] Regarding repeated oesophageal food bolus impaction for patients with eosinophilic oesophagitis (EoE), our database only captured information during the first impaction to present at our centre during the study period. This rationale was based on our primary aims, which were to report aetiologies of impaction, as well as general outcomes. Patients with EoE who had a prior impaction all had episodes prior to 2004, or presented elsewhere prior to their index impaction at our centre. For those with recurrent impaction presenting to our centre during the study period, data regarding aetiology and season were not collected.

Regarding regional pollen counts, we suspect that such a study would be valuable, as recent data demonstrate a consistent seasonal variation in EoE diagnosis, with cases occurring more in the summer.[3] Furthermore, we acknowledge that studies reporting seasonal variation in pollen counts, and whether these correlate to increased rates of impaction, are necessary. We did not collect this information, as an analysis of seasonal variation of EoE-related food bolus impaction was not our primary aim.

We did note a low frequency of erosive oesophagitis, which was a rare cause of food bolus impaction in the absence of an oesophageal stricture. Increasing the biopsy rate would likely further increase the proportion of EoE-related food bolus impaction, as the majority of non-EoE food bolus impactions do not typically require histology to establish a diagnosis.

Finally, they suggest that mandatory general anaesthesia (GA) should be employed. It is noteworthy that GA was associated with a higher risk of complications (OR 5.0). While this is confounded by indication, it demonstrates that GA does not completely mitigate periprocedural risk. Further data are necessary before imposing a practice mandating GA for oesophageal food bolus impaction, given that conscious sedation appears to be safe and effective in selected patients.

References

1. Philpott H, Royce S, Nandurkar S, Gibson P. Letter: seasonality in eosinophilic oesophagitis and food bolus obstruction – what about recurrent episodes? Aliment Pharmacol Ther 2015; 42: 936.

2.Sengupta N, Tapper EB, Corban C, Sommers T, Leffler DA, Lembo AJ. The clinical predictors of aetiology and complications among 173 patients presenting to the Emergency Department with oesophageal food bolus impaction from 2004-2014. Aliment Pharmacol Ther 2015; 42: 91–8.

3.Jensen ET, Shah ND, Hoffman K, Sonnenberg A, Genta RM, Dellon ES. Seasonal variation in detection of oesophageal eosinophilia and eosinophilic oesophagitis. Aliment Pharmacol Ther 2015; 42: 461–9.

CHAPTER 4

4.1

Overview

The diagnosis of eosinophilic oesophagitis is made at gastroscopy and biopsy, with the finding of >15 eosinophils per high power field (HPF) in one or more oesophageal region meeting diagnostic criteria. The invasive nature of current upper gastrointestinal endoscopes and the frequent need for recurrent sampling lead to our consideration of alternative methodologies. The published manuscript that represents original scientific research and forms the basis of this chapter details the use of transnasal gastroscopy in adult patients with eosinophilic oesophagitis.

4.2

Philpott, H., Nandurkar, S., Royce, S. G., and Gibson, P. R. (2016) Ultrathin unsedated transnasal gastroscopy in monitoring eosinophilic esophagitis. Journal of Gastroenterology and Hepatology, 31: 590–594. doi: 10.1111/jgh.13173.

Abstract

Background

Ultrathin unsedated transnasal gastroscopy (UTEG) has a number of advantages applicable to eosinophilic esophagitis (EoE) and has not been evaluated for this condition.

Aim

The aim of the study is to determine the feasibility of UTEG in patients with EoE and the acceptability of histological specimens obtained at biopsy.

Method

All patients with a diagnosis of EoE presenting to the outpatients department of two hospitals (Box Hill Hospital and The Alfred Hospital, Melbourne Australia) were asked to participate in the study. UTEG was performed on consenting individuals. Feasibility was determined by the success of nasal intubation, patient perception according to post procedural survey, and adequacy of esophageal biopsies was assessed.

Results

Ninety-six consecutive patients with EoE were offered UTEG, and 24 agreed to participate in the study. Seventy-four UTEGs were performed over a period of 26 months (September 2012 to December 2014). Nineteen patients had repeat procedures. Successful nasal intubation occurred in 97% (72 of 74 procedures), and 21 of 24 (86%) described high satisfaction with

the procedure and minimal discomfort, and would choose UTEG for future procedures. Mean duration was 5 min. Adverse events of epistaxis (three cases) and vomiting of liquid contents during the procedure (two cases) were recorded, cardiorespiratory parameters remaining normal in all patients. All completed procedures produced adequate histological samples.

Conclusion

In those who decide to undergo UTEG, it is a safe and well-tolerated procedure

Introduction

Eosinophilic esophagitis (EoE) is a chronic inflammatory condition of the esophagus that is diagnosed and monitored on the basis of histopathological assessment of multiple biopsies of the esophagus obtained via video endoscopy. More than 15 eosinophils per high power field is considered diagnostic of EoE or indicates a lack of response to treatment if administered[172]. In Western countries such as Australia, video endoscopy is most frequently performed via the transoral route using a standard caliber video endoscope and requires intravenous sedation[173]. The costs and inconvenience associated with intravenous sedation (e.g. employment of a specialist anesthetist, inability to work, or drive a car on the day of the procedure) are considerable[174]. Furthermore, the success or otherwise of therapy for EoE also depends upon esophageal histopathology as there are no reliable non-invasive indices of response. When complicated treatment regimens such as the six-food elimination diet are applied, repeated endoscopic assessment is required to assess response. Indeed, eight or more gastroscopies are required for the successful implementation of dietary therapy for EoE[174]. Thus, there is the need for a less cumbersome method of obtaining esophageal biopsies[175].

Improvements in technology have enabled the development of fine bore video endoscopes[176]. These devices have the advantage of greater patient comfort, and hence, the need for sedation is reduced. The application of local anesthetic spray alone is feasible[176]. The use of ultrathin unsedated transnasal gastroscopy (UTEG) for the performance of esophageal biopsies has been trialled successfully for the assessment of Barrett's esophagus[177]. In a comparative study of trans-oral versus trans-nasal video endoscopy in 32 patients with Barrett's esophagus, no differences in the quality of endoscopic vision or histological specimens were observed in either group[177].

We hypothesized that the use of UTEG specifically for the assessment of EoE would likewise be safe and accurate. The current study aimed first to assess the endoscopic success of UTEG in patients with EoE; secondly, to define the experience of patients having UTEG; and thirdly, to compare the quality of esophageal biopsies obtained from patients undergoing UTEG without sedation with those obtained in a different cohort having standard transoral gastroscopy with sedation (STOG).

Materials and methods

Participants

Patients with an established diagnosis of EoE attending the outpatients' department of two hospitals (The Alfred Hospital and Box Hill Hospital, Melbourne Australia) were invited to participate. Patients 18 years or older who were scheduled for outpatient gastroscopy were eligible for inclusion. Excluded were those with significant cardiovascular or respiratory illness; coagulopathy; ear, nose, and throat conditions; or previous surgery or those unable to give informed consent.

Protocol

Demographic data that included gender, age, country of birth, and educational attainment were recorded for those electing to undergo either UTEG or STOG after giving written, informed consent. The endoscopic procedure was performed and biopsies prepared and examined as described in the succeeding texts. Adverse events were documented by the responsible endoscopist in association with the procedure. A questionnaire was administered to the participants just prior to discharge or within 1 week just prior to being reviewed in clinic. The study protocol was approved by the Eastern Health Research and Ethics Committee and Monash University (approval number E19/1213)

Endoscopy

The procedures took place in the day procedure unit of Box Hill Hospital. Topical pharyngeal anaesthetic spray (lignocaine 100 mg/mL) was applied to the patients preferred nostril and to the posterior oral cavity 10 min prior to nasal intubation. Supplemental oxygen (2 L/min) was administered for 5 min prior to the commencement of the procedure. Oxygen saturations were recorded continuously throughout the procedure via a finger pulse oximeter.

UTEG was performed with the patient at 45°, with the head turned towards the endoscopist. Two endoscopists (HP and SN) performed all of the UTEGs, both previously having experience with the device. The Pentax EG-870 K gastroscope with an insertion tube diameter of 6 mm was inserted through the nostril following the application of a water-based lubricant. On reaching the piriform fossa, the patient was instructed to swallow to aid advancement of the gastroscope. Following visualization of the stomach, the gastroscope was withdrawn to 5 cm above the gastroesophageal junction (Z-line) and four biopsies were taken at this location ("lower esophagus"). Biopsies were repeated at two further locations, 5 and 10 cm proximal to the initial site (that is "middle" and "upper" esophagus). Two-millimeter diameter "pediatric" biopsy forceps were used.

STOG was performed by a number of different experienced and credentialed gastroenterologists. The study did not examine STOG and patient perception thereof. Biopsies were taken in a standardized manner (as per UTEG), and the histological specimens were analyzed. Sedation was administered intravenously using propofol.

Outcome measurements

Three groups of outcomes were measured:

- *Patient-reported outcomes*: These were obtained via a questionnaire that contained three questions that assessed patient satisfaction in comparison with previous transoral gastroscopy, procedural discomfort, and willingness to repeat UTEG. Responses were recorded using a Likert-type scale.

- *Endoscopic outcomes*: The successes of nasal and pharyngeal intubation and of the taking of esophageal biopsies as planned were recorded. Adverse events such as epistaxis and vomiting at the time of the procedure and, when applicable, following subsequent outpatient review were noted.

- *Histopathologist-reported outcomes*: Expert histopathologists (Eastern Health department of Pathology) estimated and recorded the size of biopsies taken in patients undergoing UTEG or STOG. The quality of the biopsies after sectioning and staining with H&E was qualitatively assessed as adequate or inadequate for diagnostic assessment. Sampling of lamina propria was assessed following additional staining of the tissue (Masson's Trichrome) and reported as present or absent. Eosinophil counts per high power field were documented. This analysis was performed blinded to the type of gastroscopy performed.

Statistical analysis

Data were arranged and analyzed using Microsoft Access. For continuous variables, distribution is described by mean. For comparison of non-parametric data, the Fisher's exact test is used

Results

Participants

Enrolment took place between September 2012 and December 2014. Of the 96 patients invited to participate, 24 consented to undergo UTEG and the remainder consented to STOG. Seventy-four UTEGs were performed in total. The characteristics of the patients are displayed in Table 1. The mean age, gender, and education status do not differ between those who elected for UTEG and the conventional procedure. Those born overseas (UK and South Africa) were more likely to choose UTEG than Australian or New Zealand born individuals ($P = 0.0233$ Fisher's exact test)

Table 1. Demographic and other features of the study participants

		Unsedated transnasal gastroscopy (UTEG) $n = 24$	Standard transoral gastroscopy (STOG) $n = 72$
Mean age (range)		38 (19–62) y	39 (18–59) y
Gender		21 male (88%) 3 female	33 male (87%), 5 female
Educational attainment	Tertiary	13	45
	High school	8	19
	Primary School	3	8
Country of birth	Europe	8 (46%)	13 (33%)
	• *UK*	*5*	*11*
	• *Continental Europe*	*3*	*2*
	Non-Europe	16 (54%)	59 (77%)
	• *Australia/New Zealand*	*13*	*56*
	• *South Africa*	*3*	*1*
	• *North America*	-	*2*
Previous transoral gastroscopy		24	72
Previous transnasal gastroscopy		2	0
Duration of diagnosis of eosinophilic esophagitis		4 (0–9) years	3.5 (0–7) years
Food bolus obstruction events		17 (70%)	56 (80%)
Treatment			
• Successful elimination diet		9 (37.5%)	16 (22%)
• Unsuccessful elimination diet - budesonide		3	7
• Corticosteroids as only therapy		12 (50%)	47 (65%)
• Esophageal dilatation		0	2

Endoscopic outcomes

Successful nasal intubation occurred in 72 of 74 procedures (97%); enlarged turbinates were described as impeding scope passage in the two failed cases. Successful esophageal intubation was possible in 71 of 72 procedures (98%), with an excessive gag reflex preventing scope passage in one patient. Once successful esophageal intubation was achieved; 70 of 71 (98%) procedures were completed including the taking of all planned esophageal biopsies, with one procedure being terminated prematurely to recurrent gagging. Those patients who were unable to proceed with UTEG all successfully completed STOG on the same day.

No major adverse events were recorded. Three episodes of minor epistaxis that did not require treatment occurred on the day of the procedure. Two patients had one episode each of gagging and then vomiting a small volume of clear liquid at the time of the procedure. One patient reported sneezing small volumes of clotted blood in the week after the procedure, and two other patients reported soreness of the intubated nostril for a few days following the procedure.

Patient-reported outcomes

A post-procedural survey was completed by all patients and was available for 63 of 74 procedures. Of the 22 patients having repeated procedures, 17 (77%) preferred UTEG compared with previous STOG following their initial UTEG (Table 2). All patients who initially successfully completed the procedure elected to undergo subsequent repeat UTEG. Patient satisfaction and levels of discomfort recorded according to the Likert score are shown in Tables 2 and 3. Minor discomfort was experienced by most patients, severe in a minority. At first attempt, the majority of patients were very satisfied or satisfied with the procedure (88%). Levels of discomfort with the procedure tended to lessen with subsequent gastroscopies, and levels of satisfaction in turn rose in those with repeat procedures (Tables 2 and 3).

Table 2. Post procedural questionnaire (initial UTEG, *n* = 24 patients)

Question	VAS[a] 0–25	VAS 25–50	VAS 50–75	VAS 75–100
1. *a*VAS = visual analogue score out of 100.				
1. "I am in general satisfied with the procedure"	Very satisfied	Satisfied	Dissatisfied	Very dissatisfied
	n = 14	*n* = 7	*n* = 1	*n* = 2
2."Please indicate the level of discomfort you experienced with the procedure"	Minor discomfort	Moderate discomfort	Severe discomfort	Very Severe discomfort
	n = 16	*n* = 6	*n* = 2	*n* = 2
3."I would prefer to have unsedated transnasal gastroscopy instead of standard transoral gastroscopy with sedation"	Strongly agree	Agree	disagree	Strongly disagree
	n = 18	*n* = 3	*n* = 1	*n* = 2

Table 3. Post procedural questionnaire (final UTEG, *n* = 22)

Question	VAS 0–25	VAS 25–50	VAS 50–75	VAS 75–100
"I am in general satisfied with the procedure"	Very satisfied	Satisfied	Dissatisfied	Very dissatisfied
	18	4	0	0
"Please indicate the level of discomfort you experienced with the procedure"	Minor discomfort	Moderate discomfort	Severe discomfort	Very severe discomfort
	16	6	0	0
"I would prefer to have unsedated transnasal gastroscopy instead of standard transoral gastroscopy with sedation"	Strongly agree	Agree	disagree	Strongly disagree
	17	5	0	0

Histological sampling

The biopsy specimens taken at UTEG and STOG were judged adequate for histological assessment in all cases and in all locations. The sizes of the specimens taken at each location (upper, middle, and lower esophagus), using 2.0 mm forceps via UTEG, compared with large capacity 2.3 mm forceps at STOG, are shown in Table 4. Lamina propria sampling was achieved in a minority of those who underwent UTEG (22%) compared with those having STOG (68%; *P* = 0.0070, Fisher's exact test).

Table 4. Comparison of esophageal biopsies when obtained via UTEG or STOG

Biopsy site		Standard Transoral Gastroscopy (STOG) $n = 188$	Unsedated transnasal gastroscopy (UTEG) $n = 74$
Lower esophagus	Mean length (range)	3.3 (1.8–4.2) mm	1.8 (1–2.4) mm
	Lamina propria sampled	124 (66%)	17 (23%)
	Mean eosinophil count	24.3 (0–80)	22.6 (0–80)
Middle esophagus	Mean length (range)	3 (2–4) mm	2.1 (1–3) mm
	Lamina propria sampled	126 (67%)	18/74 (24%)
	Mean eosinophil count	23.2 (0–80)	23.1 (0–80)
Upper esophagus	Mean length (range)	3.1 (2–4) mm	1.8 (1–4) mm
	Lamina propria sampled	126 (67%)	17 (23%)
	Mean eosinophil count	21.4 (0–80)	21.8 (0–80)

Discussion

UTEG has potential clinical and cost advantages particularly applicable to EoE, and the current study was undertaken to determine the feasibility, efficacy, and tolerance in this formerly unstudied patient group requiring repeated sampling over time. Previous data indicate that UTEG is safe and effective as a screening method for upper gastrointestinal malignancy when used in Japan and that histological sampling is adequate and well tolerated in older North American men with Barrett's esophagus [177, 178].The present study shows that UTEG is a safe and well-tolerated procedure in young predominantly white Caucasian male patients and that those electing to try the method are willing to undergo repeated procedures over time. Histological sampling is adequate for assessing the hallmark features of EoE. There was also interesting finding that an individual's country of birth was associated with the relative willingness to undergo the procedure.

The current study demonstrates that UTEG has a high degree of procedural success, with 71 of 74 procedures (96%) resulting in the requisite sampling of esophageal tissue. This is in keeping with previous studies on other patient groups[173, 179]. Subsequent UTEGs required on these patients with EoE were all successful. The obstacles to completion of UTEG, being failure of nasal intubation because of enlarged turbinate's and excessive gagging, are similar to previous data. It is notable that the technique of application of local anesthetic varies between groups. In our study, local anesthetic was applied by spray to the nostril and posterior oropharynx, which is a common method, although others advocate the use of local anesthetic gel and application with a nasal catheter[180]. Procedures were performed at 45°, although some groups prefer an upright sitting position[180]. The safety of the procedure, with three episodes of minor

epistaxis, some nasal soreness and stable respiratory observations is in keeping with previous other studies[180].

Patient perception of the procedure in the present study was similar to earlier studies in other populations[176, 178]. Minor nasal discomfort was the major side effect, and the majority of patients are satisfied with the procedure. The current study differs from others in that repeated procedures is performed over time. Of importance, all patients who underwent a successful transnasal gastroscopy were willing to have and did subsequently have at least one repeat procedure. A trend towards greater patient satisfaction and decreased procedural discomfort was observed with repeated procedures (Tables 3 and 4).

The patients in our study were reluctant to have UTEG instead of STOG. Only about one quarter of patients elected for UTEG. This is similar to the experience elsewhere, although our population was younger than those with Barrett's esophagus[181]. This would not necessarily reflect the proportion who would accept such a method in routine clinical practice, because these patients were provided a choice in an open manner that reflects good research practice. In routine practice, if sedated endoscopy was not readily available, uptake may have been greater. However, the present study was not designed to examine factors related to this choice. Nonetheless, those electing to undergo UTEG as opposed to STOG were more likely to have been born in UK, South Africa, or Continental Europe as opposed to those born in Australia or New Zealand. This may reflect cultural expectations and current medical practice. In Japan, for example 50% of gastroscopies undertaken for gastric cancer screening are via the transnasal route[178]. Age, gender, and education status did not influence the choice of endoscopic procedure in our group.

The histological samples taken via UTEG using the 2.0 mm biopsy forceps provided adequate specimens in all cases and in all esophageal locations. Estimation of eosinophil counts was possible. Tissue samples were measured to be within 1 and 3 mm in length in all UTEGs. Sampling of the lamina propria was rare. Previous work has demonstrated that the depth and length of tissue specimens obtained using 2.0 mm biopsy forceps is less than the standard 2.3 mm forceps[180]. STOG (with 2.3 mm biopsy forceps) enabled larger specimens to be taken and lamina propria to be sampled in nearly three times as many patients. Current diagnostic guidelines related to EoE do not mention analysis of the lamina propria, with treatment response guided by epithelial eosinophil count only; thus, deeper biopsies seem unnecessary in patients with EoE (as opposed to Barrett's esophagus where this may be important). Importantly, the mean eosinophil count was the same regardless of whether biopsies were taken via STOG or UTEG, discounting concerns that the depth of biopsies may influence diagnostic yield[180]. While a larger representative sample with larger forceps may have theoretical advantages as EoE is a patchy disease, the benefit of marginally more sampling of an organ whose surface area is approximately 125 cm^2 is debatable[182]. Thus, it is apparent in routine diagnosis and in planning response to treatment; biopsies taken with 2.0 mm forceps are adequate.

The financial implications of using transnasal gastroscopy and the potential savings were not addressed in the present study as patients were treated in the same day procedure unit and on the same list as those with other conditions undergoing transoral gastroscopy for other reasons. Nonetheless, future potential cost savings appear sizeable, particularly if the length of hospital admission (and required staffing) and the lack of a requirement for an anesthetist, nursing staff, and medications such as fentanyl and propofol are considered. Previous work indicates a cost saving of approximately $180 per procedure (in terms of costs to the health sector) in favour of transnasal gastroscopy[181]. Cost savings to the patient are also substantial, for example the

ability to return to work on the same day of the procedure and to drive a car. In a patient requiring eight procedures as a part of the six-food elimination diet, the cost savings are multiplicative[174]. The true benefit of transnasal gastroscopy has arguably not been realized until offered in the outpatient setting, thus abrogating the need for hospital admission. Outpatient transnasal fibreoptic laryngoscopy is routinely used by ear, nose, and throat surgeons, highlighting the potential for future implementation in managing patients with EoE[183].

Several limitations with the current study are acknowledged. Patient perception and adverse effects were not directly compared with STOG, but extensive data already exist confirming the acceptance and adequate histological sampling achieved by STOG, and the safety of UTEG. STOG and UTEG were not performed "head to head" on the same patient to determine the quality of tissue sampling, but once again, the quality of sampling in STOG is established, and as patients were undergoing treatment tissue sampling may have altered with the disease state per se. No attempt was made to determine or describe the quality of views obtained at video endoscopy using UTEG. UTEG can, however, provide adequate visualization for conditions where this is important, especially in Barrett's esophagus. In contrast, in EoE, the histological sample rather than the endoscopic appearance has diagnostic utility[24, 184].

185

The role that UTEG may play in the future management of EoE will depend on the efficacy of alternative minimally invasive techniques that sample the esophagus, or ideally novel biomarkers in, for example the blood or sputum. The esophageal string test and the cytosponge test are two newly devised methods that enable sampling of esophageal tissue without the need for endoscopic visualisation. The string test has the disadvantage of requiring the subject to leave the device in situ overnight, whilst the cytosponge requires a local anaesthetic spray prior

to swallowing (although is only swallowed for minutes prior to removal) [185, 186]. Both are well tolerated. The patchy nature of esophageal disease, with a tendency for lower esophageal infiltration arguably suggests that visually guided sampling of all esophageal areas is an advantage, as recently described[187]. Obviously, the utility to such techniques will only be clarified with systematic study.

In conclusion, UTEG is a safe, effective, and well-tolerated procedure in patients who have been diagnosed with EoE and agree to it. The advantages are particularly evident in this condition where multiple endoscopies are often required to assess the response to treatment via histological sampling. The major impediment to widespread implementation of UTEG for patients with EoE is initial reluctance to try the procedure, which relates to sociocultural expectations. Patient education and gastroenterological confidence that the technique is not compromising diagnostic ability may facilitate future widespread implementation of UTEG.

4.3

Discussion

We demonstrated that transnasal gastroscopy is a safe and efficacious method of sampling oesophageal tissue in adult patients with eosinophilic oesophagitis. Since our manuscript was published, a paediatric group mirrored our work, produced similar findings and thus consolidated our conclusions [188]. Alternative technologies that may supplant gastroscopy are considered in Chapter 8.

CHAPTER 5

5.1

Overview

Significant and rapid changes in the approach to diagnosis and treatment of eosinophilic oesophagitis have been advocated in recent years (see Figure 5.2). Two apparently disparate observations, namely that oesophageal eosinophilia can completely resolve in a significant number of patients when proton pump inhibitors are introduced, and secondly that elimination diet can resolve oesophageal eosinophilia, have led to a change in clinical guidelines. Both therapies are advocated as first line treatment in patients with eosinophilic oesophagitis. The limitations of the data underpinning these guidelines, and the need for a 'real - world' study in populations outside the United States or Spain is discussed in the published manuscript of original research and related editorial commentaries.

5.2

(Figure) History of EoE and treatment approach

1.All cases of esophageal inflammation with eosinophils are GERD or infection

2.Attwood **(1993)** distinct entity of Eosinophilic esophagitis and patients presenting for fundoplication with no GERD (pH studies are performed)

Clinicians think about EoE and use budesonide

3.Patients with esophageal eosinophilia responsive to PPI's with/without GERD (PPI-REE) (**2011**)

Clinicians think about using PPI's

4.All patients with esophageal eosinophilia should have BD PPI's for diagnostic/therapeutic purposes (**2013**)

5.3

Philpott, H., Nandurkar, S., Royce, S. G., Thien, F. and Gibson, P. R. (2016), A prospective open clinical trial of a proton pump inhibitor, elimination diet and/or budesonide for eosinophilic oesophagitis. Alimentary Pharmacology & Therapeutics, 43: 985–993. doi: 10.1111/apt.13576

Summary

Background

Elimination diets and high-dose proton pump inhibitors (PPI) are advocated as first-line treatments in patients with eosinophilic oesophagitis (EoE).

Aim

To record the treatment outcome for patients with EoE prospectively managed according to a clinical algorithm.

Methods

Patients with oesophageal eosinophilia commenced esomeprazole 40 mg twice daily for 8 weeks. Those in histological remission were re-classified as PPI-responsive oesophageal eosinophilia. Nonresponders were offered the 6-food elimination diet with a PPI, or topical budesonide monotherapy (1 mg orally twice daily as an aqueous gel). Once disease control was achieved remission was reassessed at 3 months (all modalities) and an additional 6 months (diet group).

Results

Of 107 patients who completed 8 weeks of PPI, 25 (23%) were PPI-responsive. 56 of 81 (69%) of patients with EoE chose the elimination diet with PPI. 29 (52%) had complete remission, 23 completed dietary reintroduction and food triggers were identified in 20 (36%). 25 chose budesonide with 23/25 (92%) responding. Remission was sustained in >85% of patients at 3 months with all treatment modalities. At 9 months, only 10/18 (55%) of patients who responded to the elimination diet with PPI remained compliant and sustained remission.

Conclusions

Many patients previously diagnosed with EoE will respond to PPI. Initial response >50% is possible with the elimination diet plus PPI, but many will fail to undergo food reintroduction, or will cease the diet and relapse, resulting in only one in four patient sustaining remission at 9 months. Budesonide is very effective short term, but longer term study is needed.

Introduction

Eosinophilic oesophagitis (EoE) is a recently recognised and defined condition, characterised clinically by symptoms of dysphagia and food-bolus obstruction events and pathologically by oesophageal findings of eosinophilic infiltration, epithelial and muscular hyperplasia and resultant luminal narrowing[172]. Recent studies have demonstrated a response in many patients to high dose, twice-daily proton pump inhibitors (PPI) and to elimination diets, targeting food antigens thought to drive the inflammatory process in the context of a failure of immune tolerance[1, 189, 190]. International organisations have been quick to integrate the findings of a number of therapeutic clinical trials into published management guidelines with high-dose PPI advocated for all patients with suspected EoE. Those responding to twice-daily PPI are labelled as PPI-responsive oesophageal eosinophilia rather than EoE. For those with EoE, dietary therapy is recommended as a first-line therapeutic option[1]. It is apparent, however, that the 'external validity' of this recommendation – the efficacy of such treatment outside of the closely supervised clinical trial setting, in alternative patient groups and followed over long-time periods – has yet to be established[191]. This would seem particularly important given the awkward nature of dietary therapy, the potential for region-specific allergens to alter sensitisation and thus therapeutic efficacy, and the unknown mechanism whereby PPIs exert their therapeutic effect[192, 193].

The aims of the present study were, therefore, to record the clinical outcome of patients enrolled prospectively and managed according to a structured clinical algorithm.

MATERIALS AND METHODS

Patients

This study prospectively examined all patients aged 18 years or older referred to the gastroenterology outpatient clinic of two tertiary hospitals (Box Hill Hospital and The Alfred Hospital, Melbourne, Australia) between September 2013 and January 2015 with oesophageal eosinophilia. This finding must have been made by previous upper gastrointestinal endoscopy and biopsy demonstrating an oesophageal eosinophil count of ≥15 eosinophils per HPF in at least one section. Excluded were patients with gastric or duodenal eosinophilia, and those taking medications or with medical conditions likely to produce eosinophilia or alter results (e.g. antiepileptics, inhaled corticosteroids or oral corticosteroids for asthma, lymphoproliferative conditions). All participants provided written informed consent before commencement of the study. The study protocol was approved by the Eastern Health and Monash University Human Research and Ethics Committees.

Study protocol

This was a prospective observational study. The design was quasi-experimental and involved a removed-treatment method in that patients entering the study on corticosteroids were required to cease these medications for 8 weeks prior to commencing the treatment algorithm, as shown in Figure 1. All patients were required to take esomeprazole 40 mg orally twice-daily for 8 weeks followed by gastroscopy and biopsy of the oesophagus, stomach and duodenum. If oesophageal eosinophil density was <15/HPF then PPI-responsive eosinophilia was diagnosed and PPI therapy continued. After 3 months, these patients underwent repeat gastroscopy and biopsy. They then left the study protocol irrespective of the results. Patients who were not PPI-responsive were offered the choice of one of two therapies:

1. *Dietary therapy with the six-food elimination diet*: PPI therapy was continued at the same dose. Written information about the diet was provided to all patients and consultation with a dietitian was offered. Patients who responded to dietary therapy had sequential reintroduction of foods according to the algorithm in Figure 2. Briefly, this involved food challenges (where the participant was instructed to consume at least one serve of the additional, 'culprit' food at least twice per day (e.g. one slice of bread per twice-daily, one glass of milk or one egg twice-daily) followed by gastroscopy in 2 weeks. Absence of oesophageal eosinophilia led to a challenge with the next food type, whereas recurrence of eosinophilia led to exclusion of that food type. Subsequent food challenge was given immediately when a putative food trigger failed to cause eosinophilia, while a positive response lead to food removal and a 4 week 'washout' period. Partial responders continued the culprit food for a further 2 weeks and repeated the gastroscopy, and if this persisted dietary failure was defined. Those who failed the elimination diet were offered budesonide.

2. *Oral topically acting corticosteroids*: PPIs were ceased on commencement of budesonide that was made up from 1 mg/2 mL ampoule mixed with sucralose to make a gel and administered twice-daily. Gastroscopy and oesophageal biopsy were performed 6 weeks following commencement.

Figure 1.

Process and order of dietary reintroduction in patients initially responding to the six-food elimination diet. Gastroscopy is performed 2 weeks following food introduction. If recurrence of eosinophilia is detected, the culprit food is removed and a 4 week (wash-out) interval occurs before repeat gastroscopy.

Figure 2.

Treatment outcome of patients presenting with oesophageal eosinophilia and diagnosed with proton pump inhibitor-responsive oesophageal eosinophilia or eosinophilic oesophagitis (EoE) after induction therapy following a structure treatment algorithm using combinations of proton pump inhibitors, 6-food elimination diet and to topical budesonide 1 mg twice-daily). Treatment outcome is divided into four phases:

1. *Determine if EoE or PPI-responsive oesophageal eosinophilia is correct diagnosis with esomeprazole 40 mg twice-daily and gastroscopy after 8 weeks*
2. *Patients choose if they wish to have budesonide monotherapy or elimination diet and PPI, with the results determined by gastroscopy after 6 weeks*
3. *(a) Patients responding to elimination diet undergo process of food reintroduction and repeat gastroscopy. (b) Patients failing elimination diet choose budesonide monotherapy and have repeat gastroscopy.*
4. *Determine durability of treatment. Outcomes for patients with PPI-responsive oesophageal eosinophilia (on esomeprazole 40 mg twice-daily) and EoE (budesonide monotherapy or diet with PPI) as determined by gastroscopy and biopsy were assessed at 3 months. The durability of diet with PPI therapy also determined at 9 months.*
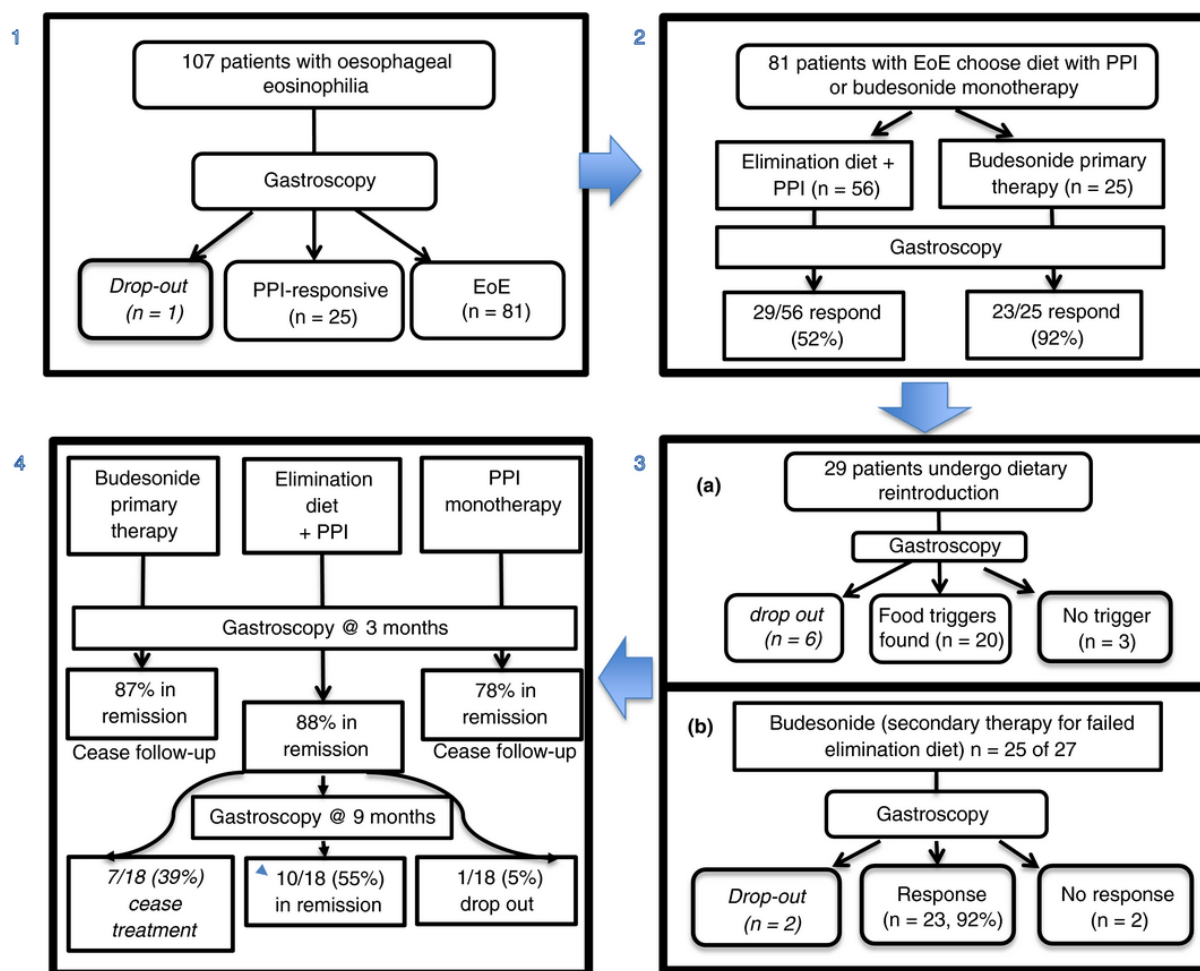
RESULTS

Endoscopy, biopsy and histological assessment

All gastroscopies were performed by gastroenterologists from the respective departments of both hospitals, the majority by HP and SN. Biopsies were taken from the lower oesophagus (defined as 5 cm proximal to the gastro-oesophageal junction) and from the middle and upper oesophagus at 5 cm intervals. Four specimens of tissue were taken at each location. Transoral and transnasal gastroscopes were used, sedation use as previously described[194]. Specimens were transported in 4% neutral-buffered formalin, then imbedded in paraffin and stained with haematoxylin and eosin. Standard histopathological analysis of gastric and duodenal biopsies occurred. The peak eosinophil count was recorded in all three areas of the oesophagus in the most densely infiltrated areas, where 10 respective areas analysed at HPF (400 times magnification, area measured in each case 0.212 mm^2) were averaged to give the mean eosinophil count. All specimens were reviewed by consultant pathologists blinded to the treatment method. The same pathologist reported the results of each individual patient over time. The accuracy of eosinophil counts independently was cross-checked by HP and SR using digital technology (Aperio imagescope), the results were almost identical and led to no change in categorisation.

Demographic data

All patients were assessed by a gastroenterologist (HP) and the following data were recorded: date of birth, country of birth, migration and date of migration from overseas, coexistent allergic conditions, previous food bolus obstruction events, date diagnosed, previous treatment and current symptoms.

Outcome measures

The success of any treatment modality was defined according to histopathology. A *complete response* was defined as <5 eosinophils per HPF in all oesophageal locations, a *partial response* as 5−14 eosinophils is one or more location, and *no response* as 15 or more eosinophils in one or more location.

Statistical analysis

The demographic data were expressed as mean and standard deviation or as a percentage of the total individuals. Only patients who had endoscopic evaluation after 8 weeks' PPI therapy were included in the analysis. For categorical data, inter-group comparisons were made using Chi-square analysis, while maintenance of disease remission among treatment groups was assessed using the one-way anova. The paired *t*-test was used to compare mean eosinophil counts post-treatment. A $P \leq 0.05$ was considered significant. Microsoft Excel was used for statistical analysis.

Patient characteristics and clinical features

A total of 156 patients were invited to participate, 115 patients were enrolled to study, seven failed to return for gastroscopy following 8 weeks of treatment with esomeprazole. Thus, 107 were included in the analysis. Demographic details are listed in Table 1. The male predominance, white Caucasian racial background and mean age of 37 years are evident. All patients had been previously treated with either a single therapy or combination therapies including PPI and topically acting corticosteroids. None had received systemically acting corticosteroids

Table 1. Demographic and clinical data on the 107 evaluable patients with oesophageal eosinophilia and according to a diagnosis of proton pump inhibitor-responsive oesophageal eosinophilia (PPI-REE) or eosinophilic oesophagitis (EoE)			
Index	**PPI-responsive oesophageal eosinophilia (*n* = 25)**	**Eosinophilic oesophagitis (*n* = 82)**	**P value**
Mean age (s.d.)	44 (14)	34 (11)	<0.01
Male gender	18 (72%)	69 (84%)	N.S.
Mean age (s.d.) at diagnosis	42 (11)	32 (10)	<0.05
Ethnicity			
White Caucasian	23 (92%)	80 (98%)	N.S.
Asian	1 (4%)	1 (1%)	N.S.
Middle Eastern	1 (4%)	1 (1%)	N.S.
Presence of atopic illness			
Seasonal rhinitis	16 (58%)	36 (44%)	<0.05
Asthma	5 (20%)	16 (19%)	N.S.
Food allergy or oral-food allergy syndrome	3 (12%)	6 (7%)	N.S.
Coeliac disease	2 (2%)	2 (2%)	N.S.
Helicobacter pylori positive (at initial endoscopy post 8 weeks of BD esomeprazole)	1 (1%)	2 (2%)	N.S.
Presenting symptom			
Food bolus obstruction	16 (64%)	31 (38%)	<0.05
FBOE and dysphagia	5 (20%)	25 (30%)	N.S.
Dysphagia alone	3 (8%)	19 (23%)	<0.05
Heartburn	1 (1%)	4 (5%)	N.S.
Other	0	4 (5%)	N.S.
Previous treatment of oesophageal eosinophilia			
PPI – daily	12 (48%)	66 (80%)	<0.05
PPI – BD	4 (16%)	8 (10%)	N.S.
Swallowed topically acting corticosteroid	8 (32%)	35 (43%)	N.S.
Diet	0	0	–
No treatment	0		

Treatment outcome

Treatment outcomes are shown in Figure 2. For clarity, patient management is considered in four phases;

Diagnosis of EoE or PPI-responsive oesophageal eosinophilia

The initial therapy with twice-daily PPI induced a complete response in 25 (23%), of whom 12 (48%) had previously received a daily PPI and 2 (8%) twice-daily PPI. Thus, 25 of the 107 patient with oesophageal eosinophilia were labelled as PPI-responsive oesophageal eosinophilia and 82 patients had a diagnosis of EoE.

Patient-directed choice of first-line therapy for EoE: trial of diet with PPI or budesonide monotherapy

Characteristics of patients choosing budesonide monotherapy or diet with budesonide are shown in Table 2. Of the 56 patients who elected to follow the elimination diet with PPI, 29 (52%) responded completely while 27 (48%) recorded persistent eosinophilia. In patients who did not respond, however, the eosinophil count did fall in all three regions of the oesophagus from a pre-diet eosinophil count in the upper, middle and lower oesophagus respectively of 36, 39 and 35 per HPF to a post-diet eosinophil count of 19, 19 and 24 per HPF respectively ($P \leq$ 0.005; paired *t*-test). A dietitian was consulted by 14/29 (26%) of responders and 14/27 (52%) of non-responders. Two were initially defined as partial responders, but both had relapsed at the repeat biopsies after 4 weeks.

Table 2. Comparison of patients who chose either dietary therapy or budesonide after failing to respond to twice-daily PPI		
Index	**Elimination diet ($n = 56$)**	**Budesonide ($n = 25$)**
Mean age (s.d.)	36 (10.4) years	39 (12) years
Male gender	40/56 (71%)	19/25 (76%)
Previous treatment		
Budesonide	27/56 (48%)	7/25 (28%)
Diet	0	0
Mean eosinophil count prior to treatment (s.d.)		
Upper	24 (9)	28 (8)
Middle	32 (9)	29 (9)
Lower	29 (7)	31 (8)
Mean eosinophil count after treatment		
Upper	0	0
Middle	0	0
Lower	0	0

Dietary reintroduction or trial of budesonide according to response to diet

Of the 29 patients who responded to the elimination diet, 23 had food triggers defined and again achieved resolution of eosinophilia following removal of the culprit food or foods. Six patients dropped out before completing the process of reintroduction and three reintroduced all foods, but still failed to define a food trigger. A preponderance of lower oesophageal eosinophilia was observed in response to culprit foods. Thus, participants who were in remission following the elimination diet and who had a disease recurrence following food introduction demonstrated isolated lower oesophageal eosinophilia in 12 of 34 such flares, lower and middle oesophageal eosinophilia in a further 14 of 34, and isolated upper oesophageal eosinophilia in only one case ($P = 0.005$; Chi-square). The focal eosinophilia was deemed secondary to food exposure as this resolved during the wash-out phase in 32 of 34 such flares (one lost to follow-up, the other failed to resolve and abandoned the diet). As outlined in Table 3, common food triggers included gluten, dairy and eggs, alone or in combination.

Dietary reintroduction or trial of budesonide according to response to diet

Of the 29 patients who responded to the elimination diet, 23 had food triggers defined and again achieved resolution of eosinophilia following removal of the culprit food or foods. Six patients dropped out before completing the process of reintroduction and three reintroduced all foods, but still failed to define a food trigger. A preponderance of lower oesophageal eosinophilia was observed in response to culprit foods. Thus, participants who were in remission following the elimination diet and who had a disease recurrence following food introduction demonstrated isolated lower oesophageal eosinophilia in 12 of 34 such flares, lower and middle oesophageal eosinophilia in a further 14 of 34, and isolated upper oesophageal eosinophilia in only one case ($P = 0.005$; Chi-square). The focal eosinophilia was deemed secondary to food exposure as this resolved during the wash-out phase in 32 of 34 such flares (one lost to follow-up, the other failed to resolve and abandoned the diet). As outlined in Table 3, common food triggers included gluten, dairy and eggs, alone or in combination.

Patient	Age (years), sex	Foods avoided	Follow-up oesophageal histology		
			3 months	6 months	9 months
1. [a]					
Gluten-containing food was avoided in according with guidelines issue for coeliac disease.					
1	55, female	Gluten[a]	Remission	Remission	Remission
2	58, female	Gluten	Remission	Not done	Remission
3	21, male	Gluten	Not done	Remission	Remission
4	44, female	Gluten	Remission	Not done	Remission
5	54, female	Gluten	Remission	Remission	Remission
6	29, male	Gluten	Not done	Remission	Ceased diet/flare
7	62, male	Dairy	Remission	Remission	Remission
8	19, male	Dairy	Remission	Not done	Remission
9	61, male	Dairy	Remission	Not done	Remission
10	19, male	Egg	Remission	Remission	Pending
11	19, male	Egg	Remission	Ceased diet	Ceased diet
12	64, male	Soy	Remission	Remission	Ceased diet
13	49, female	Dairy, egg, gluten, nut	Remission	Remission	Not done
14	27, male	Nut, fish	Remission	Remission	Remission
15	33, female	Dairy, egg	Remission	Ceased diet	Ceased diet
16	52, male	Dairy, egg	Ceased diet/flare	Ceased diet	Ceased diet
17	48, male	Gluten, egg, dairy	Remission	Remission	Ceased diet
18	19, female	Egg, soy	Remission	Remission	Remission
19	35, male	Gluten, egg, dairy	Ceased diet/flare	Ceased diet	Ceased diet
20	34, male	Gluten, dairy	Remission	Remission	Not done

Table 3. Longer term follow-up of individuals with eosinophilic oesophagitis who responded to the six-food elimination diet

Of the 27 patients who failed to respond to the elimination diet, 25 elected to commence budesonide monotherapy and 23 (92%) responded.

Maintenance and comparison of treatment durability

The durability of responses after the 'induction' therapy was assessed at 3 months in the majority of patients. Fourteen of 18 patients (78%) maintained remission after continuing twice-daily esomeprazole for a further 3 months. Twenty of 23 patients (87%) had continuing remission at 3 months while taking budesonide. Sixteen of 18 patients assessed at 3 months on a maintenance diet (88%) were in remission. Patients on the elimination diet were in addition followed up over 9 months, where 10/18 (55%) continued the diet and were in remission, 7/18 (39%) had ceased the diet and one was lost to follow-up.

DISCUSSION

This prospective study evaluated the external validity, or real-world application, of the various contemporary treatment strategies available for adult patients with oesophageal eosinophilia. Twice-daily PPIs are advocated as the initial treatment of oesophageal eosinophilia, and for the differentiation of EoE and PPI-responsive oesophageal eosinophilia[1]. Our cohort referred with suspected EoE included many previously under the care of specialist gastroenterologists, and, despite this, only a minority had received twice-daily PPIs highlighting a deviation from currently recommended practice. Subsequently, one quarter of all patients previously diagnosed with EoE were re-categorised as being PPI-responsive. Interestingly, 12 of 25 patients (48%) responding to twice-daily PPIs had a documented history of daily PPI for at least 4 weeks *before* a gastroscopy and initial diagnosis of EoE. This suggests a dose–response relationship, although other variables including the individual responsiveness to different PPIs and adherence to the prescribed regimen, as well as the number and location of oesophageal biopsies may have played roles. This is an important issue because the obvious corollary is whether or not patients with PPI-REE must be maintained on twice-daily PPI indefinitely or transitioned to daily therapy. The related issue of durability of PPI response is questioned by

the finding of relapse at 3 months in 22% of patients despite apparent adherence. Both the durability of PPI and the possible dose−response relationship was addressed in a very recent study, where patients were transitioned from twice to once daily PPI and a 18% relapse rate demonstrated[195]. PPI-responsive oesophageal eosinophilia had previously been defined using varying doses and durations of PPIs, a systematic review identifying eight studies all with different doses and variable use of pH studies (see below)[196]. Relapse of eosinophilia on PPI was identified in only six of 258 patients in a pooled analysis[196]. Closer supervision of a larger number of patients over a longer time period in further dedicated studies may better answer if durability and/or dose related effects are important considerations[191, 197].

The response to the elimination diet in the current study was numerically inferior to previous dedicated controlled trials of elimination diet (<55% compared to >65%)[24, 189]. This may relate to the relative lack of structured resources available to our patients (such as a clinical trial nurse), and the variable use of a dietitian, which was dictated by patient-choice in line with routine practice. However, the current study was not adequately powered to examine such issues. The continued surveillance of patient on dietary therapy over 9 months demonstrated that only 10/18 (55%) remained compliant and in remission. This has major implications as to the real-world application of the diet suggesting that efficacy is compromised presumably due to factors such as palatability. Our study differed from previous work in that twice-daily PPIs were continued for all patients, oesophageal pH studies were not performed, gastroscopes occurred at 2 weeks (instead of four or more weeks) after each food was introduced and biopsies were taken from the lower, middle and upper oesophagus (instead of two locations)[24, 189] However, it is unlikely that continuation of high-dose PPIs had influenced the initial success of dietary therapy and this may have in fact increased the response rate (see below). The duration of food reintroduction appears adequate as previous work has demonstrated recurrence of eosinophilia within 72 h of rechallenge[198]. Taking more biopsies

may have decreased the number of patients declared as responsive to the elimination diet, although previous work indicates five samples taken from two locations should diagnose all cases of EoE[199]. Indeed, eight of 27 patients (30%) who were determined to have initially failed dietary therapy had eosinophilia in the lower oesophagus only. Intriguingly, the combination of diet and PPIs did decrease the mean eosinophil count and in all oesophageal locations in patients deemed unresponsive to the elimination diet. This is a novel observation that we speculate may have relevance in patients refractory to monotherapies, and arguably suggests polysensitisation with some reduction of antigenic load with the elimination diet (see Figure 2). Three of the 29 patients who responded to the elimination diet continued in remission despite reintroduction of all foods. This also has not been previously reported. It is speculated that these patients have become tolerant to the putative food antigens in concert with continuation of the PPIs. Close follow-up with repeated gastroscopies over time may answer this question.

The response to budesonide therapy in our cohort is higher than attained in other adult studies and more akin to the response commonly demonstrable in paediatric centres[146, 200, 201]. Potential reasons to explain this difference might include better adherence to the therapy, though this was not formally assessed, the careful explanation of the timing of budesonide therapy (before bed, after brushing teeth and after breakfast after brushing teeth) and instructions to avoid eating or drinking for at least 2 h after the budesonide, and the use of a budesonide slurry rather than powder. Our patient cohort is very similar in terms of demographics and risk factors to previous studies[202].

The sparing of the upper oesophagus, and predominance of lower and middle oesophageal eosinophilia in our cohort of patients maintained on twice-daily PPIs and who received the elimination diet is worthy of special consideration. Our findings, which differ from previous

work where generalised eosinophilia has been noted following food reintroduction,[24, 189, 198] could potentially be related to the continuation of twice-daily PPIs in all patients in our study, the shorter duration between food exposures and biopsies (2 weeks compare to greater than 4 weeks) and the supposition that PPIs reduce eotaxin-3 predominantly in the upper oesophagus.[24, 189, 192] From a clinical standpoint, we assert that patients undergoing elimination diets must have lower oesophageal biopsies taken. We also speculate that the lower oesophagus is most important in initiating a recurrence of eosinophilia to trigger foods, possibly due to increased exposure to food antigens due to reflux of gastric contents, in a time-dependent manner. The effect of gastric refluxate in causing lower oesophageal eosinophilia, possibly by influencing barrier integrity, has been proposed previously and warrants further study[203].

Several weaknesses are acknowledged in the design of the current study. First, treatments were not randomised or placebo-controlled. Patients who did not respond to PPI were thus able to choose if they received dietary therapy or budesonide, and this limits the validity of comparative data, particularly as many patients had received budesonide (albeit in dry powder form) previously. However, our aim was to evaluate the external validity of contemporary management outside of a clinical trial setting. A related point is that the efficacy of previous treatments for oesophageal eosinophilia (prior to enrolment) was not available and thus comparable, preventing analysis of secondary treatment success. Indeed, the response to PPI therapy in defining PPI – REE is considerably lower than reported elsewhere[204]. This may be explained by the recruitment method and treatment setting, whereby patients were often referred by gastroenterologists in private practise to our specialist academic centre. Second, pH studies were not performed, potentially falsely ascribing cases as EoE that were in fact GERD. However, clinical guidelines advocate empirical use of twice-daily PPI as described, citing a failure of pH studies as a discriminative tool[1, 196]. Third, the use of frequent gastroscopies to evaluate oesophageal eosinophilia may be considered cumbersome, hazardous and expensive.

Certainly, we acknowledge the cost of this approach, although the potential for lifelong remission may offset the initial outlay. We utilised transnasal gastroscopy in some patients, and previous work by our group indicates the safety of either nonsedated transnasal or standard transoral gastroscopy with sedation[194]. Fourth, PPIs were continued for patients who responded to the elimination diet. Currently, clinical guidelines do not specify the use of PPI with dietary therapy. The notion of combination therapy is proposed and subsequent follow-up by our group may help answer this question. Patients commenced on budesonide therapy on the other hand had their PPI ceased, as described in the majority of previous studies. In contrast, PPI use has varied in association with dietary treatment[24, 146, 189, 200, 205]. Thus, our trial design we felt better reflected current reasonable clinical practice in this 'real world' study. A fifth point is that symptoms (e.g. dysphagia), endoscopic features and blood tests were not recorded or analysed. Previous data however have demonstrated a poor correlation between these features and eosinophil count at biopsy[206]. Admittedly, ongoing work in refining these relationships shows promise[187, 207]. Finally, ideally all patient groups would have been followed – up for a longer interval (at least for 9 months as for patients receiving the elimination diet). Given the invasive nature of surveillance requiring gastroscopy, and the relative abundance of data pertaining to PPI and budesonide use in EoE, this was not undertaken[205].

In conclusion, the current real-world experience suggests that re-evaluation of PPI therapy in those patients presenting with oesophageal eosinophilia may be required both in the durability of resolution of that finding in those deemed to have PPI-responsive oesophageal eosinophilia, and in its continued use in patients responding to the six-food elimination diet. The current approach as indicated by clinical practice guidelines warrant further evaluation given the limited initial response to the elimination diet, the cumbersome nature of the diagnostic process and the tendency of patients to cease the diet as determined at 9 months follow-up. In contrast, budesonide will successfully treat more the 90% of patients and few gastroscopies are required,

suggesting that the latter is a more viable first-line therapy. If dietary therapy is considered, biopsy of the lower oesophagus should be performed given the predilection for disease recurrence during food reintroduction in this area.

5.4

Discussion

Our study raises concern about the 'real-world' applicability or efficacy of elimination diets in patients with eosinophilic oesophagitis. Whilst the removal of food antigens can clearly resolve oesophageal eosinophilia in some patients (thus emphasising the importance of food antigens in disease pathogenesis), the exhaustive process of food reintroduction and maintenance of a restrictive diet severely limits the number of patients that sustain benefit using this approach. These findings are of current significant interest, and are discussed in the dialogue between ourselves and other interested parties who corresponded with letters and editorials (see 5.5 – 5.8)

5.5

Editorial: Management of Eosinophilic Esophagitis - Efficacy Versus Effectiveness

Frazier RD. (Alimentary Pharmacology and Therapeutics 2016 – in press)

Randomized-controlled trials of medications or other interventions are efficacy studies, reporting the beneficial effect of a medication, procedure or other intervention under carefully controlled conditions [1, 2]. In contrast, effectiveness is the actual benefit of an intervention under "real-world" circumstances. Unlike efficacy, effectiveness incorporates many factors related to patients' and physicians' behavior and access to the health-care system. They include, access to the intervention, accuracy of the diagnosis and thus candidacy for the intervention, acceptance of the intervention and adherence to therapy [2]. Consequently, clinical effectiveness is markedly lower than efficacy, explaining the inability to replicate many times the results of clinical trials in the community.

Philpott and colleagues [3] reported their findings from a "real world" study in which treatment outcomes of various therapeutic modalities were evaluated in patients with eosinophilic oesophagitis (EOE). They included, proton pump inhibitors (PPIs), six-food elimination diet plus PPI and budesonide. The study focused exclusively on objective clinical endpoints. The authors did not assess any subjective clinical endpoints, such as symptoms or health-related quality of life.

Initially, all patients received double dose PPI for a period of 8 weeks in order to identify those who had either gastro-oesophageal reflux disease (GERD) with eosinophils or PPI responsive esophageal eosinophilia (PPI-REE). Therapy with a PPI in this study followed the treatment guidelines for advanced erosive oesophagitis, with most experts recommending twice a day standard dose PPI for 8 weeks [4].

However, because the effect of the PPI on PPI-REE is unrelated to its anti-secretory activity, it is unclear whether double dose is the proper dose, or 2 months the proper duration of PPI treatment. Historically, using the erosive oesophagitis management - paradigm in other GERD phenotypes, hindered the development of disease specific treatment.

In the study by Philpott et al., patients who were not responsive to PPI therapy, were offered either elimination diet plus PPI treatment or budesonide. As the authors of the article mentioned, the effectiveness of dietary intervention in EOE has not been established and more importantly the long-term outcome of EOE patients on dietary therapy has not been reported.

Interestingly, approximately 69% of the participants selected elimination diet over budesonide treatment, suggesting that patients prefer non-pharmacologic intervention, where possible. More importantly, the study revealed a relatively low response rate among those receiving elimination diet as the initial intervention, with only 52% of the EOE patients demonstrating normalization of mucosal eosinophils count. In contrast, efficacy studies have demonstrated an approximately 70% response rate in a more homogeneous adult patient population with EOE [5]. The authors do not provide information about patients' symptoms, which may not always correlate with improvement, or lack of improvement, in eosinophils count.

More disconcerting was the "real world" long term clinical outcome of the elimination diet in EOE patients. Of the very small number of EOE patients available for follow-up, 39% ceased elimination diet, while only 55% demonstrated esophageal mucosal remission. The authors concluded that, although initial response to elimination diet plus PPI was greater than 50% as compared with more than 90% response rate to budesonide, only one in four patients

on elimination diet demonstrated oesophageal mucosal remission at 9 months.

While the study has several limitations, including lack of a control arm, standardization of treatment and symptom assessment, as well as a very intense endoscopy protocol, the results provide a glimpse into "real world" effectiveness of the elimination diet. As with many other disorders, requiring patients to follow restricted diets over the long term is too cumbersome, and may result in poor compliance and possibly disease relapse. Thus, studies are needed to identify patients who are able to maintain elimination diet in the long run. In those who find it very difficult to follow diet restrictions over a long duration, pharmacologic therapy should be considered.

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5.6

Reply to Editorial; Management of Eosinophilic Oesophagitis. Efficacy versus effectiveness. Philpott H, Gibson PR. Alimentary Pharmacology and Therapeutics 2016 (in press -13666)

Sirs,

We thank Frazier et al for their insightful commentary concerning our paper concerning the treatment outcome of adult patients with EoE[208]. We agree with much of the analysis, although a few clarifications appear justified.

The use of twice daily proton pump inhibitors for 8 weeks prior to the diagnosis of PPI- REE is (as eloquently summarised by Frazier et al) an arbitrary intervention based upon the GERD paradigm. Indeed, so called 'step – down' therapy, where the dose of PPI is reduced (and remission sustained in most patients) suggests that lower doses may be effective in treating the oesophageal eosinophilia[195]. The mechanism whereby proton pump inhibitors (PPI's) exert their therapeutic effect is of ongoing debate, and effects distinct from the ability to decrease gastric pH appear possible, including the ability to downregulate eotaxin -3 expression[209]. It may however be premature to conclude that the ability of PPIs to increase gastric pH plays no part in treatment response. It is not unreasonable to propose that decreasing refluxate of acidic and thus erosive gastric contents will improve barrier integrity and lessen food antigen interaction with the immune system[61]. Evolving research appears aimed at determining the significance of proposed impairments in barrier function in this patient group[61].

Frazier et al point out a number of potential limitations in study design including what was perceived as a lack of treatment standardisation, no symptom report measures and an intensive endoscopy protocol. It is our belief that treatment was standardised both in terms of dose and

duration of medication (esomeprazole 40mg Po BD or budesonide 1mg Po BD), duration and sequence of dietary reintroduction, that endoscopic surveillance was the minimum necessary and not dissimilar to previous studies and that symptom report correlates poorly with disease relapse in any case[210]. Furthermore we utilised minimally invasive transnasal endoscopy without sedation in some patients[194]. In the future, alternatives such as cytosponge may decrease the need for endoscopy[186].

 The difference between effectiveness and efficacy appears to hold particular significance when considering the steps required to institute successful dietary therapy in EoE. Previous studies had conclusively demonstrated that resolution of esophageal eosinophilia can be achieved with dietary *restriction[24]*. What had been overlooked was the need to *reintroduce* foods (requiring multiple gastroscopies) and then *maintain* a diet. A clearer perspective of the difficulties likely to be encountered is thus achievable if this process is considered to entail multiple steps. The results of our study are testament to the real world difficulties of such a regimen.

5.7

Letter; avoiding misconceptions about elimination diet for eosinophilic oesophagitis. Molina – Infante J, Lucendo AJ. Alimentary Pharmacology and Therapeutics 2016 (in press).

Sirs,

Philpott *et al.* report an interesting prospective study evaluating a stepwise clinical algorithm for adult patients with eosinophilic oesophagitis (EoE): proton pump inhibitor (PPI) therapy followed by either topical steroids or elimination diet in patients unresponsive to PPIs[1]. Some noteworthy findings are reported, such as reinforcing the idea of interchangeability of therapeutic assets[2,3] (patients initially responsive to topical steroids may also respond to PPI) and evaluating the long-term efficacy of diet therapy. Response to PPI therapy (23%) is much lower than that reported in a recent meta- analysis (50%)[4]. This is possibly related to the fact that patients were not naïve for treatment, but were included after discontinuation of previous treatment with PPI and/or topical corticosteroids. Similarly, response to a six-food elimination diet (SFED) was lower (52%) than that reported in a meta-analysis with homogenous remission rate of 72% in children and adults[5]. We have several concerns regarding the methodology and interpretation of results for elimination diet:

1. The elimination diet was combined with PPI therapy, whereas PPI therapy and budesonide were each given as monotherapy. This discrepancy may lead to unreplicable results. One can speculate why PPIs were not added to topical budesonide therapy in a similar way it was done with diet. The authors nicely address this controversial issue

along the discussion

2.   In responders to a six-food elimination diet, inflammation relapse was evaluated after 2 weeks of individual food challenge. Partial responders (not defined) had a repeat endoscopy after a further 2 weeks (data not shown). This scheme is likely based on the first study on a six-food elimination diet for adult EoE patients, in which questionably food groups were reintroduced every 2 weeks, with repeat endoscopy after the reintroduction of 2 food groups[6]. Aside from wheat and cow´s milk, a minor role for egg (5%) and not for legumes as food triggers was identified[6]. A 2-week challenge maybe too short for confirm relapse of a predominantly IgG4-mediated disease[7], strongly contrasting with 6- to 12-week period that the authors waited, to confirm EoE remission with PPI, steroids or diet. This is likely the reason why there was less dense eosinophilia during individual food challenge in the study by Philpott *et al*[1]. In the remaining studies evaluating empiric six- or four-food elimination diet so far[8-10], a minimum of 6 weeks was established after individual food challenge. Food triggers identified in these studies were extremely homogeneous (by order of frequency, by far cow´s milk, followed by wheat and eggs, and then legumes/soy[8-10]). However, gastronomy differences among distinct geographical areas, like Spain, United States and Australia, may account for different food triggers of EoE. Interestingly, the authors observed patients with no food triggers identified after the whole reintroduction process. We have also observed this phenomenon[8,9] and we can speculate that it might be associated with low intermittent intake of the culprit food, the need for challenges even longer than 6 weeks to document disease relapse in some patients, or even with the involvement of a seasonal airborne trigger.

3. The authors literally state "At 9 months, only 10/18 (55%) of patients who responded to the elimination diet with PPI remained complaint and sustained remission". This "intention-to-treat" analysis may be somewhat tricky. The real fact is that among patients responsive to diet, 100% who were re-evaluated while compliant with avoidance of culprit foods remained on remission at 9 months.

Having stated these clarifications, we are extremely excited to see that new research groups out of Spain and the United States join the endeavor for improving patient care and outcomes in EoE with empiric elimination diets.

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5.8

Authors response; Avoiding misconceptions about elimination diet for eosinophilic esophagitis. Philpott H, Gibson PR. Alimentary Pharmacology and Therapeutics 2016 (in press -APT 13644).

Dear Sirs,

We thank Molina- Infante et al for their interest and comments illustrating controversies regarding treatment allocation and histological sampling of adult patients with eosinophilic oesophagitis (EoE), and our alternative emphasis on real world clinically acceptable investigation, treatment and outcome measures.

Firstly, regarding combined use of diet with PPI, we agree that this methodology may not be employed by future researchers, but equally attest that previous work was marred by a lack of consistency in regard to pre- diet PPI trial and/or the use of pH studies[24]. The initial published study in this field arguably arose prior to the current understanding of PPI_REE, and many patients may have been re-categorised had PPI trial occurred prior to dietary therapy[24]. Continuing PPI therapy during the dietary therapy in our study not only saved precious time (that would have been required for wash- out) but also the need for repeat gastroscopy prior to commencing dietary therapy. A second and related issue is that we did not compare diet with PPI to budesonide with PPI, rather using budesonide monotherapy. Budesonide with PPI in our view would be foolish given the already significant risk of oropharyngeal candidiasis with monotherapy and because budesonide has demonstrable efficacy as a sole agent[211].

Molina – Infante et al suggest longer periods of food challenge may be required to determine individual culprit foods (e.g. 6 weeks compared to the 2-week period in our study)[189, 212]. This may be so, although Pedersen et al demonstrated recurrent eosinophilia within 3 -7 days in

patients controlled on elemental diet and PPI. The notion that EoE is and IgG mediated disease and thus of delayed onset is controversial[213]. In terms of 4 food or 6 food elimination diets, only have two groups aside from ourselves that have published data, namely that of Molina- Infante et al themselves, and a North American group[214]. The only way to answer this question would be to do repeated gastroscopies at set time intervals following the introduction of each food, e.g. at 3 days, 2 and 6 weeks! In reality the need to deliver acceptable and safe treatment regimens dictated our study algorithm. That is, we deemed an extremely restrictive diet should be used for the minimum possible period of time.

Finally, our observation that only 55% of patients remained compliant with their diet at 9 months, the rest relapsing with eosinophilia is, we believe, a fair assessment [215]. Figure 2 clearly demonstrates that 7/18 patients representing for gastroscopy had ceased treatment and relapsed, 1 patient dropped out and 10/18 (all compliant patients) sustained remission. The question 'can dietary therapy cause remission of EoE' has already been comprehensively answered. Our contribution is, that outside of expert centres the response to dietary therapy and the long term compliance and thus success is modest.

CHAPTER 6

6.1

Overview

Food antigens can cause the inflammatory infiltrate of eosinophilic oesophagitis and food antigen removal can resolve the eosinophilia in many patients. Because eosinophilic oesophagitis is thus considered a form of food 'allergy', and given that allergy tests have an established role in the apparently related conditions of oral food allergy syndrome, classical food allergy, asthma and atopic rhinitis, it is logical that such tests are proposed as useful in this condition. The previous limitations in the study of these tests (particularly in adult patients), and the need for rigorous evaluation are discussed in the manuscript, which contains a comprehensive prospective study and thus is an original scientific paper.

6.2

Allergy tests do not predict food triggers in adult patients with eosinophilic oesophagitis. A comprehensive prospective study using five modalities. Philpott H., Nandurkar S, Thien F, Royce S. G, and Gibson P. R. Alimentary Pharmacology and Therapeutics 2016 (in press – APT – 0437-2016)

Summary

Background

The use of allergy tests to guide dietary treatment for eosinophilic oesophagitis (EoE) is controversial and data are limited. Aeroallergen sensitisation patterns and food triggers have been defined in Northern Hemisphere cohorts only.

Aims:

To determine if allergy tests that are routinely available can predict food triggers in adult patients with EoE. To define the food triggers and aeroallergen sensitisation patterns in a novel Southern Hemisphere (Australian) cohort of patients

Methods:

Consecutive patients with EoE who elected to undergo dietary therapy were prospectively assessed, demographic details and atopic characteristics recorded, and allergy tests, comprising skin-prick and skin-patch tests, serum allergen-specific IgE, basophil activation test and serum food-specific IgG, were performed. Patients underwent a six-food elimination diet with a structured algorithm that included endoscopic and histological examination of the oesophagus a minimum of two weeks after each challenge. Response was defined as <15 eosinophils per HPF. Foods defined as triggers were considered as gold standard and were compared with those identified by allergy testing.

Results:

No allergy test could accurately predict actual food triggers. Concordance amongst skin-prick and serum allergen-specific IgE was high for aeroallergens only. Amongst seasonal aeroallergens, rye-grass sensitisation was predominant. Food triggers were commonly wheat, milk and egg, alone or in combination.

Conclusions:

EoE. Exclusion-rechallenge methodology with oesophageal histological assessment remains the only effective investigation. The same food triggers were identified in this southern hemisphere cohort as previously described.

INTRODUCTION

Eosinophilic oesophagitis (EoE) may be successfully treated by removing from the diet food antigens that are responsible for inciting the immune reaction and characteristic pathological changes demonstrable at gastroscopy and tissue biopsy[172]. Whilst elimination diets are successful, the requirement for multiple gastroscopies - one after each food is reintroduced requiring eight or more in the case of the 6-food elimination diet - makes this management untenable to many [189]. The need for non- invasive, once-off investigations that can accurately predict food triggers in EoE is hence obvious. To date, skin-prick and skin-patch testing combined have shown marginal benefit in guiding dietary treatment in paediatric patients with EoE, whilst skin-prick alone or in combination with specific serum IgE for adult patients was of no benefit in two prospective studies[24, 110, 216]. The allergy tests so far utilised for EoE have been borrowed from experience gained with other disease states. Thus, for conditions characterised by the development of symptoms within minutes (oral food allergy syndrome, food allergy with anaphylaxis, atopic rhinitis or atopic asthma), skin-prick or specific serum IgE are validated, clinically useful and conceptualised to measure immediate hypersensitivity (Gell and Coombe type 1) [217, 218]. EoE, a condition that has slow onset and is slow to resolve, is more akin to contact dermatitis, and thus skin-patch testing was used and validated for the latter as a measure of type 4 (cell-mediated) immunity has also been adopted[110, 219]. However, for unknown reasons, all of these investigations have so far failed to deliver acceptable accuracy such that empirical dietary therapy followed by repeat gastroscopy is advocated [42].

The basophil activation test measures acute IgE-mediated (type 1) immune responses, as well as non-IgE-mediated responses, and has a role in detecting drug allergy and anaphylaxis to food and aeroallergens, particularly when skin prick tests are unavailable or contraindicated[220, 221]. The basophil activation test is of unknown value in EoE. We hypothesised that the basophil

activation test may have utility in EoE, particularly given the central role that non-IgE-mediated basophil activation may play in controlling the inflammatory response. This has been demonstrated by the so-called 'basophil-TLSP axis' (basophil – thymic stromal lymphopoietin axis) in animal models [222, 223]. Briefly, TLSP, a cytokine produced by epithelial and stromal cells, can activate basophils, which express TSLP receptors. In a murine model, EoE will only develop in the presence of TSLP and basophils [223].

Patients with EoE may be atopic, with coexistent allergic conditions and elevated IgE levels to common aeroallergens. Recently, dense oesophageal deposits of IgG and elevated serum IgG to common food allergens have been demonstrated in EoE, raising the hypothesis that IgG and IgE may mediate the disease [216]. Serum IgG levels have been used by alternative medical practitioners to guide elimination diets targeting a range of gastrointestinal conditions, although the validity and rationale of this approach is questioned [224]. We hypothesised that serum IgG levels to common food antigens will reflect immune tolerance, and will have no predictive value in guiding dietary elimination.

Food and aeroallergens may be important in the pathogenesis of eosinophilic esophagitis (EoE). To date, studies performed in the Northern Hemisphere have concluded that seasonal exacerbations of EoE may be related to tree pollinosis, with birch-pollen sensitisation (and potential cross sensitisation with food allergens) being particularly common[225, 226]. Lacking are data relating to regions where grass pollinosis (as opposed to tree pollinosis) is predominant, with the corollary that food allergen sensitisations may in turn be influenced and be different.

In view of the apparently strong relationship between immune responses to specific antigens and the pathogenesis of EoE, and of its association with atopy in at least a northern hemisphere

population, the current study had two aims. First, we determined if skin-patch and skin-prick testing, the basophil activation test, specific serum IgE and serum IgG levels to food antigens, as available in routine clinical practice, predicts proven food allergens in patients with EoE undergoing an elimination diet. Secondly, we sought to characterise the demographic details and atopic characteristics of a novel 'southern hemisphere' cohort of patients

METHODS

Recruitment

This study prospectively examined patients aged 18 years or older presenting to the gastroenterology outpatient clinic of two tertiary hospitals (Box Hill Hospital and The Alfred Hospital, Melbourne Australia) between September 2013 and January 2015 with oesophageal eosinophilia. This finding must have been made previously by upper gastrointestinal endoscopy and biopsy demonstrating an oesophageal eosinophil count of ≥15 eosinophils per HPF in at least one section. Excluded were patients with gastric or duodenal eosinophilia, those taking medications or with medical conditions likely to produce eosinophilia or alter results (e.g., antiepileptics, antihistamines, inhaled corticosteroids or oral corticosteroids for asthma, lymphoproliferative conditions). Subjects gave written, informed consent and the protocol was approved by the Ethics Committees of Eastern Health and of Monash University (E 119/1213 and E120/1213).

Study protocol

This was a prospective observational study designed to examine the real-world outcomes in patients presenting with histopathologically proven oesophageal eosinophilia. The design of the main study is presented elsewhere in detail[208]. Briefly, after withdrawal of all corticosteroid, therapy for 8 weeks for those previously treated with topical corticosteroids, patients were asked to take esomeprazole 40 mg orally twice daily for 8 weeks followed by histopathological assessment of the oesophagus. Those with oesophageal eosinophil density >15/HPF were then diagnosed with EoE. A gastroenterologist (HP) assessed all patients and the following data were recorded: date of birth, country of birth, migration and date of migration from overseas,

coexistent allergic conditions, previous food bolus obstruction events, date diagnosed, previous treatment and current symptoms.

Patients were then offered topical corticosteroids or dietary therapy with the six-food elimination diet. Esomeprazole was continued at the same dose. Written information about the diet was provided to all patients and consultation with a dietitian was offered. Patients who responded to dietary therapy had sequential reintroduction of foods according to an established algorithm as previously reported[227]. Briefly, this involved food challenges, where the participant was instructed to consume at least 1 serve of the additional, 'culprit' food at least twice per day, e.g. 1 slice of bread per twice daily, 1 glass of milk or 1 egg twice daily). This was followed by gastroscopy with oesophageal biopsies after a minimum of two weeks. Absence of oesophageal eosinophilia led to a challenge with the next food type, whereas recurrence of eosinophilia led to exclusion of that food type. Subsequent food challenge was given immediately when a putative food trigger failed to elucidate eosinophilia, whilst a positive response led to food removal and a 4-week 'washout' period. Those who failed the six food elimination diet were offered topical budesonide.

Allergy Tests

Allergy tests were performed at specific intervals, dependent on the response to dietary modification (Figure 1)

- *Skin-prick tests:* These were performed using commercially prepared antigens (Alloystal, Stallergenes France, allergen concentration approximately 5000AU/ml) and standard methodology, where a positive result was recorded as a wheal 3 mm greater than the negative control at 15 minutes post skin prick. Antigens tested were the foods; egg, wheat,

milk, soy, peanut, hazelnut, fish and shellfish and the aeroallergens ryegrass, dust mite and birch pollen. Histamine (10 mg/ml) and saline were used as positive and negative controls respectfully.

- *Skin-patch tests:* These utilised the same commercially prepared antigens with 3 drops of allergen placed on a filter paper disc, secured with petrolatum in 12mm Finn chambers, and covered with adhesive tape, the reading performed at 72 ± 6 hours. A result was considered positive if erythema and clear infiltration with papules (+) or vesicles (++) occurred, as previously described[228].

- *Serum food- and aeroallergen-specific IgE levels:* These were assessed to the same antigens as above on peripheral blood using the Immunocap test according to the manufacturer's instructions (Pharmacia Diagnostics, Uppsala Sweden) in a single hospital laboratory. Scores recorded between < 0.35 (low or undetectable) to greater than 100 Ku/L. Moderate or strong positives were considered clinically significant and recorded.

- *Basophil activation test:* This was performed on whole blood using the same antigens via the Flow- CAST assay (Bühlmann, Schoenberg Switzerland) on the FACSverse (BD Biosciences, Melbourne Australia) flow cytometer according to the manufacturer's instructions. Specifically, venous blood was collected in K-EDTA (potassium ethylenediaminetetraacetic acid) venepuncture tubes and samples stored at six degrees Celsius before being processed within 2 hours of collection in all cases. 3.5ml polypropylene tubes were used for subsequent analysis. Stimulation controls were N – formylmethionyl – leucyl – phenylalanine (fMLP) and anti-Fcε RImAb. Stimulation buffer contained calcium, IL-3 and heparin. Staining reagent was a mix of

anti-CD-63 and anti-CCR3-PE mAb. Additional cell surface receptor antibodies to CD 203c and TSLP were also utilised. FLOW-CAST antigens were used, namely egg white

and egg yolk, wheat, milk, soy, peanut, hazelnut, fish and shellfish and the aeroallergens ryegrass, dust mite and birch pollen. Samples were incubated in a water bath, and following use of lysing reagent were centrifuged and then resuspended with wash buffer prior to flow cytometry. A positive result to a given antigen was defined as >15% CD 63 positive basophils (CCR3 positive) in a subject where one or both positive controls (fMLP or anti-FcRAb) were positive (>15%) in accordance with the manufacturer's protocol.

- *Serum food-specific IgG antibodies levels:* Healthscope Functional Pathology, Melbourne, Australia using the Genova Diagnostics Food IgG ELISA test kit (Asheville North Carolina), performed these. Specifically, the samples were stored at between 2-8 degrees Celsius for <48 hours. Trained laboratory staff performed the procedure using a manufactured (automated) standardised microplate coated with food antigens. A goat anti-human IgG conjugated to horseradish peroxidase was added prior to incubation, and a solution of 3,3,5,5-tetramethylbenzidine (TMB) is added to trace specific antibody binding before using the STOP solution (sulphuric acid) and optical densities are measured using a microplate reader at 450nm. A positive control containing human serum is used. Food-specific IgG was reported as positive if greater than 12.5 units/ml (manufacturers own arbitrary reference range and units).

Endoscopy, biopsy and histological assessment

As previously outlined, gastroenterologists from the respective departments of both hospitals performed all gastroscopies. Four biopsies were taken each from the lower esophagus (5 cm proximal to the gastroesophageal junction) and from the middle and upper oesophagus at 5 cm intervals. Transoral and transnasal gastroscopies were used, the latter with local anaesthetic spray and the former with propofol sedation. Histopathological analysis of gastric and

duodenal biopsies was performed by consultant pathologists blinded to the treatment method in sections of tissue fixed in 4% neutral-buffered formalin and stained with H&E. The peak eosinophil count was recorded in all three areas of the oesophagus in the most densely infiltrated areas. The mean eosinophil count of ten respective areas analysed at HPF were calculated.

Outcome Measures and analysis

A response to specific food elimination was defined as <15 eosinophils per HPF in all three oesophageal locations. A positive reaction to foods during a dietary challenge was defined as recurrence of >15 eosinophils per HPF in one or more location following the reintroduction of a food. The performance characteristics of the results of the allergy tests were compared to the foods identified by the elimination-rechallenge methodology, considered as gold standard.

RESULTS

Characteristics of patients with EoE

82 patients with EoE were identified following the course of twice-daily PPI therapy. The patient demographics are shown in Table 1. Most patients were male (84%), white Caucasian (98%) and presented with food-bolus obstruction events and/or dysphagia. Coexistent atopic illness was present in many, with seasonal rhinitis most common (42%).

The results of skin-prick and IgE testing are shown in Table 2. Aeroallergen sensitisation was frequent, with rye grass the predominant allergen (approximately 70% of patients), followed by dust mite (Table 2). Birch-pollen sensitisation was rare (2-7% skin prick or serum allergen specific IgE). Sensitisation to putative food allergens was more often demonstrated with serum food-specific IgE than by skin prick tests. Wheat (45%), milk (32%) and egg (19%) were most frequent as defined by serum food-specific IgE.

Timing of gastroscopies and allergy tests

The gastroscopies to determine response to the six food elimination diet were performed at a time interval closely matching the intended protocol of 42 days [mean 41.36 days (s.d. 2.82)], and the gastroscopies to determine food triggers were similarly well timed [mean 15.61 days (s.d. 0.71, protocol was 14 days]. Figure 1 shows the timing of allergy tests, with skin prick and serum food and aeroallergen specific IgE being performed at the commencement of the study immediately prior to the introduction of PPI. Skin patch testing was performed immediately before commencing the 6FED, whilst the basophil activation test and the serum IgG to food antigens were performed following a response to the 6FED [mean 43.2 days (s.d. 3.6)]
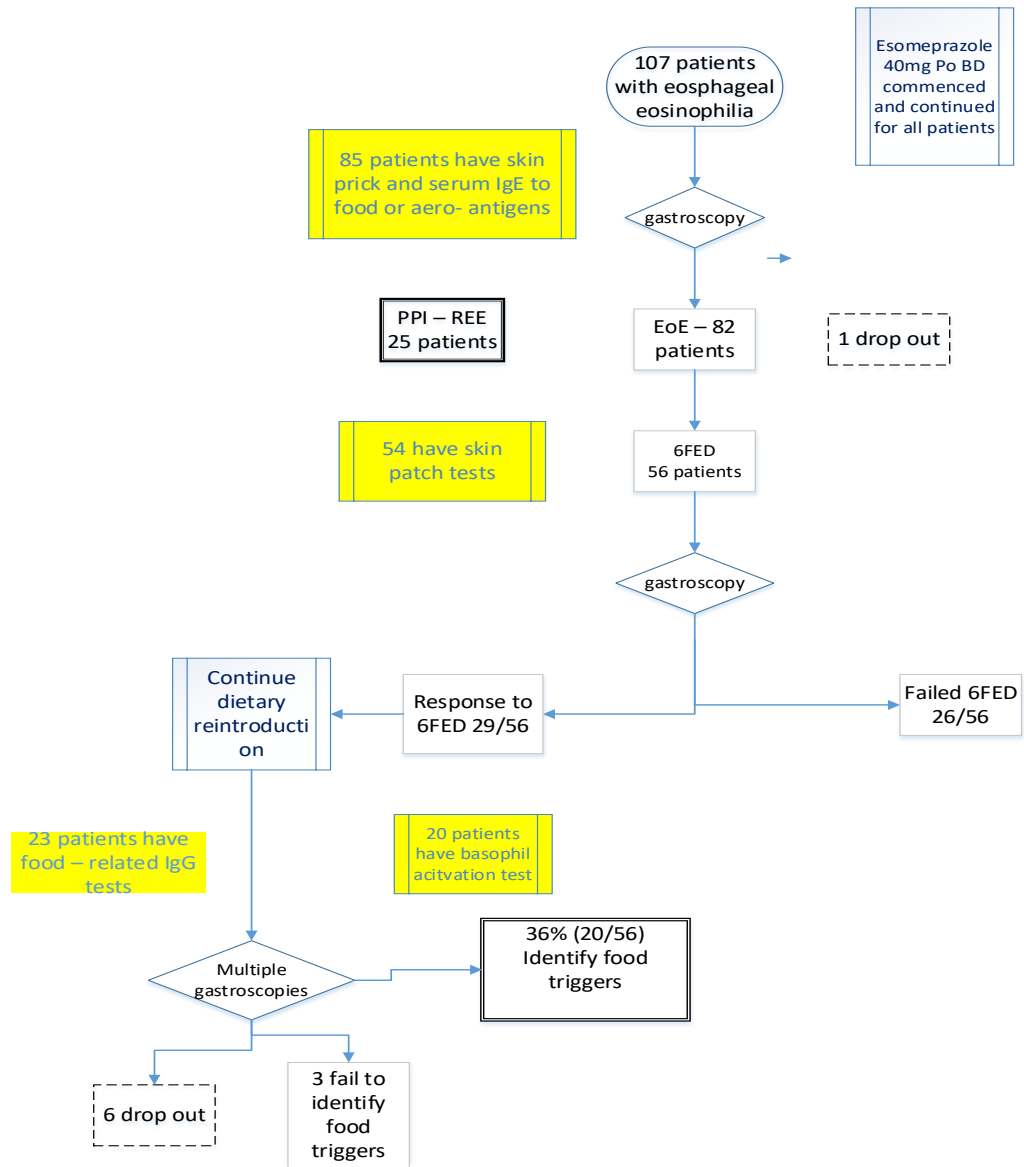
Figure 1 – Allergy tests performed on individuals who chose the six food elimination diet (6FED)

Performance of allergy tests in patients completing 6-food elimination diet

Of 56 patients who commenced the six-food elimination diet, 29 initially responded to the diet and 23 of these completed the diet. The characteristics of these patients are shown in Table 1. Food triggers were identified in 20 patients as outlined in Table 3. A recurrence of EoE following food reintroduction was caused by a single food in 12 cases, and by two or more foods in the case of 8 patients. The commonest food triggers were wheat (implicated alone or in combination in 10 cases), milk (alone or in combination in 9 cases) and egg.

None of the five allergy testing modalities could accurately predict food triggers. Skin patch testing was always negative with respect to food, and serum IgG levels to food antigens was positive to two or more foods in all cases, showing no correlation with actual triggers. Serum IgG levels to food antigens would accurately predict an individual food trigger in 13/20 patients, miss a food trigger in 11/20 and lead to an over-restrictive diet in 19/20 patients. Skin patch detected no food triggers. Specific serum IgE and, to a lesser extent, skin-prick tests were positive to a number of food allergens but were not accurate in correctly predicting dietary triggers of EoE, except in one case where childhood milk allergy (manifesting as classical anaphylaxis) was recalled by the patient and the individual had positive specific serum IgE, skin-prick test and the basophil activation test to milk. Interestingly, a patient with known classical food allergy had positive skin-patch test, specific serum IgE and the basophil activation test to the culprit antigen (soy). The basophil activation test was otherwise negative to all food antigens. As both TSLP and CD 203c were universally positive in the first 10 patients, these assays were subsequently abandoned. Given the obvious lack of utility, calculations of sensitivity, specificity, positive and negative predictive values were not

appropriate. The propensity for false positive results is demonstrated by the three patients who had no food triggers identified.

Aeroallergen sensitisation was demonstrated in 14/20 patients (70%) using specific serum IgE, in 10/20 (50%) by skin-prick testing, and in 7/20 (35%) by the basophil activation test. Rye-grass sensitisation was predominant for all test modalities, and concordance was observed for this allergen between specific serum IgE and the skin-prick test in 10/14 (71.5%). Serum IgG levels to food antigens did not measure aeroallergen sensitisation.

DISCUSSION

Elimination diets have been successfully used to treat EoE in Northern Hemisphere patient cohorts[214]. To render such an approach practical, identification of trigger foods by relatively non-invasive means is desirable, but current techniques of empirical food reintroduction and frequent gastroscopy are cumbersome. Simple allergy testing is a much more attractive option but its role in EoE to guide dietary therapy has been debated [110, 189, 229]. Thus, we systematically investigated such testing using a panel of five available techniques prospectively in a consecutive cohort of patients presenting with EoE. Crucially, none of the tests predicted the actual food sensitivity associated with EoE as defined by gold-standard elimination-re-challenge techniques.

The lack of utility of allergy tests in directing dietary therapy for EoE has been demonstrated previously in reference to skin-patch testing in one adult cohort [24] and to skin-patch testing and serum food antigen specific IgE combined in another group of adult patients[189]. Our study differed in that additional modalities of allergy test were applied prospectively to a patient cohort that was systematically followed up and subject to ongoing treatment with high-dose PPI. Notably, the skin-patch test was negative to food allergens in all cases and the basophil activation test was similarly negative, except for two cases where classical food allergy to milk and soy were correctly predicted. Skin patch testing and serum food antigen specific IgE were positive in 5 patients, (25%, a similar percentage to previous studies) but did not predict food triggers[24].

Skin-patch testing has previously demonstrated poor sensitivity in determining food triggers for EoE in a paediatric cohort, as well as an adult cohort that were treated with an elemental

diet, although the absolute inability to react to any food in our study of adult patients was novel [110, 198]. The method of SPT differed from some studies in that commercially prepared as opposed to fresh foods were employed, skin taping and stripping was not used prior to patch placement, and petrolatum was added to assist disc adhesion[110, 198]. Nonetheless, little consensus exists as to whether skin-patch testing has a use in food-related allergic disease *per se*, and the literature in adults is scarce[219]. Our experience would suggest that, as well as being a cumbersome test disliked by patients and clinicians, skin patch testing should not be used in EoE.

In an attempt to explore non-IgE-mediated mechanisms of food allergy, the basophil activation test was applied using standard markers of basophil activation in additional to cell surface markers CD 203c and TSLP [220, 221]. Activation of CD 203c is thought to predict non-IgE-mediated immune activation of unspecified type and TSLP is the relevant receptor. The presence of exogenous food- or aero-antigens did not influence expression of these cell surface receptors. Whilst TSLP may be important in EoE, the use of TSLP receptors on basophils was not differentially expressed (i.e., it was not dependent on exogenously applied food antigens) and thus, the assay as described is of no use in predicting food triggers for EoE. This observation is in keeping with recent work that demonstrated a lack of utility of serum biomarkers (including TSLP) in determining disease activity in EoE [230].

The serum IgG levels to food antigens was invariably positive to two or more foods, but did not correctly identify food triggers in EoE. The use of this assay stemmed from a recent study that demonstrated a preponderance of IgG rather IgE in oesophageal tissue from patients with EoE and from elevated serum IgG levels to food antigens to a number of foods, again in patients

with EoE, but to a lesser extent in patients with alternative diagnoses[216]. We concur that patients with EoE have elevated serum IgG levels to food antigens to two or more foods, and add that serum IgG levels to food antigens was not a useful test in predicting food triggers for EoE in the current prospective study. Serum IgG levels to food antigens use is most common in the practice of alternative and complementary medicine, and we utilised the same laboratory and methodologies that are commercially available. The validity of the so-called 'diagnostic' levels of IgG to food antigens using apparently arbitrary units is questionable given the limited studies of variable quality relating to these assays [224]. It is possible that IgG levels represent food exposure rather than being reflective of food triggers. In the context of classical IgE-mediated food allergy, it has been suggested that food-specific IgG become elevated with disease resolution and may thus facilitate immune tolerance. It is hypothesised that this mechanism of immune tolerance exists in EoE and further studies seem indicated[193].

The reasons why all of the allergy tests failed to correctly identify food triggers deserves consideration. First, it is possible that non-IgE-mediated mechanisms of immune activation are responsible for EoE, and thus skin-patch testing and serum food antigen specific IgE would be unhelpful. Nonetheless, the basophil activation test, serum IgG levels to food antigens and skin-patch test, which are considered measures of non-IgE-mediated immune activation, also lacked utility. It is also possible that the gastrointestinal immune compartment responds differently from the systemic immune compartment. Methods capable of directly exposing the gastrointestinal mucosa to putative antigens may in future prove useful. Certainly, testing for food allergy *per se*, even when considering classical food allergy is less established and more problematic compared to tests for aeroallergens[231]. Tests that directly interrogate the gastrointestinal immune compartment already have a template in studies of food antigens for

patients with IBS and/or food allergies [232, 233]. Perhaps such techniques applied to the oesophagus might also be applicable to identifying food antigens in patients with EoE.

The characteristics of our patient group in reference to age, gender, race and aeroallergen as opposed to food-allergen sensitisation are similar to those of previous studies. The predominance of rye-grass sensitisation is novel and deserves comment. Rye-grass pollinosis is very high in our region and the results of the allergy tests not surprisingly reflect this. The fact that rye-grass pollen (rather than birch pollen) is predominant, yet the food allergen triggers of EoE are the same as Northern Hemisphere cohorts, arguably counters a previously held hypothesis that birch-pollen cross-sensitisation is a potential mechanism in driving food antigen exacerbation of EoE [24, 189, 225, 234, 235]. It is also suggests that the inverse situation is also true - that rye grass cross-sensitisation with wheat is not a valid hypothesis as previously debated[236]. This may be better resolved by performing the so-called 'component-resolved diagnostics', where putative shared antigen epitopes of aeroallergen and vegetable/fruit allergens such as profilins are analysed, but this was beyond the scope of our research[237].

Several limitations are evident with our study design. First, the reluctance of patients to undertake the program in the first instance, along with the high rate of dropout limited the numbers and ultimately the power of our analysis. However, this is readily understood, as the burden of eight or more gastroscopies is considerable, and emphasises the need for less invasive measures for identifying food triggers in EoE. Similarly, the variable use of a dietitian (16 out of 29 responding to dietary therapy elected to utilise this service), may have decreased the efficacy of dietary therapy and thus indirectly the results of allergy tests (although the study was not designed or adequately powered to examine this, as discussed previously). Indeed, the

response to diet was inferior to earlier studies (52% in our cohort compared to >65% elsewhere) and may reflect the 'real – world' nature of the study where the utilisation of structured resources including a dietician was limited [24, 189]. Secondly, the timing of the basophil activation test and serum IgG levels to food antigens may have influenced the results. Both were performed following the 6-week removal of food antigens from the diet. Ideally, they should have been performed at the commencement of the study with the other assays. Countering this assertion is that antibodies of the IgE and IgG sub-classes are both traditionally thought to be formed as a result of immunological memory, and that this time interval is relatively short in any case. Thirdly, commercially-prepared as opposed to fresh food antigens were used for skin prick and patch testing, the latter being preferred and affording improved accuracy according to some authorities[217, 238]. Fourthly, the serum IgG levels to food antigens was performed by an alternative or 'functional' laboratory, albeit overseen by a mainstream organisation and IgG subclasses were not specified. Our choices of assays were governed by a need for reproducibility, external validity and practicality. Ideally, IgG4 to putative food antigens should have been used in line with the finding that this subclass is deposited in oesophageal tissue[216]. Thus the results of the assay we used which measured IgG antibodies to food antigens per se (as opposed to the IgG4 subclass) need to be viewed with caution. Finally, the timing of oesophageal biopsies post food ingestion (2 weeks), and the acquisition of tissue (using either 2.0 mm transnasal forceps or standard 2.3mm transoral forceps) deviates from previous practice where variable (2-4 weeks) or more prolonged intervals (6 weeks) were allowed and standard forceps used[24, 189]. Nonetheless, we have previously demonstrated that tissue acquisition was adequate with smaller forceps, and another group found disease recurrence at between 3-7 days post food reintroduction [194, 198]. We took biopsies in the upper, middle and lower oesophagus in all patients, thus a minimum of 12 tissue fragments was obtained which is far in excess of the suggested approach (5 biopsies with inclusion of the

upper and lower oesophagus only) for optimum diagnosis of EoE [1, 199]. Of the 56 patients who underwent the six food elimination diet with PPI, 12 chose to use transnasal gastroscopy and 10 of these patients had a response to dietary therapy and made the decision to continue to use transnasal gastroscopy. All of these had a food trigger identified. It is thus unlikely but not impossible that putative food triggers would have been missed as a result of this approach.

In conclusion, none of the commercially-available allergy tests that measure systemic immune responses can accurately predict food triggers for EoE and should not be applied for this indication. Such findings emphasise the need for less invasive methods of identifying food triggers if dietary manipulation is to establish itself as the first-line therapy for EoE. The food antigens found responsible for causing EoE are similar to previous research, despite the predominant aeroallergen sensitisation to rye grass (and absent tree - allergen sensitisation) consistent with the Australian location of our cohort.

6.3

Discussion

The conclusion that currently available measures of food 'allergy' do not predict the food triggers responsible for causing the inflammatory response measurable at gastroscopy in patients with eosinophilic oesophagitis can be made confidently based on the results of our study. The patients were well characterised, treated prospectively and surveyed at consistent time intervals and the range of allergy tests used was broad. Therefore, this work remains of significant current interest and provides useful clinical data.

Two related studies were not discussed in the manuscript. Both utilised a *selective* diet (based on the results of allergy tests) rather than comparing the results of allergy tests to empirical elimination and reintroduction (as considered by ourselves and the relevant cited works). Firstly, Molina-Infante et al implemented a selective elimination diet (based on skin prick, skin patch and serum food specific IgE) in 15 adult patients with EoE, with only 26% achieving histological remission [239]. Secondly, van-Rhijn et al studied component resolved diagnostics (CRD), a technique whereby multiple epitopes to food antigens are considered (with the recognition to that cross-sensitisation between foods and to aero-allergens is possible) [240]. Fifteen adult patients were commenced on a selective diet based on CRD, and the futility of this approach was demonstrated, given that eosinophilia persisted (as demonstrated by esophageal biopsy) in 14 out of the 15 patients studied[240]. It is apparent that these studies reiterate the message that allergy tests do not reliably predict food triggers in EoE

CHAPTER 7

7.1

INTRODUCTION

Two new treatment strategies for adult patients with oesophageal eosinophilia have emerged over the last 5 years, which have at once broadened the therapeutic arsenal, but have also raised apparently disparate models of disease pathogenesis. First, the observation that some patients may respond to an elimination diet strongly supports food antigens as central to disease pathogenesis and thus posits that eosinophilic oesophagitis (EoE) is a form of food 'allergy'[24, 189, 212]. Secondly, many patients with oesophageal eosinophilia may have a complete response to proton-pump inhibitors (PPI), raising the possibility that acid reflux causes esophageal eosinophilia[196]. Patients responding to PPI are termed PPI-responsive oesophageal eosinophilia (PPI-REE). Debate as to how PPI induce remission, and if patients with PPI-REE are distinct from those with EoE (that by definition fail to respond to PPI) is ongoing[192, 241]. The injury to oesophageal mucosa caused by gastric acid is well documented, can result in eosinophilia and be at least partially restored in using PPI in gastro-oesophageal reflux disease (GERD)[242]. Effects of PPI unrelated to acid suppression, involving downregulation of chemokine eotaxin-3, and the possibility that patients that respond to PPI may also respond to diet have raised the possibility that PPI-REE and EoE may be one condition as opposed to two distinct entities. Such a contention is supported by limited data[192]. Further controversy exists regarding the relative importance of upper oesophageal inflammation as a defining characteristic of EoE as opposed to GERD highlighting the need to address anatomical regions specifically in studies[203].

Determining the mechanism whereby food antigens cause EoE and dissecting this process from alterations in barrier integrity are thus of central importance in understanding the condition. Decrements in barrier function have been demonstrated in patients with active EoE, with

decreased desmoglein-1 expression a feature [61]. Dietary studies to date have demonstrated some benefit with monotherapy, but potentially have overlooked a major confounding factor of fluctuations in gastric refluxate of acid contents[24, 189, 212]. Admittedly, guidelines now suggest that PPI should be used first line and a gastroscopy with biopsy repeated to determine if PPI-REE exists[1]. Nevertheless, the pivotal adult study of dietary therapy was performed prior to the conception of PPI-REE and thus patients were heterogeneous in reference to PPI use[24]. Subsequent research has examined patients on dietary therapy alone after a trial of PPI therapy[189, 212]. Furthermore, even if PPI-REE has been excluded, fluctuations in the amount and type of gastric refluxate with changes in diet, body weight and posture would suggest that PPI maintenance should be considered if singular examination of the nature and timing of food antigen exposure in causing EoE is to occur[243, 244]. A final consideration in the role of food allergen elimination for treating oesophageal eosinophilia is the 'histological fingerprint' or characteristics of this therapy with reference to treatment modalities such as corticosteroids (budesonide) or PPI (in the case of PPI-REE).

The mechanism whereby food antigens may cause EoE, or, moreover, the characteristics of the inflammatory process are further subjects of debate. So far, the studies of elimination diet have utilised arbitrary fixed (e.g., 6 weeks) or sometimes heterogeneous (e.g., 2-4 weeks) time intervals between food-antigen exposure and oesophageal biopsy to define relapse[24, 189, 212]. Timing of biopsies is important in defining the disease process given other atopic conditions also manifesting tissue eosinophilia (particularly food allergy) are characterised by an acute inflammatory response and mast cell degranulation (within minutes)[245]. Patients with EoE by contrast appear to present in a subacute fashion with dysphagia, although acute massive exposures of aero-allergens (as opposed to food allergens) have been described[47]. Several alternative theories of pathogenesis have been proposed to explain this difference. IgE

antibodies, which are considered central players in food allergy, have been observed in oesophageal tissue, although the lack of therapeutic response to monoclonal antibodies to IgE and the recent finding of dense IgG infiltrates in the oesophagus of patients, but not in controls, arguably suggest a role for IgG rather than IgE in disease pathogenesis[213, 246]. Indeed, IgG-mediated disease may be conceived as more likely to present sub-acutely. An alternative and unifying hypothesis is that EoE is a T-helper type 2 cytokine (Th-2) mediated disease typified by an eosinophil rich infiltrate accompanied by both IgE and IgG deposition[193].

In considering the potential role of food antigens in EoE, it is thus apparent that dedicated study of patients maintained on a PPI (to eliminate the confounding factor of variable acid reflux), surveyed with biopsy at consistent time intervals after exposure to a food antigen, with biopsies taken throughout the oesophagus, and with a range of biomarkers to define newly conceived disease models is required.

7.2

AIMS

In light of the above discussion, the current study had the follow two major aims:

1. To characterise the nature, distribution and variation with time of the inflammatory infiltrate (including IgG and IgE antibody deposition) and changes in barrier integrity of patients with EoE treated successfully with dietary therapy and PPI combined, and then re-exposed to food antigen; and

2. To compare the integrity of the oesophageal barrier and the oesophageal inflammatory infiltrate between patients with oesophageal eosinophilia treated with a range of modalities, and normal controls.

7.3

METHODS

Patients

Four groups of patients were studied, namely patients diagnosed with EoE and who responded to treatment with elimination diet and PPI (primary study group), patients with EoE who responded to budesonide, patients with oesophageal eosinophilia who responded to PPI (by definition PPI-REE), and finally normal control patients with dysphagia and subsequent biopsy demonstrating a normal oesophagus. All subjects were $\geq$ 18 years old and were recruited from two hospitals (Box Hill Hospital and The Alfred Hospital, Melbourne Australia).

Patients treated with diet and PPI, budesonide monotherapy or PPI monotherapy were involved in a prospective clinical study described in detail elsewhere[227]. Control patients presented to outpatients with dysphagia, had oesophageal biopsies taken and were histologically normal. These individuals were identified by retrospective case note review. They must not have been using PPI at the time of endoscopy, and biopsies of the upper, middle and lower oesophagus must have been taken.

Excluded were patients with gastric or duodenal eosinophilia, those taking medications or with medical conditions likely to produce eosinophilia or alter results (e.g., antiepileptic medications, inhaled corticosteroids or oral corticosteroids for asthma, lymphoproliferative conditions). Written, informed consent was obtained and the protocol was approved by the Ethics Committees of Eastern Health and of Monash University (E 119/1213 and E120/1213).

Study design and endpoints

The study was designed to determine the correlation of the expression of a range of biomarkers measured in oesophageal tissue in untreated EoE compared with that when there was a response to food elimination and then to food reintroduction when a dietary trigger was found. A secondary endpoint was to compare the histological appearance of patients with oesophageal eosinophilia treated successfully with alternative treatment modalities namely budesonide or, in the case of PPI-REE, proton pump inhibitors.

In patients with EoE undergoing dietary treatment, tissue acquired from oesophageal biopsies on three separate occasions was chosen for analysis: (a) at the time of *diagnosis* (following 8 weeks of twice-daily PPI therapy); (b) following 6 weeks of dietary therapy with ongoing PPI use (*remission*); and (c) following the *first* food-induced *recurrence* of oesophageal eosinophilia (Figure 1). Similarly, patients treated with budesonide monotherapy had tissue taken after 8 weeks PPI (diagnosis) and following 6 weeks of budesonide therapy. Patients with PPI-REE had oesophageal biopsy tissue acquired prior to and following 8 weeks of BD PPI. The biopsy results of these patients were compared within individuals and to those of normal control patients.

Immunohistology

Biopsies of the oesophagus - 4 each in the lower, middle and upper oesophagus, hence 12 in total were taken at 5 cm intervals proximally from a starting site 5 cm above the gastroesophageal junction during gastroscopy performed via the transoral or transnasal route, the latter with local anaesthetic spray and the former with propofol sedation. The biopsies were immediately placed in 4% neutral-buffered formalin. Sections were processed in three ways.

- *H&E staining:* This was performed to assess the peak eosinophil count in all 3 areas of the oesophagus. The mean eosinophil counts of ten respective areas analysed at HPF were calculated.

- *Immunohistochemical (IHC) staining:* This was performed sections mounted on glass slides that were deparaffinised with xylene, endogenous peroxidase activity blocked and steam treated for antigen retrieval. The concentration of the primary antibody was optimised with titration. The primary antibody was omitted from 2 slides during the titration process to serve as a negative control. Incubation with a secondary antibody, staining with diaminobenzidine chromagen (DAB: Dako) and counterstain with haematoxylin was performed. The primary antibodies included anti-human mast cell tryptase (Clone AA1; 1:500 dilution, Dako), desmoglein-1 (rabbit, 1/50 dilution, Novusbio), caveolin 1 (rabbit, 1/1000 dilution, Santa Cruz). IHC glass slides were scanned and converted to digital slides, and viewed with Aperio Imagescope (Aperio Technologies, Vista CA). The maximum density of tissue that stained intensely positive for each antibody of interest was quantified (cells and or tissue/mm^2) using Aperio positive Pixel Count Algorithmn (version 9.1, Aperio Technologies) and was measured in 5 microscope fields at 40 times magnification in the lower, middle and upper oesophagus respectively, choosing areas of maximal staining, in accordance with methods described elsewhere[190]. A semi-quantitative grading was also applied and numbers were assigned as; 1= not present, 2 = minor positive, 3= moderate and 4 = strong positive (again using methods previously described)[247]. HP performed the analysis and results were cross - checked by an experienced scientist of histopathology (Dr Simon Royce) with guidance from a clinical histopathologist (Professor Prithi Bhathal).

- *Immunofluorescence:* The methodology was the same as immunohistochemistry with the exception that the secondary antibody was Alexa Fluro 488-labelled goat-anti-rabbit

antibody (Thermofisher Scientific NYSE USA). Flouroshield with DAPI (4, 6 diamino-2-phenylindole) counterstain (Sigma) was used as a mounting medium. The stained glass slides were photographed using a digital camera (Diagnostic Instruments, Stirling Heights USA) using a R600 fluorescence microscope (Nikon Instruments, Melville USA). TIFF files were then viewed with Aperio Imagescope. A semi-quantitative grading was applied as described below.

Statistical Methods

Continuous variables were expressed as mean and standard deviation. Categorical variables were expressed as frequencies. The t - test was used to compare continuous variables, the Mann-Whitney test for ordinal variables and Fisher's exact test was used for categorical variables. Statistical analyses were performed using Microsoft excel and Graph-pad. A p value of $<.05$ was considered significant.

7.4

RESULTS

Patient characteristics and pre- treatment histopathology

Biopsies from 20 patients with EoE and PPI, 18 patients with PPI-REE, 10 patients managed with budesonide and 10 normal control patients were analysed. Characteristics of patients in each group are shown in Table 1. Patient groups with oesophageal eosinophilia were well matched, but controls were a mean of 10 years older and significantly more presented with dysphagia. Other demographic and clinical features were not significantly different. Regional distribution of biomarkers prior to treatment, and markers in control patients is shown (Table 2).

Technical issues

Staining intensity for each biomarker was analysed using quantitative and semi-quantitative methods as described. When immunostaining was intense, very high values were generated by Positive Pixel count that show variability across samples that was not discernible using semi-quantitative (visual) analysis. Because the semi-quantitative analysis generated data that was considered more practical and also comparable across different biomarkers, this method was preferred and is displayed.

Comparison to control

The expression of biomarkers in untreated patients with EoE and PPI-REE was compared to that of controls as shown in Table 2. Patients with EoE had a higher mean eosinophil count in all oesophageal regions and the intensity of staining of mast cell tryptase was also increased except in the mid-oesophagus. Expression of IgG and IgE was increased in all oesophageal

regions (p=<.05) (Table 2). The expression of caveolin was lower throughout the oesophagus (p=<.05), but desmoglein was only reduced in the lower oesophagus (Figure 1 and Figure 4).

Treatment of esophageal eosinophilia

Diet with PPI

By definition, the mean eosinophil count fell markedly in the patients treated successfully, the eosinophil count decreased such that the mean eosinophil count in all oesophageal regions across treatment groups was <5, and, as shown in Figure 1, eosinophils were undetectable in most biopsies, as previously described [215]. There was heterogeneity of response to the biomarkers examined in association with this reduction of the density of eosinophils as is shown in Figures 1 a-e. Inflammatory biomarkers in general fell with treatment. Mast cell tryptase intensity decreased with treatment, the mean scores at diagnosis being 3.1 in the upper oesophagus, 3.3 in the middle oesophagus, and 3.8 in the lower oesophagus, compared to post-treatment scores of 2.0, 2.2 and 2.2 respectively (p <.05 in all areas). IgG antibodies fell in response to treatment in the lower oesophagus only (p<.05), the scores being: 2.7, 3.1, 3.7, compared to post-treatment 2.2, 2 and 2.6 (Figure 6). IgE antibodies did not change significantly with treatment: 2.4, 2.8 and 2.5 compared to post-treatment 2.2, 2.1 and 2.1 (p >.05 all areas). The expression of both measures of barrier integrity, caveolin and desmoglein, increased significantly with successful elimination dietary therapy (Fig 3c). Caveolin intensity increased in all areas: 1.7, 2.2 ,1.1, to post treatment 3.4, 3.9, 2.9 (p <.05 all areas). Desmoglein increased significantly with treatment in the lower and middle oesophagus only: 2.8, 2.2, 1.4 to post treatment 3.6, 3.4, 3.1 (p <.05 lower and middle oesophagus only) (Figure 1).

Budesonide monotherapy

Declines in eosinophil count were symmetrical across oesophageal regions. As shown in Figure 2a, in the lower oesophagus, the eosinophil count pre-treatment of 37 (12) fell to 1.4 (1.4) post treatment (p<.001), and intensity of mast cell tryptase fell from 3.5 to 2 (p<.01). Intensity of caveolin increased from 1.5 to 3 (p< .04) and desmoglein from 1.2 to 2.8 (p<.01). The intensity of IgG did not significantly change (2.5 to 1.9, p >.57) and that of IgE was similarly unaltered (2.1 to 2, p > 0.8).

PPI alone in the group with PPI-REE

Decline in eosinophil count were symmetrical across oesophageal regions. As shown in Figure 2b, in the lower the oesophagus the mean (SD) eosinophil count pre –treatment was 39 (9) and fell to post treatment 1.8 (1.4) (p <.001) and the mean intensity of mast cell tryptase fell from 3.7 to post treatment 2.2(p <.001). Intensity of caveolin increased from 1.7 to post-treatment 3.3 (p <.001) as did desmoglein from 1.1 to post-treatment 2.9 (p <.002). No significant decline in IgG, pre-treatment 2.8 to post treatment 2.2 (p >.57), or IgE pre-treatment 2.3 to post-treatment 1.9 (p >.5) were recorded.

Effect of food antigen reintroduction

As per definition of a positive food antigen challenge, the eosinophil count increased across the three regions of the oesophagus, as shown in Figure 1. Densities of eosinophils returned to levels similar to those observed prior to the elimination diet. The inflammatory biomarker, mast cell tryptase increased in all areas (post food introduction values were: upper oesophagus 3.2, middle oesophagus 3.8, lower oesophagus 3.7, p<.05 all areas). IgG antibodies did not significantly increase (2.9, 2.9, 3.3). IgE antibodies (2.7, 2.8, 2.6) did not significantly increase. Desmoglein decreased significantly in the lower oesophagus [mean score upper oesophagus

3.1, middle oesophagus 2.2, lower oesophagus 1.4 (p=<.05 lower oesophagus only), and caveolin decreased, mean 1.6, 1.4, 1.4 in all regions (p= <.05) (Figure 1).

7.5

DISCUSSION

Previous studies have considered barrier integrity to be important in EoE and the related entities of PPI-REE[61, 248]. Other investigators have recently separately considered the etiopathogenesis of EoE in terms of newly-conceived, disparate inflammatory disease 'models' including IgG-mediated pathology[216]. These studies were predominantly retrospective, with heterogeneous treatment and time intervals. We thus conducted a prospective study of well characterised patients (surveyed at consistent time intervals, biopsied in all oesophageal regions) with the objective of defining the inflammatory response and variations in barrier integrity in response to food antigens. A secondary objective was to compare the response to various treatment strategies, namely diet with PPI, budesonide monotherapy or PPI alone.

Patients diagnosed with oesophageal eosinophilia (both EoE and PPI-REE) had increased expression of mast cell tryptase as well as IgG and IgE antibodies compared to controls[246, 249]. Barrier integrity was compromised in both groups with lower expression of desmoglein (previously described) and caveolin (only previously shown in asthma) compared to controls [61, 250]. Response to the various treatment protocols (diet and PPI, budesonide monotherapy, or PPI alone in those diagnosed by convention as PPI-REE) was accompanied by similar immunohistological changes. Thus, whilst eosinophil count fell, along with mast cell tryptase, and both caveolin and desmoglein increased, the deposition of IgG and IgE antibodies did not substantially alter with treatment. We speculate that this may relate to the duration of treatment, that antibody deposition may take time and be a secondary phenomenon to Th-2 mediated

inflammation. This could be viewed as being supported by the similar failure of IgG and IgE to rise following food antigen exposure (see below).

Time-dependent and region-specific observations were facilitated by the introduction of food antigens and subsequent gastroscopy at 2 weeks. The introduction of a food antigen and subsequent flare of disease induced a more florid lower oesophageal eosinophilia. IgG and IgE antibodies did not significantly rise, and likewise the marker of barrier integrity, desmoglein, fell in the lower oesophagus only. These observations should be tempered by a recognition of a relatively small samples size and also may be explained by more preserved desmoglein expression in the upper oesophagus in patients with active disease in our cohort. It is also notable that significant disparity, although of a lesser degree, was evident between the upper and lower oesophagus in patients from various subgroups pre-treatment. Previous studies have not demonstrated this regionality following dietary reintroduction, although regional differences (with respect to eosinophil count) have been variably shown in untreated patients[24, 189, 203].

The apparent focus of inflammatory activity and barrier integrity impairment in the lower oesophagus can then be considered. We speculate that this may relate to the greater exposure of the lower oesophagus by refluxate of food from the stomach, with subsequent migration of inflammation over time. Alternative explanations could include that PPIs mitigate the expression of the chemokine eotaxin-3, preferentially in the upper oesophagus, or, alternatively, that physiological or pathophysiological properties of the lower oesophagus favour this regional disparity[192]. Previous studies have not used, or have variably used PPIs during dietary reintroduction. The lower oesophagus has increased number of antigen-presenting Langerhans cells, and increased numbers of goblet cells that produce mucus and may perhaps trap and

present antigen (although mucus has also been also shown to limit antigen interactions with the mucosa in the intestine which contradicts this hypothesis)[251-253]. Another explanation could be related to coexistent GERD. Patients with GERD have impairments in barrier integrity of the lower oesophagus, and this could potentially facilitate antigen exposure[242]. Interestingly however, is the observation that even dilute acid exposure of the lower esophagus can subsequently lead to decrements in barrier integrity of the upper esophagus over time [254]. It is thus apparent that understanding what is cause vs effect ('the chicken and the egg') poses a significant future challenge. These hypotheses could be addressed by studies of patients exposed to food antigens without concomitant PPIs and patients with untreated GERD, as well as dedicated molecular biological studies examining protein expression (including for eotaxin-3) and antigen-presenting cells such as Langerhans cells.

The question as to which disease model most aptly describes EoE and to the timing of the inflammatory model can be considered with reference to our study, and remains unanswered. Features of a T helper-2-mediated inflammatory condition are present, with the presence of mast cells, eosinophils as well as IgG and IgE antibodies[193]. Deficits in barrier function (as observed in our patients) have also been extensively detailed in the prototypical T helper-2-mediated conditions of asthma and atopic dermatitis[255, 256]. Our findings differ from one recent study that found no significant IgE, but florid IgG deposition in oesophageal tissue of patients with EoE[216]. Other groups have found IgE in oesophageal tissue. Our results, therefore, can neither confirm nor refute the claim that EoE is an IgG and not an IgE mediated disease. What role either antibody in fact plays remains to be determined. The fact that IgE in particular failed to increase following two weeks of food antigen exposure despite florid eosinophilia arguably counters any assertion that EoE is an IgE-mediated disease, based on experience with other clearly defined IgE-mediated entities characterised by acute manifestations following antigen exposure[257].

Several limitations in the current study are recognised. First, the number of control subjects is small, and they are not matched for factors such as age, gender or atopic status. Additional information concerning the role of barrier integrity may have been gained by studying patients with GERD. Secondly, slide processing was manual rather that automated. Nonetheless, our laboratory has ample experience in the former techniques. Similarly, the immunohistochemistry of oesophageal tissue was studied using semi-quantitative methods, which is subjective. This technique has been used, however, in similar studies previously, was cross-validated by two researchers, and utilised both manual generation of deposition density (scored from 1 to 4) and computer-generated density measurements. Fourthly, patients did not have oesophageal pH studies, potentially erroneously including patients with predominant GERD in the study. Countering this argument are guidelines that indicate twice-daily PPIs followed by oesophageal biopsy at 8 weeks (as followed in the present study) as the preferred means of diagnostic allocation[1]. A fifth concern is the variability in duration of remission, given that individual patients responded to different food antigens that were introduced at 2 weekly intervals in a pre-specified order. This could potentially influence the degree of inflammatory response to the eventual food trigger. A major aim of our study was to consider time-related changes in oesophageal inflammation following food antigen exposure. The suggestion that repeated biopsy over staggered time intervals (e.g. 3 days, 2 weeks and 6 weeks) and longer periods of food antigen exposure has thus been made. We had to balance the need of safe, well-tolerated clinical care with scientific hypothesis generation and hence, this was not deemed a practical process given the need for invasive sampling (gastroscopy). Finally, the measurement of IgG and IgE antibodies in tissue is technically difficult and the use of tissue digestion may have allowed greater biological sampling and thus discrimination between groups and

following treatment. IgG in particular may be deposited preferentially in the deeper lamina propria which was only sampled in approximately 60% of cases in our study[216].

In conclusion, an inflammatory infiltrate typical of a Th-2 mediated disease process is present in patients with EoE and those with PPI-REE. The inflammatory infiltrate and impairment in barrier integrity as determined by immunohistochemistry is produced by food antigen exposure. Treatment with budesonide, diet and PPI (for EoE) or PPI alone (PPI-REE) all largely resolve the immunohistochemical changes associated with eosinophilia. The lower oesophagus appears particularly important in initiating inflammation, whilst density of IgG and IgE deposition appear to relate to the duration of the inflammatory response.

Table 1. Characteristics of patients with eosinophilic oesophagitis and the control group [control compared to diet/PPI group using two tailed t test (continuous data) or fisher exact (categorical)]

		Eosinophilic oesophagitis		PPI-REE	Control	P-
		Diet + PPI	Budesonide			value
Number studied		20	10	18	10	
Mean age (SD) y		40 (11)	38 (14)	44 (12)	60 (14)	.002
Male gender		13/20 (65%)	7/10 (70%)	12(64%)	7/10 (70%)	1.00
Presence of atopic illness		12/20 (60%)	6/10 (60%)	8 (44%)	3/10 (30%)	0.37
Presenting symptom	Food bolus	15 (75%)	6(60%)	8(44.5%)	4 (40%)	0.10
	Dysphagia	12 (60%)	5(50%)	10(55%)	10 (100%)	0.028
	other	3 (15%)	1(10%)	4(22%)	0	0.30
Additional medications		2/20 (10%)	2/10 (20%)	4 (11%)	6/10 (60%)	0.17
Cigarette Smoking		0/20	1/10 (10%)	2(11%)	3/10 (30%)	0.30
Mean eosinophil count (prior to treatment) (SD)	upper	24 (9)	29 (12)	35 (9)	0	<0.001
	middle	32 (9)	35 (13)	37 (8)	0	<0.001
	lower	29 (7)	37 (12)	39 (9)	0	<0.001

Table 2. Pre – treatment biomarkers in patients with oesophageal eosinophilia and controls (p-values denote comparison of diet with PPI patients, and control patients using two tailed t test)

Biomarker	Eosinophilic oesophagitis		PPI-REE	Control	P-value
	Diet/PPI (mean)	budesonide			
Eosinophil count	(n=20)	(n=10)	(n=18)	(n=10)	
• upper	29 (9)	29 (12)	35 (9)	0 (0)	<.001
• middle	32 (9)	35 (13)	37 (8)	0 (0)	<.001
• lower	34 (7)	37 (12)	39 (9)	0 (0)	<.001
Mast cell tryptase	(n=16)	(n=7)	(n=14)	(n=10)	
• upper	4	3.5	3.8	1	<.002
• middle	3	3.7	3.4	2	<.12
• lower	4	3.5	3.7	1	<.002
IgG antibodies	(n=16)	(n=10)	(n=14)	(n=10)	
• upper	2.7	1.7	3	1.4	<.003
• middle	3	2.7	2.7	1.3	<.001
• lower	3.7	2.5	2.8	1.8	<.001
IgE antibodies	(n=16)	(n=10)	(n=14)	(n=10)	
• upper	2.4	1.9	2.8	1.4	<.002
• middle	2.9	2.6	2.4	1.8	<0.03
• lower	2.5	2.1	2.3	1.8	<0.06
Caveolin	(n=16)	(n=7)	(n=14)	(n=10)	
• upper	1.7	2.1	1.7	3.5	<.05
• middle	2.2	2	1.6	3.7	<.05
• lower	1.1	1.5	1.7	3.4	<.05
Desmoglein	(n=16)	(n=7)	(n=14)	(n=10)	
• upper	2.8	1.6	1.4	3.3	0.32
• middle	2.2	1.3	1.3	3.2	0.55
• lower	1.4	1.2	1.1	3.3	<.05

Figure 1 (a-f) – Biomarker intensity (or eosinophil count) at diagnosis, following treatment with diet and PPI and following reintroduction of food antigen.

1 a

Eosinophil count per HPF
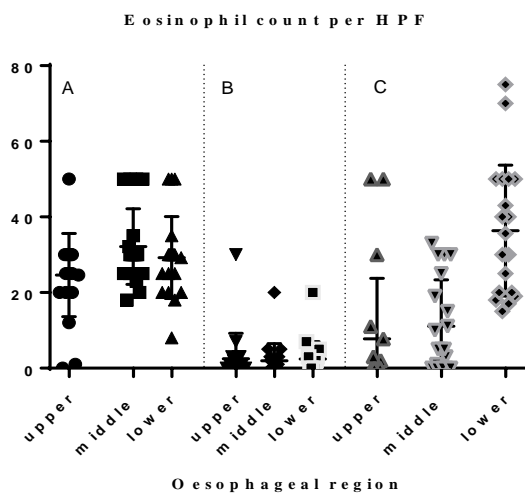
The eosinophil count fell in all oesophageal regions with treatment (diet with PPI, that is A to B, p <.001)). Food reintroduction lead to an increased in eosinophil count in all regions (that is B to C, p <.001).

1 b
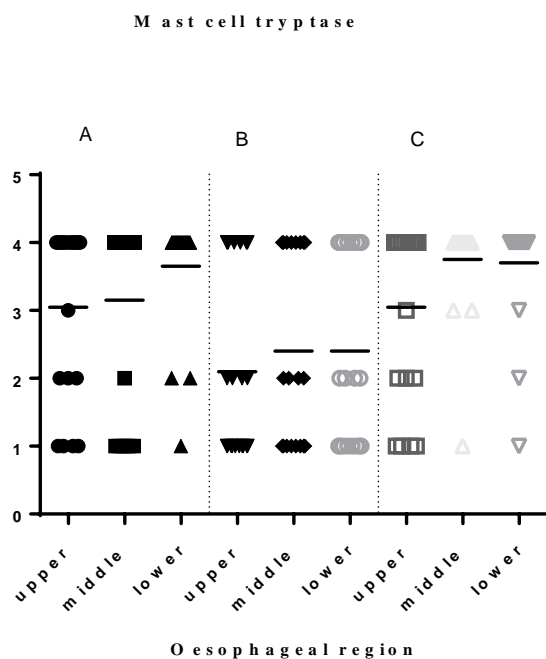
Mast cell tryptase

Mast cell tryptase intensity decreased in the upper, middle and lower oesophagus from 3.1,3.3,3.8 to 2.2,2.2,2 (that is A to B, p <.05 all areas). Dietary reintroduction lead to an increase in all areas to 3.2,3.8,3.7 (that is B to C, p <.05 all areas)
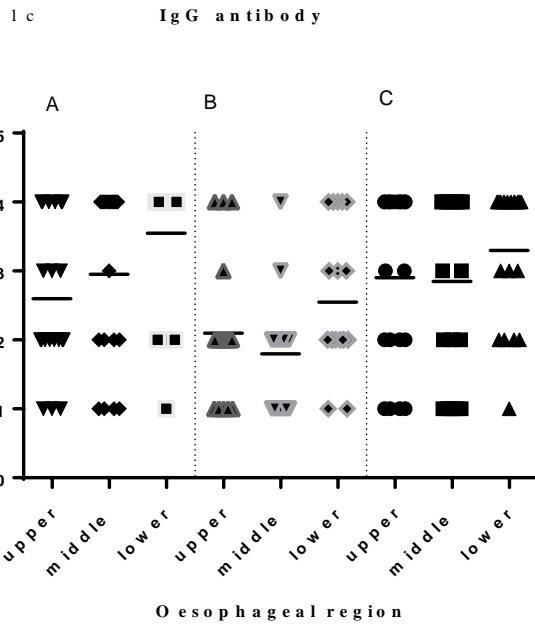
1 c   **IgG antibody**

IgG antibodies fell in response to treatment (diet with PPI) in the lower oesophagus only, from 2.7,3.1,3.7 to 2.2,2 and 2.6 (p <.05, lower esophagus only, that is A to B)

Food reintroduction did not significantly change IgG antibodies, to 2.9,2.9,3.3 (p>.05, B to C)
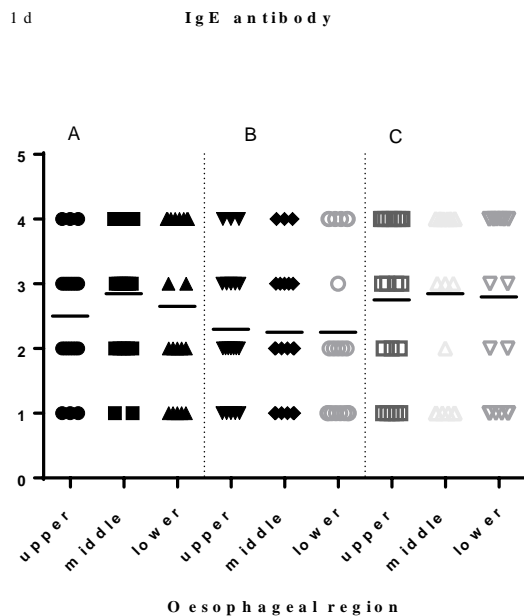
1 d   **IgE antibody**

IgE antibodies did not change significantly with treatment, from 2.4,2.8 and 2.5 to 2.2,2.1,2.1 (p >.05, that is from A to B)

Food reintroduction did not significantly alter IgE, to 2.7,2.8,2.6 (p >.05, that is B to C)

1 e
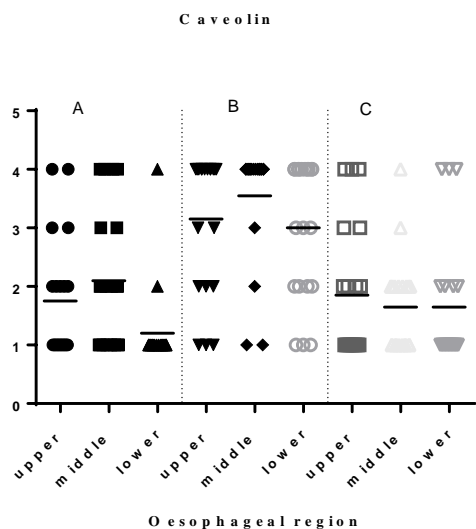
C a v e o l i n

With dietary and PPI therapy (combined) the caveolin intensity increased in all areas:1.7,2.2,1.1 to post treatment 3.4,3.9,2.9 (p <.05 all areas), that is from A to B. With food reintroduction (C), caveolin decreased in all areas to 1.6,1.4,1.4 (p <.05)

1 f
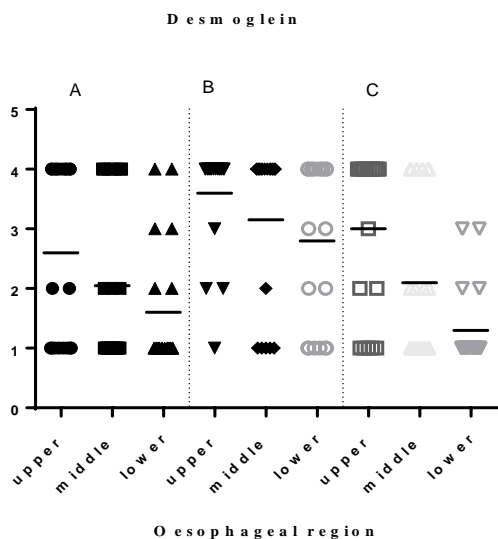
D e s m o g l e i n

With diet and PPI (combined), desmoglein increased significantly in the lower and middle oesophagus only:2.8,2.2,1.4 to 3.6,3.4,3.1 (p < .05 lower and middle oesophagus only, that is from A to B)

With food reintroduction, desmoglein decreased significantly in the lower oesophagus only, to 3.1, 2.2,1.4 (p <.05 lower oesophagus, that is from B to C)

Figure 2a and 2b – Biomarker intensity (lower oesophagus) in patients treated with budesonide and PPI monotherapy respectively. 't' =treatment

2 a

Budesonide treatment

The intensity of mast cell tryptase fell from 3.5 to 2 (p <.01). Intensity of caveolin increased from 1.5 to 3 (p < .04) and desmoglein from 1.2 to 2.8 (p <.01). The intensity of IgG did not significantly change (2.5 to 1.9, p >.57) and that of IgE was similarly unaltered (2.1 to 2, p > 0.8).

2 b
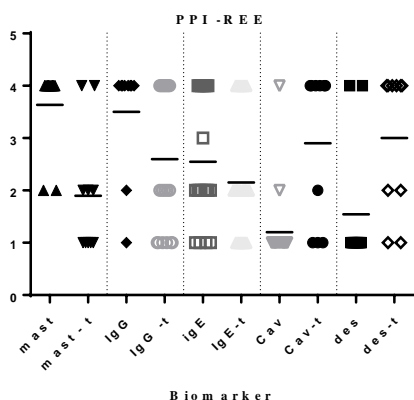
PPI-REE

The mean intensity of mast cell tryptase fell from 3.7 to post treatment 2.2 (p <.001). Intensity of caveolin increased from 1.7 to post-treatment 3.3 (p <.001) as did desmoglein from 1.1 to post-treatment 2.9 (p <.002). No significant decline in IgG, pre-treatment 2.8 to post treatment 2.2 (p >.57), or IgE pre-treatment 2.3 to post-treatment 1.9 (p >.5) were recorded.

Figure 3. Semi quantitative analysis of mast cell tryptase for a patient with active EoE using positive – pixel count via the Aperio digital imaging platform (panel on right).
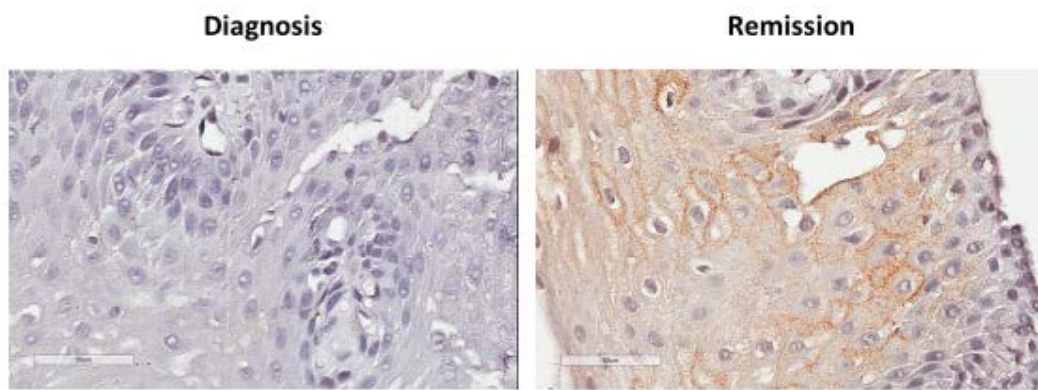
Figure 4. Increase in desmoglein expression (barrier integrity) in a patient with EoE treated with diet and PPI.
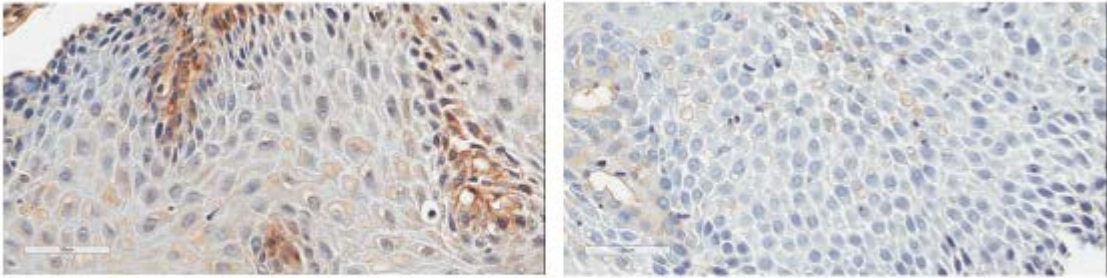
Figure 5 – Caveolin expression in the lower oesophagus pre – treatment (panel on the left) and following dietary reintroduction (panel on the right). A decline in inflammatory biomarker intensity was demonstrated with successful treatment.
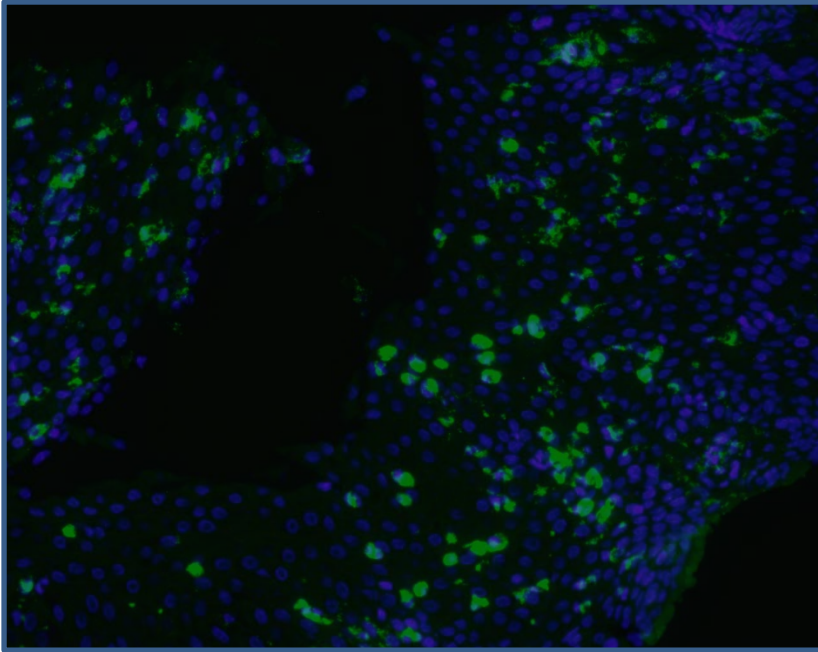
Figure 6 – IgG deposition in a patient with active eosinophilic oesophagitis.

(blue is DAPI nuclear counterstain, green is IgG)

CHAPTER 8

8.1

Food but not aeroallergens are the major cause of EoE

In Chapter 3, it was demonstrated that FBOE, themselves the commonest presenting feature amongst patients with EoE, are no more likely to occur in the pollen season. The ability of the 6FED to cause complete histological remission in more than 50% of patients was shown in Chapter 5. It is our contention that the accumulated body of research points to food antigens playing a major role, with aeroallergens possibly causing exacerbations in a small subset of individuals[24, 47, 54, 189, 258].

EoE is an antigen driven disease, predominantly but not exclusively triggered by food antigens. In the first recent meta-analysis evaluating the efficacy of dietary treatment for EoE, elemental diet (90%) and SFED (72%) were highly effective in inducing histological remission of the disease. The other way around, 10% and 28% of patients did not achieve disease remission despite no food at all or eliminating six major food groups, respectively.

Understanding how and where the food antigens incite the immune response remain key challenges for the future. Exposure of the oesophageal mucosa to food antigens may be responsible. However, the rapid transit of food bolus and the relatively thick layer of squamous epithelium and lack of abundant antigen presenting cells and lymphoid follicles challenges this theory[43, 89]. An alternative hypothesis is that antigen presentation occurs in the duodenum and subsequent selective trafficking of eosinophils to the oesophagus takes place[38, 259]. Promising methodologies to facilitate further study could be use of confocal endomicroscopy to determine real-time changes in oesophageal mucosa with antigen exposure in patients in remission from dietary therapy, or duodenal (and not oesophageal) antigen exposure with encapsulated food antigens in the same population[233].

8.2

Aeroallergens may exacerbate EoE in a small subset of individuals

Formerly, the assertion had been that aeroallergens were a major factor in causing adult EoE. This hypothesis was based partly on indirect observational data lacking a control group, and on the finding that sensitisation to aeroallergens was more common than food allergens in adult patients with EoE. Moawad et al, and Almansa et al demonstrated increased rates of diagnosis of EoE in the pollen season[50] [33]. As discussed in Chapter 2, there was the lack of a control group or standardisation in the presenting symptoms, diminishing the quality of these studies. Our study by contrast compared patients with EoE to others presenting with FBOE and found no difference[51]. The distinction between sensitisation and true allergy is also important. Individuals with EoE are often atopic - by definition, they have elevated levels of IgE or positive skin prick tests to common environmental antigens[111]. Patients with EoE are also more likely to have allergic conditions including rhinitis and asthma. Thus, the finding of aeroallergen sensitisation alone does not imply causation in relation to EoE. Indeed, the limitations of allergy tests have been clearly demonstrated most comprehensively by ourselves (see Chapter 6).

A subgroup of our patients with EoE and resultant FBOE had recurrent events and these occurred with increased frequency in the pollen season, particularly at the beginning of the pollen season[51]. It is plausible that aeroallergens may further exacerbate the oesophageal inflammation in these individuals. Fahey et al recently reported a retrospective study of children with EoE, and similarly demonstrated a peak prevalence of symptoms (and diagnosis of EoE) at the commencement of the grass pollen season[260]. Related observations supporting this theory are case reports of dysphagia and/or FBOE following sudden massive aeroallergen exposure (e.g. during lawn mowing) and of development of the condition after commencing

allergen immunotherapy[47]. How the aeroallergens incite the EoE (whether by direct contact with the esophageal lumen, or by causing inflammation indirectly after first inciting rhinitis and then swallowing inflammatory secretions) is a subject of ongoing debate[261].

Studies of large numbers of individuals with oesophageal eosinophilia have recently demonstrated significant geographical disparity in the prevalence of EoE. People living in temperate as well as dry-arid environments and undergoing oesophageal biopsy are more likely to demonstrate oesophageal eosinophilia[41]. Interestingly, this difference in prevalence is demonstrated in young to middle aged adults only, raising the hypothesis that novel and/or dose-related (increased) exposure to aeroallergens may precipitate the condition. Our studies were not powered to examine the effect of migration in terms of the diagnosis of EoE and lacked a control group (e.g. those with alternative pathology such as gastroesophageal reflux disease). Nonetheless, a number of individuals who had migrated from overseas were included in our cohort (Chapter 5). Another interesting and novel observation in terms of adults with EoE was that birth in winter was more common than summer in our patient cohort, although sufficient numbers were not achieved to power this enquiry rigorously. Borrowing from a hypothesis generated from other atopic conditions (rhinitis and asthma), it could be proposed that this relates to aeroallergen exposure in the early weeks of life[262]. Contradicting this theory is the fact that aeroallergens are present only for a few months in spring in most areas of the world and would be at low atmospheric concentration during summer[263]. More plausible perhaps is the theory that maternal sunlight exposure during the latter part of pregnancy results in higher vitamin D levels, that in turn have an epigenetic effect in decreasing allergic disease including EoE[55, 264]. This is supported by studies of umbilical cord blood demonstrating that higher levels of serum vitamin D at birth are protective for latter development of atopy and moreover food allergy[264]. Certainly, study of large numbers of patients who have migrated to

Australia together with longitudinal follow-up of patients with EoE based on birth records is a promising area of enquiry. Ideally, a disease registry and databank should be established, Australia as an isolated continent being suited particularly in generating migration-related data. Practical and ethical considerations have limited the study of aeroallergens in EoE, and hence applicable data to date are indirect, observational or from animal models. To directly determine if aeroallergens incite oesophageal eosinophilia via respiratory and/or gastrointestinal deposition, future studies would ideally involve patients in remission from dietary therapy being exposed to high concentrations of aeroallergen. This could be administered to the nasal mucosa or bronchial tree[52]. Careful patient selection to ensure safety (and minimise risk of, for example, acute severe asthma) would be critical.

8.3

Allergy tests are not useful in guiding dietary therapy in EoE

Our research provides the strongest evidence to date that allergy tests are unable to predict food triggers of EoE. Previously, skin-prick and skin-patch demonstrated marginal benefit in a large retrospective paediatric study, but a lack of efficacy in adult studies[24, 189, 265]. We performed 5 different modalities of allergy test and none were able to predict food triggers.

The reasons why allergy tests cannot detect food allergy and thereby guide dietary therapy may be numerous. First, it is possible that EoE has a different and non-IgE-mediated pathogenesis compared to classical food allergies. If this is the case, then certainly skin-prick and serum-specific IgE) would be of little utility, which was indeed demonstrated. However, we performed additional tests aimed at detecting cell-mediated immune response (skin-patch test), activation of the TSLP-basophil axis (the basophil activation test with a range of cell-surface markers) and IgG4-mediated disease (serum food specific IgG) and none could accurately

predict food triggers. A second hypothesis is that the gastrointestinal immune compartment is relatively isolated and thus measures of systemic immune activation such as those described are not useful. Nonetheless, recruitment of bone marrow-derived (myelogenous by definition) eosinophils strongly suggests a systemic signal is manifest[266]. An alternative approach was recently trialled, namely the search for serum biomarkers (particularly cytokines including eotaxins) by comparing patients with active EoE to controls[267]. Again no test could distinguish patients with EoE from control.

Improved future understanding of the cytokine signature and genetics of EoE may translate into more targeted and thus accurate allergy tests. In the interim, it is evident that histological sampling is required and this has led to the development of less invasive techniques including transnasal gastroscopy (see below). Major drawbacks of histological sampling aside from the invasive nature include the cost of histological sampling and time delays incurred in this process. From a theoretical standpoint, oesophageal skin-prick test via video endoscopy is a tantalising prospect that could both provide information about the likely site of immune exposure and rapid information concerning likely food triggers. This has been attempted in the human colon previously, apparently successfully, but has not been taken up in clinical practice[232]. Ethical considerations as well as technical hurdles, including what a positive test really means without a reference group, perhaps limit this enquiry.

8.4

Transnasal gastroscopy is a safe and well tolerated procedure in consenting individuals

EoE currently requires histopathological sampling both for diagnosis and in the assessment of treatment response[42]. In the Australian setting, patients undergoing standard video-endoscopy demand sedation, and there is associated inconvenience and the cost of prolonged hospital admission, as well as the inability to drive on the day of the procedure. We demonstrated that unsedated transnasal gastroscopy (UTEG) is a safe, well tolerated procedure capable of taking adequate samples in consenting individuals[268]. The major impediment to widespread implementation of this technique is the reluctance of patients to undergo the procedure. The notion of awake nasal and oesophageal intubation is not acceptable to many potential subjects leading to an uptake by only 1 in 4 patients. Another drawback of UTEG is that the instrument requires the standard processing (cleaning), image management system and monitor as per standard transoral gastroscopy. To make UTEG a rapid, inexpensive outpatient delivered procedure, a compact fibre-optic system with an external biopsy channel that could facilitate office based housing and cleaning is needed. Current standard practise in the field of Ear, Nose and Throat Surgery is to use a transnasal endscope to inspect the vocal cords, but this device does not have the capacity to biopsy[269]. Two similar alternatives under development (the cytosponge and the esophageal string test) appear to deliver acceptable tissue sampling and are well tolerated[185, 186]. We have some concerns regarding the ability of this technique to detect disease activity given the focal nature of inflammation particularly following food reintroduction (see below) although a direct head-to-head study with video-endoscopy is currently underway and will answer the question[186].

8.5

Highly restrictive diets are effective, but may not be implemented in clinical practice

The American College of Gastroenterology (ACG) issued guidelines in 2014 stating that dietary therapy for EoE be considered a first-line treatment by clinicians managing patients with the condition [1]. This recommendation appears premature based on our real-world, patient-driven study, and if greater scrutiny is applied to existing research and comparative therapies. Critically, only 3 previous studies (2 of which were performed at 1 centre) have been performed using heterogeneous treatment protocols and in a closely supervised setting[212, 214].

The question of whether dietary therapy induce remission in EoE can certainly be answered in the affirmative, but the more important consideration is what percentage of patients who attain remission actually undergo complete food reintroduction, determine triggers and maintain a diet. In our study, only 1 in 3 patients initially attaining remission on the 6FED went on to determine food triggers and then maintain a diet at 9 months[215].

Until less invasive means of determining food triggers are developed, dietary therapy for EoE needs to be undertaken with great caution. In the interim, in a practical sense there is a compelling argument that, should dietary therapy be pursued, then fewer foods should be restricted. The 4-food diet elimination diet has already been trialled and found to offer only a minor reduction in efficacy compared to the 6FED[212]. Our experience mirrors that achieved elsewhere in that wheat, eggs, milk and (to a lesser extent soy) are the predominant identifiable food triggers in patients responding to an elimination diet[215].

Pharmacotherapy remains the most feasible option for the management of EoE (and oesophageal eosinophilia per se – see below) Budesonide is a highly efficacious treatment for many patients. In Chapter 5 it was demonstrated that budesonide administered as an oral

viscous solution (1mg Po BD) resulted in histological remission in >90 % of patients. This was evident both for patients electing to use budesonide 'first line' after failing to respond to PPI, and also in patients attempting but failing to respond to dietary therapy and subsequently using budesonide. Notably, no significant side effects were reported by our patient cohort, including those followed up over 3 months.

Proton pump inhibitors are effective for some patients and are well tolerated. We demonstrated that 25% of patients previously diagnosed with EoE could then be labelled as PPI-REE. This is a lower response rate than some previous studies. Two very recent observations may lead to a reconsideration of the somewhat arbitrary distinction of EoE or PPI- REE that is applied to patients with oesophageal eosinophilia, and suggest that lower dosage of PPI is effective thereby arguably making maintenance therapy more appealing[195, 270]. Thus, patients responding to diet may respond to PPI and vice - versa implying that the distinction between these two 'diagnoses' is not based on disease pathogenesis (and indeed most studies suggest both conditions have similar immunohistology and even genetic expression, see also chapter 7)[271-273]. Also, more than 80% of patients responding to twice daily PPI sustain complete histological remission on daily dose over 3 months[195]. PPI's therefore must be viewed increasingly as a feasible maintenance treatment. The suggestion that EoE and PPI-REE are in fact the same condition that may respond to multiple therapies, (and even that dual therapy can be considered as a strategy) is therefore raised, but requires validation with rigorous, well powered studies (utilising cross – over methodology) in future.

8.6

EoE is characterised by a Th2- mediated cellular inflammation with abundant IgG deposition

In Chapter 7, the use of histopathology and immunohistochemistry in well characterised patients, and the subsequent induction of disease activity with food antigens in patients treated with elimination diet facilitated consideration of region-specific and time-related changes in esophageal tissue. Recently it has been suggested that EoE is an IgG- and not an IgE-mediated disease[213, 216]. Our study demonstrated prominent IgG deposition during active disease only and IgE deposition that was indistinguishable from control subjects regardless of disease activity. Thus, our findings add credence to the previous study of IgG, although the pathophysiological significance of IgG remains to be determined. That is, whilst the monoclonal anti-IgE antibody, omalizumab, failed to suppress disease activity, such a therapy has not been developed with reference to food-related IgG[216]. IgG deposition itself could be a consequence of Th2-mediated cellular inflammation and has been observed in other disease states including atopic rhinitis[213]. It would seem premature for a paradigm shift away from current model of disease pathogenesis where a Th2-mediated inflammatory signature, characterised by an infiltration of mast cells, eosinophils, lymphocytes, which were all demonstrated in our study, to a model akin to autoimmune pancreatitis[274]. Future enquiry could sensibly include animal models using IgG 'knock-out' techniques using, for example, antibodies to IgG itself or to cell surface receptors to determine if oesophageal eosinophilia can occur in the absence of IgG. Ready templates exist to guide this endeavour[223].

8.7

Barrier integrity decreases with eosinophilic infiltration and increases with treatment

Alterations in barrier integrity have been proposed as both a cause and a consequence of eosinophilic inflammation in EoE. This pathophysiological alteration has previously been considered in more detail in the conditions of asthma and atopic dermatitis[172, 255]. We considered barrier integrity by measuring the expression of desmoglein and caveolin by immunohistochemistry in patients with active and quiescent disease. Near normalisation of their expression equating near complete resolution of barrier dysfunction was achieved in the various treatment groups, leading to tentative speculation that impairments were a consequence rather than a cause of eosinophilic infiltration. Indeed, comprehensive studies of patients with EoE treated with fluticasone, and of patients with PPI-REE (pre and post PPI administration) have demonstrated similar decrements in barrier integrity that were reversed with treatment and corresponded to a decline in eosinophil count [275, 276]. Recent studies of patients with GERD have also suggested that an immune reaction (characterised by lymphocyte infiltration and inflammatory cytokine expression) precedes alterations in barrier integrity, thus highlighting the complexity of these purported pathophysiological models [277, 278]. Future longitudinal studies are warranted, given the limitations of any single biological assay and the fact that PPIs were used concurrently with diet in our study. Alternative methodologies could include use of electron microscopy and oesophageal impedance in a patient cohort treated with and without PPIs[279]. Furthermore, the use of pharmaceutical agents that may improve barrier integrity independent of gastric pH (e.g. sucralfate or larazotide) could be considered[280, 281].

8.8

Conclusion

Eosinophilic oesophagitis is predominantly caused by food antigens and characterised by a Th2-mediated inflammatory infiltrate with abundant IgG antibody deposition. Allergy testing cannot predict food triggers, and thus oesophageal sampling is currently required to make management decisions. Unsedated transnasal gastroscopy offers a less-invasive and potentially more patient-friendly approach. Highly restrictive elimination diet can achieve disease remission in many patients, although the complexity of food reintroduction and need for strict compliance limits efficacy. Currently, PPIs should be considered for all patients with oesophageal eosinophilia, and budesonide used first line for those failing to respond to PPI. We propose future research be directed to determine the site and mechanism of antigen presentation, the action of proton pump inhibitors, the validity of PPI – REE as a distinct entity, and the utility of combination therapy.

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APPENDIX 1

OPINION

Risk factors for eosinophilic esophagitis

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Summary

Eosinophilic esophagitis (EoE) is a chronic antigen driven disease, whereby food and/or aeroallergens result in inflammation and luminal narrowing, and the clinical symptoms of dysphagia and food bolus obstruction events (FBOE). Established risk factors are male gender, Caucasian race and atopy. Increased risk amongst family members, and a single nucleotide polymorphism (SNP) in a gene coding thymic stromal lymphopoietin (TSLP) on the pseudoautosomal region of the X and Y chromosomes supports a genetic predisposition. Environmental factors including the timing and nature of food and aeroallergen exposure to the developing immune system may be important, whilst esophageal barrier function integrity and the influence of microbiota are worthy of future research.

Keywords allergy, eosinophil, esophagus, antigen, remodelling
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Introduction

Eosinophilic esophagitis (EoE) is conceptualised as a chronic antigen-driven disease, whereby food and/or aeroallergens stimulate an eosinophil-rich infiltrate in the oesophagus that produces the clinical syndrome of dysphagia, feeding difficulties (in young children) and food bolus obstruction [1, 2]. EoE was first recognised as a distinct clinical entity only recently, in 1993 [2]. The epidemiology, basic scientific and clinicopathological data are hence somewhat limited, and to date, the most clearly defined risk factors for EoE are gender (male predominance), race (mainly a disease of white Caucasians) and atopy (elevated serum IgE to common aeroallergens and other allergic conditions (asthma, seasonal rhinitis and atopic dermatitis). Other putative risk factors include alterations in barrier function (e.g. from gastroesophageal reflux disease (GERD), variation in the nature and timing of oral antigen exposure (e.g. secondary to infant feeding practices, proton pump inhibitor use and commercial food processing) and variation in the nature and timing of aeroallergen exposure (seasonal, geographical and secondary to migration and factors relating to fibrous remodelling (e.g. ACE gene polymorphisms and TGF-β polymorphisms).

The reasons for this gender and racial difference are not known, but could include genetic factors transferred on the sex chromosomes, mitochondrial DNA or possibly the pathoplastic effects of sex hormones on inflammation and fibrosis [3–6]. A history of EoE in a first-degree relative has been reported in 23–37% of cases (adult vs. paediatric), supporting a genetic basis, although a more modest familial trend was demonstrated recently (about 3%) [7, 8]. Genetic studies have been useful in correlating single nucleotide polymorphisms (SNPs) with a propensity to develop atopic conditions, including EoE. Notably, thymic stromal lymphopoietin (TSLP), eotaxin 3 and filaggrin SNPs appear to predispose to EoE, and a SNP for TGF-β predicts response to corticosteroids [9–11].

This review examines the basic scientific, epidemiological and clinical studies relating to the pathogenesis of EoE and attempts to highlight unexplored risk factors with a view to future research and therapeutic innovation.

Epidemiology – A Western disease on the increase?

The history of EoE is short. Prior to 1990s, only a few case reports of oesophageal eosinophilia had been reported, with Attwood, Levine and Saul, and Vitellas being amongst the first to suggest the existence of a distinct clinicopathological entity [2, 12, 13]. What is *not* known is if EoE is a disease of modern life, or if the

apparent increase in the last 20 years is a result of awareness by clinicians and researchers armed with modern endoscopic equipment able to make the diagnosis with the mandatory oesophageal biopsy. For example, some patients who were once assumed to have gastroesophageal reflux disease (GERD) with stricture formation may now be called EoE. To date, several large uncontrolled retrospective studies from North America, western Europe and Australia demonstrate an increasing incidence and/or prevalence of EoE [14–16]. Interestingly, other atopic conditions that are better characterised such as atopic dermatitis and food allergy *per se* have increased amongst children in the USA in the last 14 years [17].

Theories relating to the apparent increase in EoE are broad. They are mostly borrowed from research on other atopic conditions and relate to recent changes in living conditions and medical treatments, such as exposure to bacterial pathogens, moulds and animal antigens (i.e. the hygiene hypothesis), or a decrease of *Helicobacter pylori* infection in Western populations [18, 19]. Furthermore, use of proton pump inhibitors, a decrease in consumption of fresh fruit and vegetables and in increase in processed foods have also been suggested as causes for the apparent rise in allergic conditions including EoE [20, 21]. Whilst hypothesis abound, good quality prospective data collection in relation to EoE is needed before conclusions can be reasonably made.

Age, gender and genetics

EoE is a disease of both children and adults. The majority of cases diagnosed in childhood are between 5 and 10 years of age, although cases in very young children are seen [22, 23]. In adults, the mean age of diagnosis is in the late 30's, with almost all cases diagnosed before the age of 50 [15, 24]. It is notable that amongst both children and adults, EoE mainly afflicts males with a male to female ratio of approximately 3 : 1 in most series [1, 25].

The distinction between adult and paediatric EoE may relate to the increased recognition of the condition, rather than two rigidly distinct entities. In other words, in the past, paediatric cases may have been missed and are now being diagnosed in adulthood. A recent prospective study suggests this is the case, demonstrating that EoE in more than 70% of paediatric cases remains active on transition to young adulthood over a period of 5 years' observation [26]. The authors suggest that both paediatric- and adult-onset conditions exist (as is the case with asthma), but that a significant number of current paediatric-onset cases will progress to adulthood in what is increasingly viewed as a chronic condition.

The male predominance of EoE is unique when compared to other apparently related conditions, such as asthma, atopic dermatitis and seasonal rhinitis, that share key similarities such as the eosinophilic infiltrate and atopy defined as an elevated IgE to common food or environmental allergens [17, 27, 28]. Classical food allergy (characterised by anaphylaxis or angioedema within minutes to hours of food ingestion) demonstrates a male predominance in early life that disappears or even slightly favours females in adulthood according to some studies [17]. Asthma again shows a male predominance in childhood that disappears later in favour of females [28]. Finally, atopic dermatitis favours females in both children and adults [27].

Thymic stromal lymphopoietin (TSLP) is a cytokine produced by epithelial cells that is central to the pathogenesis of EoE, as demonstrated by animal models and recent human studies [6, 9, 29]. Intriguingly, the gene coding for the TSLP receptor (TSLP – R, that is, cytokine receptor – like factor 2 – CRLF2) is found on a pseudo-autosomal region of the X and Y chromosomes (Xp22.3 and Yp 11.3), and statistical analysis has demonstrated that a single nucleotide polymorphism of this region predisposes male patients to develop EoE [6]. Furthermore, a SNP in the region coding TSLP itself (5q 22.1) predisposes to EoE [6]. Thus, TSLP-related inflammatory pathways may in part contribute to the gender predominance.

Other factors related to gender predominance can be considered, but remain unstudied. First, mitochondrial DNA is inherited from the mother, and it is notable that mitochondrial dysfunction has been attributed to result from allergen exposure in an animal airways model [3]. Furthermore, a maternal history of atopy may predispose to asthma to a greater extent than a paternal one, and a SNP in a region of mitochondrial DNA has been linked to elevated IgE levels and atopy [3]. Secondly, relaxin is a hormone present in large amounts in pregnancy, in small amounts in non-pregnant females of reproductive age and possibly also in males [30]. It may have the potential to decrease fibrosis in a range of organs, as demonstrated by animal models [31]. It is acknowledged that most patients with EoE never become pregnant (many paediatric patients and a male predominance in adulthood). A study of relaxin in human bronchial biopsies suggests an association with the remodelling process in asthma, whilst a greater expression of relaxin receptors in female compared with male cruciate ligament tissue has been cited as an explanation for greater tissue laxity in females [32]. Further research may allow the therapeutic use of relaxin in the future.

SNPs unrelated to gender have been implicated as risk factors for EoE [33]. Eotaxin-3, a cytokine expressed by epithelium, plays a central role in eosinophil recruitment

to the oesophagus, and a SNP (+2496 GG on chromosome 7) correlates with EoE development [34]. A SNP coding for filaggrin, an epithelial structural protein, has also been implicated in EoE, whilst a SNP coding for TGF correlates with response to inhaled corticosteroids (see below) [35, 36]. Gene expression studies have also detailed a 'genetic signature or thumbprint' of EoE, useful in differentiating this condition from the more common GERD, although arguably such research may document the response to inflammation as opposed to causative sequences [10].

The timing and nature of food and aeroallergen exposures

The dominant theory pertaining to the likely pathogenesis of EoE is that food antigens are causative [37]. The use of an elemental diet can eliminate eosinophilic infiltration in the oesophagus in up to 90% of children and 75% of adults, and the less restrictive six-food elimination diet is successful in approximately 65% of adults and children [1]. Gonsalves et al. [24] not only demonstrated endoscopic and histological recurrence of the condition following successful treatment with the six-food elimination diet, but also histological and in many cases observable endoscopic recurrence after reintroduction of the putative foods.

EoE can be viewed as a form of food allergy with distinct features, lacking the acute 'allergic' features (anaphylaxis or angioedema characteristic of classical food allergy or oral food allergy syndrome) but sharing the atopic profile of the suffers (that is elevation in serum IgE to aero and/or food allergens, and frequent co-morbid atopic conditions such as asthma or rhinitis) [38]. As food allergy may be considered a defect of immune tolerance, and antigen exposure is a factor in the development of tolerance, the timing and magnitude of antigen exposure in shaping the immune system (e.g. the type of extent of food and aeroallergen exposure) may be important in disease pathogenesis.

Much ongoing research is dedicated to factors influencing sensitisation and tolerance to food antigens with reference to classical IgE-mediated food allergy [17]. This may be of relevance to EoE, particularly given the observed increase in incidence over time (see below). For several decades, allergy-prevention guidelines have stressed late introduction of food antigens to infants. However, food allergy amongst children has increased during this period. Animal models have since suggested that both oral and cutaneous exposure to antigens in early life may be integral to achieving immune tolerance [17]. As a result, guidelines issued in the last few years have advocated cautious exposure to a wide range of foods from 4 months of age, whilst maintaining breastfeeding as the primary source of nutrition

[39]. Breastfeeding during the first 6 months of life may decrease the risk of food allergies, although the mechanisms have not be delineated [39]. Also, the use of acid-suppressing medication may increase food allergen sensitisation, possibly by decreasing protein degradation of food antigens and increasing intestinal exposure to intact antigens [40].

It has also been proposed that aeroallergens may cause or contribute to the pathogenesis of EoE. The supportive data are limited to uncontrolled observational studies and an animal model. Case reports detail sudden symptomatic worsening following seasonal aeroallergen exposure, and sublingual immunotherapy has been hypothesised to both cause (precede the diagnosis) and cure (disappearance of EoE following the treatment of rhinitis) the condition [41–43]. Mishra et al. [44] showed that ovalbumin-sensitised mice developed oesophageal eosinophilia in response to airway but not gastrointestinal rechallenge. Almansa et al. [45] and Moawad et al. both demonstrated a seasonal peak of EoE diagnosed at gastroscopy. The assertion of these studies is that patients with EoE present in spring/summer when aeroallergens are at their peak atmospheric concentration. Notable weaknesses of both studies are the lack of a control group and the fact that the case definition included all-comers (i.e. both newly diagnosed and past cases). Both were also retrospective and, hence, susceptible to recall bias.

In an attempt to address some of the methodological issues related to the study of potential seasonality in presentation of EoE, our group performed a retrospective study of all food bolus obstruction episodes occurring across six metropolitan hospitals over a period of 10 years. As food bolus obstruction events (FBOEs) are one of the key clinical features of EoE, and as FBOEs are caused by EoE or GERD in similar proportion, a control group as such (i.e. GERD) is hence included. In this study of 1082 individuals, cases of GERD and EoE were both evenly distributed across the year. Thus, amongst the 88 patients who were diagnosed with EoE, the FBOEs were evenly distributed throughout the year. Again, the notable weaknesses are the retrospective nature of data collection. Also, disappointingly, few patients (5–45%) underwent biopsies at gastroscopy [46].

The role of aeroallergens in EoE pathogenesis may foreseeably be as a cofactor in some or many patients, with food antigens playing a more dominant role. The ability of an elemental diet to induce complete histological remission in a majority supports this assertion. As many patients with EoE have coexistent atopic disease (especially seasonal rhinitis), it is also possible that seasonal worsening of that disease may contribute to EoE by facilitating secondary trafficking of eosinophils to the oesophagus. This has been demonstrated both in the research setting, where exposure of the bronchioles

to aeroallergens at the time of bronchoscopy can induce nasal mucosal eosinophilia, and clinically in relation to the control of asthma and rhinitis [47, 48]. The 'common airway' hypothesis asserts that control of rhinitis improves asthma therapy.

Geographical disparity in the prevalence of EoE has been suggested by a retrospective epidemiological study examining climatic regions in North America [49]. Arid/temperate zones manifested higher rates of diagnosis of EoE on endoscopic biopsy. Tropical regions manifested the fewer relative diagnoses of EoE, using total cases undergoing oesophageal biopsy as the reference [49]. The reason for a geographical disparity is not known, but could include variations in atmospheric pollen counts, which are higher in low humidity zones, and/or regions where putative tree or grass pollens are abundant. Alternatively, the role of serum vitamin D levels has been proposed as a protective factor in tropical environments in atopic conditions per se, using adrenaline auto-injectors or hospital admissions for anaphylaxis as a surrogate marker [50]. It is notable that conflicting data concerning the geographical influence on atopy exist; a recent Australian study demonstrated that distance away from the equator and, therefore, sunlight actually predisposes to atopy [51]. Much remains to be clarified.

The effects of migration on the development of EoE have not been explored. Migration between countries and climate zones has been proposed as a factor influencing the development and severity of other atopic conditions, particularly rhinitis. Asian immigrants to Melbourne, Australia were observed to develop asthma more commonly than Australian-born non-Asian and Australian-born Asian populations [52]. The length of stay positively correlated with the likelihood of symptom development. The new occurrence of rhinitis amongst children who had migrated to Italy also correlated with the length of time living in the new region. We have observed in our own patient group of adult patients a disproportionately large number who have migrated from overseas in young adulthood. The scientific validity of this observation cannot be asserted; however, it is conceivable that exposure to novel (to the migrant) region-specific aero or food allergens may precipitate the disease. Further study seems warranted.

Barrier function and microbiota

Impairments in the structural integrity of the oesophageal mucosal barrier may predispose to and/or perpetuate EoE. Research focussed on EoE directly is lacking, but much attention and importance have been placed on variations in epithelial barrier function in patients with atopic dermatitis. Filaggrin is a structural protein of critical importance in the development of dermatitis. In health, filaggrin is found in the stratum corneum

Established risk factors for EoE

Risk factor	Proposed Mechanism/s
Male gender	TSLP on sex chromosomes [29], Relaxin* [73]
Caucasian	Non-X linked SNP's, e.g. for Filaggrin, Eotaxin 3 [35]
Atopy	IgE-mediated inflammatory infiltration [25]

Putative risk factors for EoE

Risk factor	Proposed Mechanism/s
Impaired barrier function (may due to GORD, be genetic, e.g. filaggrin, or due to altered microbiota)	Increased antigen exposure to oesophageal mucosa [20]
Aeroallergens in spring/summer	Exposure of air passages leading to an inflammatory reaction and trafficking of eosinophils to the oesophagus [33]
Impaired tolerance to food antigens*	Immune reaction may be increased in infants not exposed to a wide range of foods, in those born by C section [74]
Commercially prepared foods*	Agglutinated proteins incite immune reaction [21]
Proton pump inhibitor use	Gastric pH is higher, and hence, proteins are not denatured and greater antigen exposure may results [59]
Migration as an adult*	Novel antigens incite immune reaction
Increased fibrotic remodelling	Decreased relaxin expression, SNP's for TGF B, SNP's for ACE [32, 66]
Living in a temperate or arid climate	Low vitamin D levels and/or higher aeroallergen exposures [75]

*Hypothesis generated from other related disease processes or anecdotal observation.

and binds keratin and intermediate filaments [11]. The gene for pro-filaggrin, which is subsequently phosphorylated to filaggrin, is located on chromosome 1. Specific mutations within this locus have been described to cause or greatly increase the risk of ichthyosis vulgaris and, of great relevance to EoE, atopic dermatitis [11]. As human skin is composed of stratified squamous keratinising epithelium, the importance of filaggrin in keratin attachment is apparent. However, the squamous epithelium of the oesophagus does not undergo keratinisation to a significant extent. Yet, both of these conditions, along with asthma, correlate with an SNP coding for filaggrin (R501X and 2282 del4), albeit to a lesser extent in the case of asthma and EoE [53, 54]. Blanchard demonstrated that whilst SNPs for filaggrin correlated with the development of EoE (independent of the risk of atopic dermatitis), that filaggrin expression measured by mRNA analysis was decreased in all patients, and was influenced by interleukin-13 production. Hence, it remains to be seen if the association between filaggrin and EoE is more than a confounding factor, simply demonstrating the coexistence of EoE with atopic dermatitis, or occurring as a secondary response to eosinophilic inflammation, or if instead there is a hitherto unknown role for filaggrin in the oesophagus that contributes to the pathogenesis of EoE. The evidence thus far suggests that filaggrin is not of central importance in EoE as immunohistochemical staining of the oesophagus has failed to reveal filaggrin expression [11].

Proteins aside from filaggrin, such as occludins, claudins and cadherins, may be important in EoE. Patients with atopic dermatitis have demonstrable decreases in the production of tight junction proteins, claudin-1 and claudin-23 [55]. Furthermore, single nucleotide polymorphisms in claudin-1 have been demonstrated in patients with AD, implying that decreased protein expression results in disease, rather than being a result of the dermatitis [55]. A deficiency of the cadherin desmoglein 1 may result in impaired barrier integrity in patients with EoE, and the expression of desmoglein 1 is influenced by interleukin 13, that is, in turn commonly elevated in this patient group [56]. Patients with connective tissue disease have been noted to have an eightfold increase in prevalence of EoE, the cause unknown, but conceivably could relate to barrier function or may relate to allergic inflammation (see TGF-β). It is interesting to note that disruption of tight junction proteins is thought to contribute to the increase in size of intracellular spaces in patients with GERD and that treating patients with EoE using proton pump inhibitors improves the histology in many patients and is considered a first-line therapy [57, 58]. It could be hypothesised that GERD may cause or precipitate EoE [59]. Abnormalities in tight junction protein and hence barrier function warrant further study.

Microbiota of the oesophagus and possible effects on barrier function and immune tolerance may be important in the pathogenesis of EoE. Limited studies in patients with GERD, Barrett's oesophagus and oesophageal adenocarcinoma have reported a decrease in the microbial diversity in these diseases, with a propensity to be colonised with *Clostridium consisus* in one study [60, 61]. It is not known if the difference in microbial diversity represents a causal role for some bacteria, or rather a secondary change to altered local conditions. Little is known about the microbial profile in patients with EoE.

Microbiota of the large intestine (and presumably the oesophagus) is established in the days and weeks following delivery of the infant and may influence the development of food allergy [62]. Children born via caesarean section and those administered probiotics in the first 6 months of life may have a lower rate of food allergy [17]. Bacterial colonisation of the skin is important in atopic dermatitis, with different populations in those with the condition compared with healthy controls and demonstrable improvement with antibiotics in some patients [63]. Defensins are proteins that a secreted at the mucosal surface and play a role in maintaining microbial homoeostasis, being considered part of the innate immune system. It has been noted that patients with atopic dermatitis and, more recently, those with EoE have a decreased expression of defensins (using techniques *in vitro*) [64]. Further, studies *in vivo* appear warranted.

Are all cases of EoE the same? – Fibrosis and stricture formation

A key question in managing any chronic illness is prognostic evaluation, counselling and (potentially) appropriate management selection. A spectrum of clinical, endoscopic and histological pictures emerges across patients with the condition, including those with recurrent, frequent food bolus obstruction, those with severe oesophageal narrowing limiting scope passage and those with variable eosinophil counts or lamina propria thickness at biopsy [15, 65]. To date, neither the endoscopic appearance nor the eosinophil counts have been predictive of symptom severity or response to therapy [66, 67]. Notably, the diagnostic validity of endoscopic visualisation alone (in the absence of biopsy) in diagnosis is poor. Furthermore, the absolute eosinophil count does not correlate with symptom severity as measured by questionnaire [1]. Schoepfer in a retrospective study of 200 patients demonstrated increased stricture formation in those patients whose diagnosis was delayed; for example, 17% had strictures that were diagnosed within 2 years of the onset of symptoms, compared with > 60% when the diagnosis was delayed > 14 years [69]. Hence, it is apparent that diagnosis (and presumably treatment) alters the natural history of the condition.

Moreover, a mandate to treat patients not only to alleviate symptoms but also to avoid future pathological oesophageal narrowing is present. Current first-line treatments for EoE include swallowed corticosteroids (dry powder or gel) as well as dietary therapy. Short-term prospective studies have shown that both may result in a complete or near complete disappearance of eosinophils and reduction in epithelial as well as lamina propria thickness [1, 24, 36]. It is logical to assume that maintenance of these therapies will alleviate symptoms and minimise stricture formation long term, although data are not yet forthcoming.

The search for prognostic variables that will predict severe progression with stricture formation or response to treatment is a worthy research goal. Aceves et al. demonstrated that oesophageal remodelling in EoE was reversed with topical steroids in some patients and that a SNP coding for TGF-β (19q13) predicted treatment response in this group [9, 70]. It has also been observed that TGF-β receptor (9q 22) mutations occur in the childhood-onset Loeys Dietz syndrome (characterised by high IgE levels, multiple atopic conditions and eosinophilia including the gastrointestinal tract) suggesting that mutations in this receptor may strongly predispose to human atopic conditions, and demonstrating the complexity of the relationship between allergic inflammation and mediators thought to promote fibrosis [71]. Furthermore, Abonia et al. [68] demonstrated TGF-β expression by mast cells and eosinophils again highlighting this complexity. It is conceivable that such an approach correlating genetic alterations in terms of key pathological processes of inflammation and fibrosis and response to therapy may result in improved understanding and therapeutic advances. Polymorphisms in the angiotensin converting enzyme (ACE) gene (17q 22.3 – more than a dozen mutations noted), already implicated in cardiovascular disease and pulmonary fibrosis, are one such example [72]. Already, human trials on the angiotensin 2 receptor antagonist (losartan) in the treatment of EoE have commenced.

Conclusion

EoE is disease that presents with dysphagia and/or food bolus obstruction events. Gender (male), race (Caucasian) and atopy confer increased risk. The elemental diet and to a lesser extent the six-food elimination diet result in complete remission in many, implying that food antigens are causative. The timing and nature of food antigen exposure may be important in inducing or reversing immune tolerance and may explain the apparent increasing incidence of the condition. Aeroallergens may play a role. Oesophageal barrier function, microbiota and the clarification of factors influencing fibrous remodelling appear important areas for future study and understanding of this newly recognised condition.

Conflict of interest

The authors declare no conflict of interest.

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APPENDIX 2

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Eosinophilic esophagitis: A clinicopathological review

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ABSTRACT

Eosinophilic esophagitis (EoE) is considered to be a chronic antigen-driven disease whereby food and/or aeroallergens induce a chronic inflammatory infiltrate in the esophagus, resulting in pathological hyperplasia of the epithelia and muscular layers, and fibrosis of the lamina propria (referred to collectively as remodelling) and the symptoms of dysphagia and food impaction. EoE shares features with other atopic conditions of asthma and atopic dermatitis, such as a TH2 cytokine milieu and a mixed inflammatory infiltrate of eosinophils, mast cells and lymphocytes. Relatively distinct features include the strong male predominance amongst adult patients, and the expression of the eosinophil chemokine eotaxin 3. Current first line treatments such as strict dietary modification and corticosteroids fail many patients. Looking forward, clarification of distinct genotype/phenotype associations, determining the reversibility of remodelling following treatment, and the development of new pharmacotherapies that target fibrotic pathways (as opposed to eosinophilic inflammation per se) or specifically improve barrier integrity appear relevant.

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Contents

1. Introduction

Eosinophilic esophagitis (EoE) presents in adults as dysphagia and food impaction. The pathophysiological correlates of these symptoms are thought to comprise (1) acute narrowing of the esophageal lumen by inflammation and oedema, (2) fixed narrowing and limited distensibility of the lumen by remodelling and (3) dynamic and variable narrowing caused by muscular contraction or spasm (Fontillon & Lucendo, 2012; Liacouras et al., 1998; Read & Pandolfino, 2012). The relative contribution of these three pathological processes to the clinical syndrome is not known, although the focus of research and treatment relates to remodelling. (See Figs. 1 and 2) (See Tables 1 and 2.)

Eosinophilic esophagitis (EoE) is considered to be a chronic antigen-driven disease, whereby food and/or aeroallergens induce an eosinophilic infiltration in the esophagus (Mulder & Justinich, 2011). Remodelling refers to the structural changes caused by acute and chronic inflammation, namely epithelial hyperplasia, fibrosis of the lamina propria and muscular hypertrophy (smooth and longitudinal) of the esophagus resulting from an inflammatory infiltrate typical of a TH2-mediated milieu (Cheng et al., 2012). The mechanism of injury has been demonstrated using

Abbreviations: EoE, eosinophilic esophagitis; TSLP, thymic stromal lymphopoietin; IL (e.g. IL-3), interleukin; AD, atopic dermatitis; VCAM-1, vascular cell adhesion protein 1; APC, antigen presenting cell; MBP, major basic protein; TGF-β, transforming growth factor beta; ICAM, intracellular adhesion molecule; IFN, interferon; VEGF, vascular endothelial growth factor; EMT, epithelial mesenchymal transition.
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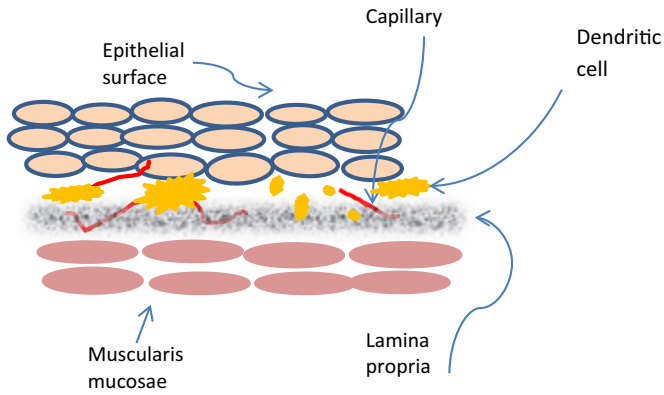
Fig. 1. Normal esophagus. Esophageal barrier function is maintained by an orderly arrangement of epithelial cells maintained by gap junction proteins. The muscularis is striated (upper 1/3 of the esophagus) and smooth (lower 2/3) of the esophagus. Antigen presentation may occur by dendritic cells or possibly epithelial cells.

animal models and in vivo human studies (before and after disease-modifying treatments) and may be conceptualised to involve cells (e.g., eosinophils, mast cells, epithelial cells and fibroblasts) cytokines (e.g., interleukins IL-4,5 and 13, and the chemokine eotaxin 3) and adhesion molecules (e.g., integrins and vascular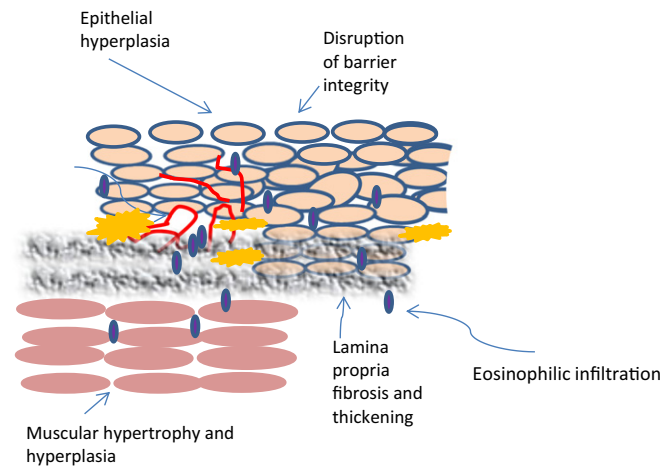 cell adhesion protein 1 (VCAM-1))(Akei et al., 2005; Liacouras et al., 2011). The precise sequence of events and the dominant cellular signalling or adhesional molecules involved are not yet fully elucidated. Importantly, however, treatments such as topical corticosteroids and dietary modification may at least partially reverse the pathological changes in a significant number of patients, with resultant improvements in swallowing reported in some studies (Aceves et al., 2010; Lieberman et al., 2012). Other atopic conditions, such as asthma and atopic dermatitis (AD) manifest tissue remodelling, again typified by cellular infiltration and fibrosis, and the significant body of research in these fields provides valuable potential clues to the pathogenesis of EoE, and may direct future research (Boguniewicz & Leung, 2011; Royce et al., 2012). The role of epithelial barrier function, epithelial defence, and repair and bacterial colonisation (both crucial in the pathogenesis of AD) are neglected areas of research. The genetic and racial predilection of EoE as a disease predominantly

Fig. 2. Eosinophilic esophagitis. Epithelial barrier integrity is disrupted, allowing greater contact between antigens and dendritic cells. The epithelial layer is thickened and disorderly, and an inflammatory infiltrate rich in eosinophils extends throughout all layers, and may contribute to dysmotility. Angiogenesis is present; the esophagus is friable and bleeds easily at endoscopy. The lamina propria is thickened and fibrotic. The clinical sequelae of the pathological changes are dysphagia and food bolus obstruction due to luminal narrowing, limited distensibility and disturbance of peristalsis.

Fig. 3. There are significant shortcomings in the current treatment options.

of white male Caucasians also deserves careful consideration (Spergel et al., 2009).

The natural history of EoE in the era of disease-modifying treatments (elimination diets and corticosteroids), and the clinicopathological correlations between remodelling, acute inflammation and esophageal dysmotility remain to be determined (Dellon et al., 2013; Gonsalves et al., 2012). The clinical and research tools that determine symptoms (such as dysphagia scores) and esophageal function (manometry) and structure (endoscopic biopsy) have limitations. Improvements in technical utilisation (such as measuring esophageal distensibility instead

Fig. 4. The 6 food elimination diet.

Table 1
Pharmacological treatments for EoE.

Agent	Pharmacological targets	Evidence Base	Efficacy
Corticosteroids	Multiple	Randomised controlled trial (Straumann et al., 2010)	50–90% response
Monteleukast	Leukotriene receptor	Open label trial (Attwood et al., 2003)	Ineffective
Sodium cromoglycate	Mast cell	Case report (Spergel et al., 2009)	Ineffective
Thiopurines (azathioprine)		Case report (Netzer et al., 2007)	Effective
Mepolizumab	IL-5	Randomised controlled trial (Straumann et al., 2010)	Ineffective
Reslizumab	IL-5	Randomised controlled trial (Walsh, 2013)	Ineffective
Omalizumab	IgE	Randomised controlled trial (Rocha et al., 2011)	Ineffective
Infliximab	TNF	Case report (99)	Ineffective
0C00459	CRTH2 receptor	Randomised controlled trial (Straumann et al., 2013)	Ineffective
Losartan	AT-2 receptor	Under study	?

of contractility and obtaining deeper endoscopic specimens including the lamina propria and muscularis) hold considerable promise (Lucendo et al., 2011; Read & Pandolfino, 2012). Clarifying the relationship between mucosal inflammation, esophageal motility, esophageal distensibility and the symptoms of dysphagia and food bolus obstruction will enable useful treatment end-points to be determined and potentially drive therapeutic innovation.

2. Antigen presentation

EoE is viewed as an antigen-driven disease. The striking success of dietary therapy (up to 65% of patients improve on a 6 food elimination diet and 95% improve on an elemental diet) suggests that direct contact with the esophageal mucosa leads to antigen presentation and a localised inflammatory infiltration (Gonsalves et al., 2012; Lieberman et al., 2012). It is also possible that the exposure of the small bowel, which is rich in lymphoid follicles and is immunologically active, may lead to immune activation and subsequent migration of the eosinophils to the esophagus. This is the implication of a recent study, that demonstrated increased intestinal permeability in patients with EoE, that was reversible with treatment (using diet or corticosteroids) (Katzka et al., 2014). Another hypothesis, suggested by the observation that there is a seasonal peak of patients presenting with clinical symptoms of EoE (correlating with high aeroallergen levels in the atmosphere) is that distant contact with the respiratory epithelium of the nose or airways leads to trafficking of eosinophils to the esophagus (Moawad et al., 2010). This hypothesis is supported directly by a murine model, in which antigenic exposure of the nasal and not the esophageal mucosa leads to esophageal infiltration with eosinophils in ovalbumin sensitised mice, and indirectly by human studies of asthma and rhinitis, where stimulation of the nasal or distal airway mucosa leads to an infiltration in the opposing mucosal surface respectively (Braunstahl et al., 2001; Mishra et al., 2001).

If it is assumed that direct exposure of the esophageal epithelial surface to ingested food leads to the eosinophilic infiltration, the question remains as to how the inflammatory cascade then proceeds. The esophageal mucosa is stratified squamous and partially-keratinising in type, whereby up to 30 layers of epithelium separate the luminal contents and, therefore, potential food antigens from the lamina propria, where mast cells and (transiently) eosinophils may reside. There is only

secretion of mucus from submucosal glands in the lower esophagus. This contrasts with the airway epithelium, where a layer of ciliated epithelial cells are interposed with goblet cells that secrete mucus and thereby potentially trap antigen. It is hence apparent that, in health, the physical interaction of the food antigen with inflammatory cells such as mast cells residing in the lamina propria will be limited. Several alternative mechanisms may facilitate the interaction of antigen with inflammatory cells, including the potential of the esophageal epithelium and/or the eosinophils themselves to function as antigen presenting cell (APCs) (Akuthota et al., 2010; Mulder et al., 2011). It is interesting to note that the same physical barrier – multiple layers, lack of mucus to trap antigen – exists in the skin, yet the exposure to aeroallergens such as dust mite is thought to play a role in the pathogenesis of atopic dermatitis (AD dendritic cells, lying within the epithelial layers present antigen in the skin of patients with AD), potentially explaining the immune activation in spite of the barrier function mentioned (Boguniewicz & Leung, 2011). Dendritic cells that are present in the lamina propria of the normal esophagus, are found in increased numbers in patients with Barretts esophagus and esophageal adenocarcinoma but are not found in increased number in patients with EoE, suggesting a role for non-professional APCs such as epithelial cells (Lucendo et al., 2007). Abnormalities in the barrier function of the skin have been proposed as factors in the pathogenesis of AD, both predisposing and perpetuating the condition by enabling increased antigen exposure (Boguniewicz & Leung, 2011). The barrier function of the esophagus is discussed below.

3. Inflammatory cell infiltration

3.1. Eosinophils

Eosinophils define EoE, in name, diagnosis (>15 eosinophils per high power field following endoscopic biopsy is required to confirm the condition) and response to treatment (Odze, 2012). Furthermore, eosinophils are key players in the process of remodelling. The normal esophagus does not contain eosinophils, although a non-specific eosinophilic inflammatory reaction may occur, for example, in patients with gastroesophageal reflux disease (GORD) and viral esophagitis (Odze, 2012). Eosinophils are derived from myeloid precursors in the bone marrow and mature in response to IL-5, subsequently circulating in

Table 2
Potential future pharmacotherapies for EoE.

Agent	Pharmacological targets	Evidence base
Anti TSLP antibody	TSLP or TSLP receptor	Monkey (Cheng et al., 2013)
Siglec 8	Siglec receptor	Mouse (Kiwamoto et al., 2012)
Relaxin	Relaxin receptor	Mouse (Royce et al., 2009)
Sphingosine kinase	Sphingosine kinase	Mouse (Price et al., 2013)
Antibiotics	Multiple	Human studies of atopic dermatitis — RCT (Huang et al., 2009)
Methotrexate	Multiple	Human studies of atopic dermatitis — case series (Weatherhead et al., 2007)
Calcineurin inhibitors	IL-1, multiple	Human studies of atopic dermatitis — RCT (Reitamo et al., 2000)

the blood for up to 20 h and residing in the tissues for between 2–14 days. Eosinophils remain increased in number in the esophagus of patients with EoE (who are untreated), but the absolute density or numbers may fluctuate over time (Mishra, 2009).

Cytokines and chemokines drive the migration of eosinophils into the esophagus. Cytokines such as IL-5, IL-9 and IL-13 and the chemokines, eotaxins 1, 2, and 3, are of central importance, as determined by elevated circulating levels and mRNA expression of esophageal tissue in human patients with EoE, and as demonstrated in murine knockout models (see below)(Blanchard et al., 2007; Konikoff et al., 2006; Niranjan et al., 2013). Studies in vitro of human lung and endobronchiolar tissues suggest a role for vascular adhesion mediated by VCAM-1 and P-selectin on the endothelial surface along with P-selectin glycoprotein and very late protein-4 on eosinophils in facilitating eosinophil attachment and migration into tissues, a process governed by integrins (Aceves et al., 2007).

Once in the esophagus, eosinophils may reside in the intraepithelial spaces where they can form microabscesses when clustered, in the lamina propria and, in some cases, muscular layers although the prevalence of the last two locations is difficult to determine given the limited sampling capability of standard endoscopic biopsies (Lucendo et al., 2011; Odze, 2009). Eosinophils release a range of mediators stored in secondary granules. These include major basic protein (MBP), eosinophil peroxidase, eosinophil cationic protein and eosinophil-derived neurotoxin the release of which causes local tissue damage and esophageal dysmotility, and may secondarily activate mast cells (Kephart et al., 2010; Mavi et al., 2012; Mueller et al., 2006). MBP comprises a major component by volume of the secondary granules and putatively plays a significant role in causing fibrotic remodelling of the esophagus (Cheng et al., 2012; Rothenberg, 2009). MBP may act on esophageal epithelial cells, leading to production of fibroblast growth factor-9 (FGF-9) that in turn promotes epithelial hyperplasia (Mulder et al., 2009). Transforming growth factor-β (TGF-β) is also produced by eosinophils. This growth factor promotes activation of quiescent fibroblasts to myofibroblasts and, in turn, the production of fibrotic tissue in the lamina propria (Abonia et al., 2010). TGF-β also may contribute to smooth muscle contraction, hyperplasia and hypertrophy.

Eosinophils are both attracted and activated by cytokines and interleukins secreted by other cell types. They are capable of cytokine secretion themselves, which influences the inflammatory process, as demonstrated by studies in vitro of human cells (Hogan & Rothenberg, 2006). Recently, the importance of IL-9 production by eosinophils in patients with EoE and of the ability of this cytokine to attract mast cells were demonstrated (Otani et al., 2013). Further clarification of the role of eosinophils in EoE appears warranted. Eosinophils have, for instance been demonstrated in vivo to have an immunoregulatory role, eosinophil granule proteins decreasing the proliferation of lymphocytes (using donor eosinophils from healthy controls) and have also been shown to influence T cells, skewing development toward a Th 2-like profile (Odemuyiwa et al., 2004; Peterson et al., 1986).

It is apparent that eosinophils contribute to the key pathological processes of tissue remodelling, namely epithelial hyperplasia, subepithelial fibrosis, and muscular hypertrophy and hyperplasia. Eosinophil density may be decreased by administering corticosteroids or instituting dietary therapy, and this reduction has been found in some studies to correlate with a reduction in remodelling or a return to a more histologically normal esophagus (Aceves, Newbury, et al., 2010; Lieberman et al., 2012). Determining if the eosinophil count following endoscopic biopsy is a reliable marker of successful treatment and correlates closely with the reversal of pathological remodelling and symptom resolution needs clarification.

3.2. Mast cells

Mast cells (MCs) are found in small numbers in the normal esophagus, residing only in the lamina propria (Odze, 2012). In EoE, increased density of MCs is found, both in the connective tissues and also within the intraepithelial and muscular layers (Fontillon & Lucendo, 2012). These cells are derived from CD34[+] progenitors in the bone marrow, but mature in the tissues and do not circulate in the bloodstream (E. Cheng et al., 2012).

Mast cells are classically associated with the type 1 hypersensitivity reaction, whereby an antigen comes into contact with specific IgE bound to mast cells leading to activation, degranulation and release of a range of mediators such as histamine, eicosanoids and cytokines (Lucendo et al., 2009). Mast cells bearing IgE have been demonstrated in the esophagus of patients with EoE in a human study, as well as in animal models of disease (Konikoff et al., 2006; Vicario et al., 2010). The evidence that mast cells are important in disease pathogenesis is furthered by the correlation between successful treatment with corticosteroid therapy, dietary therapy and mast cell density (Konikoff et al., 2006; Peterson et al., 2013). Some have suggested that 'mastocytic esophagitis' would be a better term for EoE, given the relatively greater specificity of histological techniques such as mast cell tryptase in determining the diagnosis of EoE, as compared to eosinophil density, which is non-specific (Abonia et al., 2010). Mast cells may modulate remodelling via the production of TGF-β, which in turn governs connective tissue production and also possibly smooth muscle contractility (Aceves et al., 2010). Importantly, mast cells, but not eosinophils, may cause smooth muscle spasm (Aceves et al., 2010). In models of asthma, mast cells may release TGF-β and, in turn, increase the expression of adhesion molecules, intercellular adhesion molecule (1CAM) and VCAM (Chai et al., 2011). The role of mast cells in inflammation, remodelling and esophageal dysmotility is an area worthy of further research.

3.3. B-lymphocytes and IgE antibodies

B cells, identified by staining for CD-20 (human B cell lymphocyte restricted differentiation antigen), are found in the esophageal mucosa of patients with EoE. IgE is also demonstrable, suggesting, along with the observation that skin prick tests and serum-specific IgE to food and/or aeroallergens are frequently positive, that class switching to IgE antibody production by B cells occurs in response to TH2 cytokines (Vicario et al., 2010). Furthermore, a growing body of literature suggests that the removal of the putative food allergens by, for example, elemental or six-food elimination diets, reverses the eosinophilic infiltration and remodelling in these patients (Gonsalves et al., 2012; Lieberman et al., 2012; Spergel et al., 2012).

Whilst EoE is considered an antigen-driven disease, the significance of IgE antibodies in the pathogenesis is debatable. The variably reported positive and negative predictive values of skin prick and patch tests, and antigen-specific IgE antibodies in determining a response to food elimination diets, and the heterogeneity of the responses to food or environmental allergens despite a florid infiltrate of eosinophils, hamper a definitive assertion to this regard (Gonsalves et al., 2012; Lieberman et al., 2012; Spergel et al., 2002). Binding and removing IgE from the circulation with the monoclonal antibody, omalizumab, may greatly decrease IgE levels (Steiss et al., 2012) which has had limited success in EoE (Rocha et al., 2011). The failure of this medication to deliver universally positive results also supports the suggestion that a TH2-mediated cytokine production, rather that B cell antibody production is of greater importance in the pathogenesis of EoE.

3.4. T lymphocytes

Patients with EoE have an increased density of T cells (CD8[+], CD4+ and CD3[+]) compared to the normal esophagus (Rajavelu et al., 2012). The assertion that EoE is a TH2-mediated disease is upheld by the finding that the cytokines, IL-4, IL-5 and IL-13, are produced in greater quantities by monocytes following antigen exposures in the peripheral blood of patients with EoE compared to healthy controls, and that mRNA for these cytokines is overexpressed in esophageal biopsies. Furthermore,

the mRNA levels decrease following corticosteroid therapy (Blanchard et al., 2007; Yamazaki et al., 2006). It is interesting to note that TH-1 cytokines, TNF-α and IFN-γ are also increased following antigen exposure of monocytes and are found in increased quantities in esophageal mucosal biopsies, and that skin patch tests (generally considered a measure of cellular or delayed hypersensitivity) are often positive to common food and aeroallergens (Spergel et al., 2002; Yamazaki et al., 2006).

The cytokines IL-4, IL-5, IL-13 and possibly IL-9 are produced by TH2 and TH-9 cells, and drive the eosinophilic and mastocytic infiltrates characteristic of EoE (Otani et al., 2013). It has been proposed that the expression of eotaxin-3 by the esophageal epithelium, VEGF by the endothelium and integrins by the interstitium attracts these cells to the esophagus, whereby activation and degranulation occur, modulating local tissue damage by MBP, histamine and other mediators (Cheng et al., 2012; Rothenberg, 2009). Furthermore, growth factors such as TFG-β and FGF-9 (fibroblast growth factor 9) are released by eosinophils and mast cells that activate quiescent fibroblasts to myofibroblasts, and drive hyperplasia of epithelium and smooth muscle that complete the cycle of remodelling (Fuentebella et al., 2010). It can hence be appreciated that T helper cells are central to the pathogenesis of EoE and the resultant esophageal remodelling. Accordingly, murine T helper deficient mice do not develop EoE (Rajavelu et al., 2012).

Given the role of T helper cells in EoE, some focus of research has been directed at T-regulatory cells that express immunomodulatory cytokines such as IL-2 and IL-10. A small study of paediatric patients with EoE or GORD revealed an increase number of T regulatory cells compared with those of control patients, but no difference between those with different forms of esophageal inflammation (Fuentebella et al., 2010). Murine models have suggested a reduction of regulatory T cells, and a reduction in the esophageal eosinophilia with the infusion of T regulatory cells. Further research appears warranted in humans (Tantibhaedhyangkul et al., 2009).

3.5. Basophils and the TSLP basophil axis (see also gender differences)

An exciting recent hypothesis, supported by a murine model and a single human study is that basophils and the epithelial cytokine thymic stromal lymphopoietin (TSLP) are integral to the development of EoE, and that disease development can occur in the absence of IgE and IL-5. Noti et al., in a landmark paper demonstrated that mice that were sensitised to ovalbumin and then re-exposed would develop changes representative of EoE, whereas mice that were treated in addition with antibodies to basophils and TSLP respectively did not develop esophageal eosinophilia (Noti et al., 2013). Remarkably, mice did develop disease even when IgE antibodies were administered, strongly supporting a non-IgE mediated, TSLP/basophil mediated pathogenesis. A further human study demonstrated increased expression of TSLP (immunohistochemistry of esophageal biopsies) and cells resembling activated basophils in these biopsy specimens (flow cytometry)(Noti et al., 2013). This alternative disease model would appear to offer great promise in understanding and potentially treating at least a subset of patients with EoE in the future, with monoclonal antibodies against TSLP representing one possible option (Kim et al., 2013).

4. Remodelling

4.1. Epithelial cells

The esophagus is lined by squamous partially keratinised epithelium. Patients with EoE develop epithelial hyperplasia, possibly in response to MBP and TGF-β produced by eosinophils. A complex positive feedback loop has been proposed to explain the recruitment and maintenance of eosinophilic and mastocytic infiltration and epithelial hyperplasia (Abonia et al., 2010). Eotaxin 3 is produced by esophageal epithelium in response to IL-13, which in turn attracts eosinophils expressing the CCR-3 (eotaxin 3) receptor, promoting the remodelling

described. Finally, it has been proposed that mast cells may produce IL-9 in turn causing increased IL-13 production by TH2 cells, thus completing the loop (see Figs. 1 and 2) (Abonia et al., 2010). Eotaxin 3 appears to be of central importance in the pathogenesis of EoE, with histological and mRNA studies of biopsy specimens demonstrating a specificity of this chemokine in patients with EoE compared to those with GERD, and some (but not all) studies demonstrating a correlation between disease activity, successful treatment and eotaxin 3 levels (Bhattacharya et al., 2007; Cheng et al., 2012). Furthermore, a single nucleotide polymorphism in the gene encoding eotaxin 3 has been demonstrated in some patients with EoE (Abdulnour-Nakhoul et al., 2013; Spergel, 2010).

As well as participating in the process of inflammation and remodelling characteristic of EoE, it is possible that inherited or acquired defects in esophageal epithelial barrier function may contribute to the development and/or perpetuation of EoE. Filaggrin is a structural protein of critical importance in the development of dermatitis. In health, filaggrin is found in the stratum corneum and binds keratin and intermediate filaments (De Benedetto et al., 2008). The gene for profilaggrin, which is subsequently phosphorylated to filaggrin, is located on chromosome 1. Specific mutations within this locus have been described to cause or greatly increase the risk of icthyosis vulgaris and, of great relevance to EoE, atopic dermatitis (De Benedetto et al., 2008). Since human skin is composed of stratified squamous keratinising epithelium, the importance of filaggrin in keratin attachment is apparent. However, the squamous epithelium of the esophagus doesn't undergo keratinisation to a significant extent. Yet both of these conditions, along with asthma correlate with the filaggrin genetic loci albeit to a lesser extent in the case of asthma and EoE (Boguniewicz & Leung, 2011). It remains to be seen if the association between filaggrin and EoE is more than a confounding factor, simply demonstrating the co-existence of EoE with atopic dermatitis, or if instead there is a hitherto unknown role for filaggrin in the esophagus that contributes to the pathogenesis of EoE. The evidence thus far suggests that filaggrin is not of central importance in EoE, as immunohistochemical staining of the esophagus failed to reveal filaggrin expression (De Benedetto et al., 2008).

Proteins aside from filaggrin, such as occludins and claudins, may be important in EoE. Patients with atopic dermatitis have demonstrable decreases in the production of tight junction proteins, claudin-1 and claudin-23(De Benedetto et al., 2011). Furthermore single nucleotide polymorphisms in claudin-1 have been demonstrated in patients with AD, implying that decreased protein expression results in disease, rather than being a result of the dermatitis (De Benedetto et al., 2011). It is interesting to note that disruption of tight junction proteins is thought to contribute to the increase in size of intracellular spaces in patients with GERD, and that treating patients with EoE using proton pump inhibitors improves the histology in many patients, and is considered a first line therapy (Molina-Infante et al., 2011; Tobey et al., 1996). Abnormalities in tight junction protein and hence barrier function may precipitate and perpetuate EoE and warrant further study.

4.2. Esophageal muscle

The esophageal muscle layer is predominantly striated in the cervical esophagus (the upper third), a mixture of striated and smooth muscle in the middle third and smooth muscle alone in the lower third. The muscle layers themselves are oriented in a circular (inner) and longitudinal (outer) fashion (Mittal, 2013).

The inflammatory infiltrate of EoE that involves the muscular layer includes both mast cells and eosinophils, the former predominating in one study (Aceves et al., 2010). The availability of muscle tissue for study has hampered research as standard gastroscopy forceps will sample muscle tissue in <20% (Lieberman et al., 2012). It is possible that both structural alterations (myocyte hypertrophy and hyperplasia along with inflammatory infiltration) and dynamic changes (muscular contraction) contribute to the clinical syndrome of dysphagia and food

bolus obstruction. Mediators released from mast cells, including histamine, have the ability to cause muscle contraction and hyperplasia according to animal studies and one study of humans, and a correlation between the number of mast cells and the expression of TGF-β in the esophageal muscle layer has been demonstrated (Aceves et al., 2010), suggesting a role for mast cells in driving remodelling. Future studies aiming for systematic sampling of the muscular layer across a range of patients with variable disease severity seem necessary. It is yet to be determined, for example, whether the refractory dysphagia typical of some patients with EoE represents ongoing inflammation in the muscle layer or muscular dysmotility, or simply subepithelial fibrosis.

4.3. Epithelial mesenchymal transition

Epithelial mesenchymal transition (EMT) refers to the process whereby epithelial cells may lose their typical histological and immunohistochemical appearance, and functional properties to instead acquire the structure and function of mesenchymal cells, such as motility (instead of adherent tight junctions) and depolarised cytoskeletal arrangements (vimentin instead of cytokeratin in epithelial cells)(Kagalwalla et al., 2012). Myofibroblasts, the quintessential mesenchymal cells characteristic of the remodelling process in asthma, can both synthesise extracellular matrix such as collagen and express alpha-smooth muscle actin (αSMA), possessing contractile properties relevant to airway narrowing (Doerner & Zuraw, 2009). Furthermore myofibroblasts may differentiate to smooth muscle cells and contribute to the muscle thickening typical of chronic asthma (Doerner & Zuraw, 2009).

The same process of EMT, and the resultant fibrosis and smooth muscle hyperplasia observed in asthma may occur in EoE (Kagalwalla et al., 2012). Histological as well as sonographic endoscopic assessments demonstrate thickening of the lamina propria and esophageal muscular layer (Stevoff et al., 2001). Immunohistochemistry and mRNA of tissue in studies of patients with EoE pre- and post-treatment with corticosteroids demonstrate reversible EMT as defined by expression of mRNA or cell surface protein of cytokeratin or vimentin (Kagalwalla et al., 2012). These changes correlated with a reduction in eosinophil number and immunohistochemical stains for TGF-β (Kagalwalla et al., 2012). It is hence apparent that the EMT of EoE is an important step in the remodelling of EoE, and that this area warrants further study in line with asthma research (Muir et al., 2013).

4.4. Production of extracellular matrix (ECM) — the process of subepithelial fibrosis

Subepithelial fibrosis is characterised by an increase in the thickness and density of collagen bundles, and an increase in fibroblast density (E. Cheng et al., 2012). Several studies have employed a three-point scoring system to denote the severity of fibrosis according to these three indices (Lieberman et al., 2012; Lucendo et al., 2011). The production of the ECM by myofibroblasts that have been activated by TGFβ secreted by a range of cells (e.g., eosinophils, mast cells, epithelial cells) leads to SMAD-dependent signalling, upregulating fibrogenic genes such as collagen, alpha-SMA and periostin (Bhattacharya et al., 2007). Indeed mRNA studies have found that patients with EoE express SMAD-2 and -3 and periostin at high levels in the esophageal tissues compared to patients with GERD (Aceves et al., 2007). Subepithelial fibrosis is dynamic and does respond in some patients who receive either dietary restriction (the six-food elimination diet or the elemental diet) or corticosteroid therapy (Abu-Sultaneh et al., 2011; Gonsalves et al., 2012; Lieberman et al., 2012; Spergel et al., 2012). The reversal of the fibrous remodelling appears to correlate with the disappearance of eosinophils (Abu-Sultaneh et al., 2011). Determining which patients will respond to therapy remains a research question worthy of consideration. Periostin is traditionally viewed as a cell adhesion molecule regulating extracellular matrix deposition. However, considerable research focus on its role in mediating a range of biological processes involved in the fibrous

remodelling of EoE, such as binding and facilitating cross-linking of collagen, potentially functioning to increase eosinophil adhesion to integrins and inducing epithelial mesenchymal transition, is ongoing (Sherrill & Rothenberg, 2011). Intriguingly, the upregulation of periostin is only partially reversed by corticosteroid administration, suggesting a causative role for this protein in EoE, and reinforcing the potential importance in disease pathogenesis (Sherrill & Rothenberg, 2011).

4.5. Angiogenesis

Angiogenesis, the formation and development of new blood vessels, is a feature of eosinophilic esophagitis. The angiogenic factor vascular endothelial growth factor alpha (VEGF-A), angiogenin and IL-8 have been implicated in promoting this pathological process, and it is notable that eosinophil-depleted mice have decreased angiogenesis (Persad et al., 2012; Rubinstein et al., 2011). The abnormal vascularisation of the diseased esophagus typical of EoE may contribute to the friability and propensity to bleed at the time of endoscopy, and circulating inflammatory cells including eosinophils would have ready access to the site due to the angiogenesis, potentially perpetuating the disease process. A decrease in VEGF has been demonstrated following treatment with corticosteroids and dietary therapy respectively (Lieberman et al., 2012; Lucendo et al., 2011). It is interesting that Siglec F, sialic acid immunoglobulin-like lectin, a protein that is highly expressed on eosinophils, may facilitate eosinophil adhesion and contribute to angiogenesis according to a murine model. Anti-Siglec F was shown in a mouse model to reverse remodelling, eosinophilia and angiogenesis, raising the possibility of the use of this agent as a novel therapy in EoE (Rubinstein et al., 2011).

4.6. Microbiota

Microbiota of the esophagus and possible effects on barrier function may be important in the pathogenesis of EoE. Limited studies in patients with GERD, Barrett's esophagus and esophageal adenocarcinoma (Blackett et al., 2013) have reported a decrease in the microbial diversity in these diseases, with a propensity to be colonised with *Clostridium consisus* in one study (Fillon et al., 2012). It is not known if the difference in microbial diversity represents a causal role for some bacteria, or rather a secondary change to altered local condition. Little is known about the microbial profile in patients with EoE. This is arguably an important area given the ability of skin infection to modulate the severity of atopic dermatitis and for antibiotics to improve the condition. Defensins are proteins that a secreted at the mucosal surface and play a role in maintaining microbial homeostasis, being considered part of the innate immune system. It has been noted that patients with atopic dermatitis and, more recently, those with EoE have a decreased expression of defensins (using techniques in vitro) (Schroeder et al., 2013). Further, studies in vivo appear warranted.

4.7. Gender differences

There is a male predominance of EoE with a male:female ratio of 3 or 4:1, but this remains unexplained. This differs from asthma where a bimodal gender distribution pattern occurs where asthma is more common in young male children, but adult females are more likely to suffer from asthma (DunnGalvin et al., 2006; Furuta, 2011). Interestingly sensitisation to common food and environmental allergens as determined by skin prick or serum-specific IgE tests shows no sex difference (DunnGalvin et al., 2006). It could be hypothesised that a further factor may then modulate the response to allergen exposure.

TSLP is a protein product mainly of epithelial cells that closely resembles IL-7 in structure and function, and promotes a characteristic TH2-mediated milieu via the activation of dendritic cells in AD and asthma (Zhang et al., 2012). Single nucleotide polymorphisms in the gene

coding for TSLP at 5q22 are associated with predisposition to asthma, AD and EoE (Zhang & Zhou, 2012). Intriguingly, the gene coding for the TSLP receptor is located at Xp22.3/Yp11.3, and polymorphisms at both sex linked loci may predispose male but not female patients to EoE (Sherrill et al., 2010). Recently, the 'TSLP–basophil axis' was studied and found to play a central role in eosinophilic inflammation of the esophagus in both mouse and human models, whereby disease did not develop in the absence of TLSP, and single nucleotide polymorphisms associated with gain of function correlated with increased disease activity (Noti et al., 2013). Further clarification concerning the role of TSLP is clearly warranted.

Nitrous oxide-mediated relaxation of smooth muscle in the bladder is reduced in the presence of testosterone in a rodent model. Effects of TGFβ in causing cardiac fibrosis and mediating adverse outcomes following myocardial infarction in males are attributed to testosterone (Chung et al., 2013; Vignozzi et al., 2012). Relaxin is a hormone present in large amounts in pregnancy, small amounts in non-pregnant females of reproductive age and also in males. It may have the potential to decrease fibrosis in a range of organs, as demonstrated by animal models (Mookerjee et al., 2006; Royce et al., 2009). A study of relaxin in human bronchial biopsies suggests an association with the remodelling process in asthma, whilst a greater expression of relaxin receptors in female compared with male cruciate ligament tissue has been cited as an explanation for greater tissue laxity in females (Galey et al., 2003; Royce et al., 2012). Further research may allow the therapeutic use of relaxin in the future.

5. Alterations in esophageal function (biomechanics)

From a theoretical standpoint, it is apparent that the dysphagia and the food bolus obstruction events that occur in EoE may be caused by fixed narrowing (remodelling), acute narrowing (inflammation and oedema) and esophageal dysmotility or spasm, alone or in combination. As fixed narrowing is demonstrable as focal strictures at endoscopy or barium swallow, the emphasis in research has been on remodelling. Routine esophgeal manometry has not uniformly demonstrated abnormalities in patients with EoE, although high-amplitude abnormal distal contractions have been reported in some patients and findings consistent with nutcracker esophagus found in a subset of patients with a high eosinophil count (Lucendo et al., 2007; Moawad et al., 2011; Read & Pandolfino, 2012). Manometry findings in another study were similar in patients with EoE to those with GORD, both having limited peristaltic activity, perhaps demonstrating the importance of a control group (Roman et al., 2011). Research methodologies utilising the barostat or planimetry, which assess esophageal distensibility, however, have defined abnormalities in a significant percentage of patients, with decreased distensibility and pan-esophageal pressurisation characteristic (Compare et al., 2004; Roman et al., 2011). Further research, particularly delineating the change in distensibility with treatment, and correlation with traditional measures of treatment success such as patient reports of dysphagia and eosinophil count at gastroscopy are needed.

Acute or subacute infiltration by inflammatory cells may also explain the dysphagia and food bolus obstruction events. Significant improvement in these features has been demonstrated within six weeks of the commencement of dietary modification, inhaled corticosteroids and even oral corticosteroid therapy. It remains to be determined if complete symptom resolution can be achieved using these treatments and many patients notably do not respond. The presence of eosinophils and mast cells may contribute to the esophageal dysfunction via the production of products of degranulation such as tryptase, major basic protein and eosinophil derived neurotoxin (see below), as well as causing inflammation and oedema (Kephart et al., 2010). Correlations between eosinophil and mast cell densities and dysphagia have been noted, and future definition of this potential pathophysiological

phenomenon appears important in establishing valid treatment goals (Gonsalves et al., 2012).

5.1. Treatment

There are compelling reasons to advocate aggressive early treatment of EoE, and there are significant shortcomings in the current treatment options. It is now apparent that EoE is a chronic fibro-stenosing disease that likely benefits from prompt recognition and therapy (Dellon et al., 2014; Schoepfer et al., 2013). It is possible that broadening the treatment goals to include measurable changes and return of normal esophageal distensibility may guide treatment type, duration and potentially define a need for additional agents to address not just eosinophilic inflammation (the current sole focus of treatment) but also lamina propria and muscular thickening and disturbances in physiological peristalsis. It should be noted that it is not clearly established that controlling the eosinophilic inflammation will correct all of the pathological changes and return the esophagus to normal structure and function, although some studies are suggestive (admittedly hampered by the lack of lamina propria and muscularis sampling).

Existing guidelines advocate the use of dietary therapy such as the six-food elimination diet or corticosteroids as first line in the management of EoE, and suggest esophageal dilatation at endoscopy as an alternative in refractory cases (see Figs. 3 and 4) (Dellon et al., 2013). Unfortunately, many patients do not respond or have an inadequate response to either or both therapies, as determined by symptoms and/or subsequent endoscopy and biopsy. The response to a six-food elimination diet in adult and paediatric patients approaches 65%, albeit in a controlled trial situation, whilst swallowed corticosteroids as dry powder or gel solutions result in histological improvement in 50% to 90% of patients (Gonsalves et al., 2012; Straumann et al., 2010). Dietary therapy is by definition restrictive and hence potentially cumbersome. Neither strategy is curative. Patients rapidly relapse following cessation of treatment. Basic scientific research supports this — that is after treatment with corticosteroids eosinophils do not revert to a normal phenotype, rather the capacity of eosinophils to adhere to epithelium is diminished (Lingblom et al., 2014). Corticosteroids may also cause oral candidiasis and a hoarse voice in up to 25% of patients' long term, whilst suppression of the hypothalamopituitary axis and reduction of bone mineral density are potential issues (Lipworth, 1999).

Immunotherapy (subcutaneous or oral/sublingual) could be used as treatment for EoE, and one case series supports this approach (Ramirez & Jacobs, 2013). However the fact that immunotherapy for seasonal rhinitis has potentially caused EoE suggests great caution should be exercised, and further research occurs before such therapy can be advocated (Miehlke et al., 2013). Furthermore immunotherapy for classical food allergy (anaphylactic) is not an established treatment, and the efficacy of the elimination or elemental diets in EoE suggests that food antigens are the likely trigger, again raising questions about the rationale of this approach.

The mast cell stabiliser (sodium cromoglycate) and the leuokotriene receptor antagonist (monteleukast) have both been used to treat EoE but without success. Monteleukast does not result in a reduction in esophageal eosinophil count, nor does it sustain histological remission in those treated with corticosteroids (Attwood et al., 2003). Sodium cromoglycate has been used in a number of paediatric patients and found to be ineffective (Spergel et al., 2009). Extrapolating from other gastrointestinal diseases characterised by chronic inflammation (e.g. Crohn's disease and ulcerative colitis) the thiopurines have been used successfully in a case series of 3 patients, with relapse of the condition following the cessation of this medication supporting a treatment effect (Netzer et al., 2007). Nonetheless, the potential side effects of this medication class (increased risk of non-melanomatous skin cancer, increased risk of lymphoma, immunosuppression) make this an unattractive option, given that EoE is not in itself associated with a decreased life expectancy.

Esophageal dilatation at endoscopy is an effective treatment in some patients, providing immediate relief of dysphagia. Initial safety concerns relating to the potential to cause esophageal perforation have been moderated by the publication of a retrospective cohort of 207 adult patients, in which there were no reported perforations although significant chest discomfort and odynophagia occurred in 45%(Schoepfer et al., 2010). In this same study, swallowing was normalised in 50% of patients. Other smaller and prospective studies have suggested clinical remission of dysphagia in up to 90% of patients, which is durable in 50% of these patients at 24 months (Bohm et al., 2010).

It is hence apparent that current treatments have variable and suboptimal success rates, and may be poorly tolerated. The need for alternative management approaches is evident. Biological agents have been trialled in EoE, although to date the efficacy has been disappointing and the cost remains prohibitive. Monoclonal antibodies to IL-5 (mepolizumab and reslizumab), have been trialled, with reslizumab demonstrating a dose related ability to decrease esophageal intraepithelial eosinophil counts in comparison to placebo in a large study of paediatric patients (Walsh, 2013). Unfortunately, even at maximal dose, reductions in the eosinophil count were modest (the study expressing the change in eosinophil count as a percentage, rather than the ability to decrease the eosinophil count to less than 5 per high power field as is customary). Furthermore all groups, including placebo demonstrated an improvement in symptoms, and the difference between groups was not significant. Mepolizumab was used in a small placebo controlled trial of adult patients, and like reslizumab achieved a significant reduction in eosinophils, but not symptoms with between 4 and 13 weeks of follow-up (Straumann et al., 2010).

The CRTH2 receptor antagonist OCOO459 has been trialled in a group of 26 adult patients with EoE. After 26 weeks of therapy, there was a modest decrease in the eosinophil count, but with the mean eosinophil count at the conclusion of therapy in this group of patients with severe EoE decreasing only to 75 per hpf, the utility must be questioned, despite an improvement in the physician rated disease activity index (Straumann et al., 2013). Omalizumab, a monoclonal anti-IgE antibody has been trialled, and has not proven successful according to a randomised controlled trial, as well as case reports (Rocha et al., 2011). Neither histology nor clinical symptoms were improved. Similarly, infliximab (an anti-TNF agent) was studied in a single series of adult patients with EoE, and was not effective (Straumann et al., 2008).

Borrowing from established treatments of other related conditions (asthma and atopic dermatitis) may potentially be extended to EoE, with likely benefit. The calcineurin inhibitors (tacrolimus and the related pimecrolimus) have been used topically in atopic dermatitis, as well as orally in refractory cases (including with cyclosporine) (Reitamo et al., 2000). It is foreseeable that a gel suspension of pimecrolius or tacrolimus could be used (these agents are fortunately free of the side effect of gum hyperplasia that occurs with cyclosporine). Methotrexate is another option, case reports indicating potential efficacy in atopic dermatitis (Weatherhead et al., 2007). Disturbances in the esophageal microbiota have not been characterised in EoE, but potential abnormalities could be treated with antibiotics, once again an established treatment in AD (Huang et al., 2009).

TSLP may activate basophils and drive a TH2 like mediated disease milieu independent of IgE antibodies (see below). A trial of a monoclonal antibody against TSLP in allergic airways disease in cynomolgus monkeys was successful (Cheng et al., 2013). This is particularly pertinent to EoE, given the SNP coding for TSLP and TSLP receptor that correlate with EoE in particular (Sherrill et al., 2010). Siglecs are cell surface proteins expressed on eosinophils, basophils and mast cells. Siglec F, and the related Siglec 8 in humans govern eosinophil apoptosis, and hence the observation that Siglec F may decrease eosinophilic inflammation in murine airways may lead to human trials in the future (Kiwamoto et al., 2012). Finally sphingosine kinase is an enzyme implicated in IgE mediated mast cell responses. Inhibition of sphingosine kinase-1 decreases airway hyper-responsiveness and allergic inflammation in murine inflammatory airways disease models (Price et al., 2013). Again EoE, as a mast cell rich inflammatory disease, may foreseeably be helped by this approach and research appears warranted.

All of the pharmacological treatments discussed work by reducing the (mainly) eosinophilic infiltrate. Thinking more broadly, agents that may modulate barrier membrane integrity, and thus minimise antigen exposure, or those that address the end result of eosinophilic infiltration, namely subepithelial fibrosis can be considered. Barrier integrity is a considerable focus of treatment for atopic dermatitis, where factors that denude the epithelium of the protective moisture retaining lipid surface layer, such as harsh soaps, are minimised, and moisturisers and emollients are used extensively. Furthermore, using antibiotics may improve barrier integrity (Boguniewicz & Leung, 2011). One existing treatment for eosinophilic esophagitis, namely PPIs, may act by improving barrier function of the esophagus (Dohil et al., 2012). Patients with coeliac disease have impairments in intestinal permeability and barrier function, and the agent lazerotide, that promotes tight junction assembly has been shown to return epithelial integrity to a more normal phenotype (Gopalakrishnan et al., 2012). As antigen presentation may occur in the duodenum as opposed to the esophagus in EoE, targeting intestinal barrier function may have a role in reducing antigen presentation. It remains to be seen if this agent will have a similar effect in the duodenum of patients with EoE.

Treatments that address fibrosis of the lamina propria are needed. Borrowing from research in cardiovascular medicine and hepatology, angiotensin 2 receptor blockers inhibit TGF-B production, that is significantly expressed in EoE (see above). For example ACE inhibitors and AT 2 blockers have been demonstrated to influence myocardial remodelling positively post-myocardial infarction (Uh et al., 2013). A trial has been commenced examining the role of losartan in paediatric EoE patients. Relaxin has been used and demonstrated positive effect in murine asthma models of bronchiolar remodelling (Royce et al., 2009). As expert clinical commentators suggest that the fibrosis and hence dysphagia induced by EoE may persist long after the resolution of inflammation, and currently only responds to dilatation at endoscopy, further therapeutic agents are needed.

6. Conclusion

EoE is a chronic antigen-driven disease characterised clinically by dysphagia and recurrent food bolus obstruction events. Despite a great deal of recent research interest, many questions remain. Apparently related conditions such as asthma and atopic dermatitis provide clues as to the likely mechanisms and promising research and therapeutic targets for the future. It is likely that both inherited (gender and individual genes) and acquired factors (e.g. dietary allergens, microbiota) are significant. Clarifying the relationship between the cessation of inflammation, and potential improvements in symptoms and reversal of remodelling will enable treatment end-points to be established and guide therapeutic advances.

Conflict of interest statement

The author states there are no conflicts of interest to declare.

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APPENDIX 3

Seasonal recurrence of food bolus obstruction in eosinophilic esophagitis

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Abstract

Background: Eosinophilic esophagitis (EoE) is a newly recognised condition that is apparently increasing in prevalence, and the aetiology is poorly understood. The role of aeroallergens in EoE is controversial, given the success of dietary therapy. Massive aeroallergen exposure leading to food bolus obstruction events (FBOE) has been described, and the diagnosis of EoE by esophageal biopsy noted to be more common in the pollen season according to previous case series.
Aim: To determine if a seasonal variation and a geographical variation occurred in EoE presenting as FBOE in adults, and to track the prevalence of FBOE and EoE over time.
Method: A retrospective case–control study analysis was performed from January 2002 to January 2012 to identify all FBOE in adults presenting to five tertiary hospitals in Melbourne, Australia. Endoscopy, histopathological reports, case notes and blood tests were examined, and postcodes recorded. Records of pollen counts were obtained. Cases were defined according to esophageal biopsy and grouped based on month of diagnosis. All other causes of FBOE served as controls.
Results: One thousand, one hundred and thirty-two FBOE were identified. Biopsies were only performed in 278 of these cases, and 85 patients were found to have EoE after biopsy. Patients with EoE were younger (mean age 38 years, range 18–72) compared with those with alternative diagnosis (mean age 64.4 range 22–92), more likely to be male (M : F = 4:1 compared with 1.68:1) and had a higher eosinophil count in venous blood. Overall no seasonality was demonstrated in FBOE secondary to any diagnosis, although the six cases of recurrent FBOE secondary to EoE mainly occurred in the grass pollen season in subsequent years. FBOE cases were evenly distributed throughout metropolitan Melbourne irrespective of population density. EoE as a percentage of FBOE increased over time.
Conclusion: Seasonal aeroallergens may be important for a subgroup of patients with EoE presenting as recurrent FBOE. Esophageal biopsies are performed in a minority of patients, representing a significant departure from ideal management and contributing to recurrent unnecessary FBOE. EoE is an increasingly important cause of FBOE.

Introduction

Eosinophilic esophagitis (EoE) has short history, being formally defined in 1993, and may be increasing in prevalence.[1,2] EoE is a chronic antigen-driven disease manifesting clinically as dysphagia and FBOE, and pathophysiologically is characterised by luminal narrowing and limited distensibility secondary to a mixed eosinophil-rich inflammatory infiltrate, epithelial hyper-

plasia, lamina propria fibrosis and muscular dysmotility.[3] Esophageal eosinophilia is a non-specific finding and may occur, for example, in gastro-esophageal reflux disease (GERD). The significance of proton-pump inhibitor (PPI)-responsive esophageal eosinophilia is debated.[4] The role of food allergens is well established; an elemental diet is effective in >90%, the six-food elimination diet effective in >65%, and culprit food allergens are identifiable on rechallenge and re-biopsy at endoscopy.[5,6] Aeroallergens as a contributor to disease pathogenesis are, in contrast, supported only by case studies and case series involving adults, showing increased diagnoses during the pollen season, a finding refuted by a recent

paediatric study.[7–10] A large cross-sectional study of pathology records also supports a role for aeroallergens; more cases of esophageal eosinophilia were found in climatic zones and regions where pollen counts are known to be high, such as temperate as opposed to tropical locations and areas that are less densely populated and hence possibly more vegetated.[11] Thus, in the absence of a control group, and the inability to distinguish esophageal eosinophilia related to GERD or at least responsive to PPIs from a key indicator event of disease activity, the validity of these studies must be questioned.

FBOE can be secondary to EoE in up to 50% of cases, while other causes include GERD, benign strictures and esophageal malignancy.[12] The suggestion that FBOE may be used as a surrogate marker of EoE disease activity is supported by case reports describing these events following massive aeroallergen exposure. Furthermore, apparently new cases of EoE in adults are more commonly documented in the pollen season, and often present for diagnosis with FBOE.[8] It is acknowledged that food bolus impaction may result from chronic rather than acute pathological changes in the esophagus (such as lamina propria fibrosis), and that factors unrelated to disease activity (such as the consistency of food ingested) may also play a role. Nonetheless, it is our hypothesis that the inhalation or ingestion of aeroallergens may lead to increased disease activity with resultant luminal narrowing and may in turn cause food bolus obstruction. With this in mind, and considering the limitations of previous studies relating to aeroallergens including the lack of a control group, heterogeneity of clinical presentation, unknown significance of esophageal eosinophilia, the current study aimed to determine if a seasonal and geographical pattern exists in patients with EoE presenting with FBOE and to examine the seasonality of representation of FBOE. Additional aims were to determine if this presentation is becoming more common using all other cases as a control and to define the quality of diagnostic processes in patients presenting with FBOE.

Methods

A retrospective review of the computer databases of five large tertiary hospitals in Melbourne, Australia, was undertaken to identify patients with FBOE, using the International Classification of Diseases (ICD) 9 code CM 935.1. The databases were searched between February 2002 and February 2012. Case files (electronic or hard copy) were retrieved and analysed. Included were patients aged 18 years and over who underwent an upper gastrointestinal endoscopy and had biopsies of the esophagus performed during the admission. Excluded were those who did not receive an endoscopy and biopsy

and those with oropharyngeal or tracheal obstruction miscoded as an esophageal event. Cases were defined as having >15 eosinophils per high power field (area 0 .19 mm^2) anywhere in the esophagus at endoscopy as determined by the relevant histopathology report. Controls were those with an esophageal biopsy and any diagnosis other than EoE that explained the FBOE. Patient age, gender, date of birth, postcode, clinical diagnosis, co-morbidities, medications, endoscopic diagnosis, histopathology report, eosinophil count and specific IgE to common food or aeroallergens in the serum (where available) was recorded. The postcodes were characterised according to the regional population density with reference to data from the Australian Bureau of Statistics. A higher frequency of cases per head of population from postcodes with lower population density was anticipated to define a geographical effect. The date of presentation with FBOE was recorded and compared with Melbourne pollen counts recorded by the Melbourne University Department of Botany. As the predominant aeroallergen in Victoria is Rye grass, seasonality was expected to be defined as increased numbers of EoE presenting as FBOE in the months September through to January when high levels of Rye grass pollen are present (although a small amount of annual variation does occur and could be tracked with the pollen count data). The study was approved by local hospital ethics committees.

Microsoft access database and statistical software were used to collate and analyse the data. Continuous data were expressed as means, and categorical data as percentages. Actual and expected frequencies between groups (EoE vs other) were compared using the Chi-squared test. Continuous data were compared with the Student's *t*-test. A probability value ≤0.05 was considered statistically significant.

Results

A total of 1132 patients was admitted with FBOE to the five hospitals over a 10-year period. As shown in Figure 1, 854 were excluded due to the presence of oropharyngeal pathology (*n* = 57) or the absence of esophageal biopsy (*n* = 797). Thus, 278 patients met the inclusion criteria, of whom 85 were diagnosed with EoE. The diagnoses in the other 193 patients are shown in Table 1, the most common alternative being GERD. Comparison of the characteristics of the two groups of patients showed that those with EoE were younger and more likely to be male (Table 1).

Dividing the data into two periods, 2002–2006 and 2007–2012, the number of cases of FBOE seen at the hospitals had increased over time, as shown in Table 2.

Figure 1 Identification of food bolus obstruction events (FBOE) subgroups.
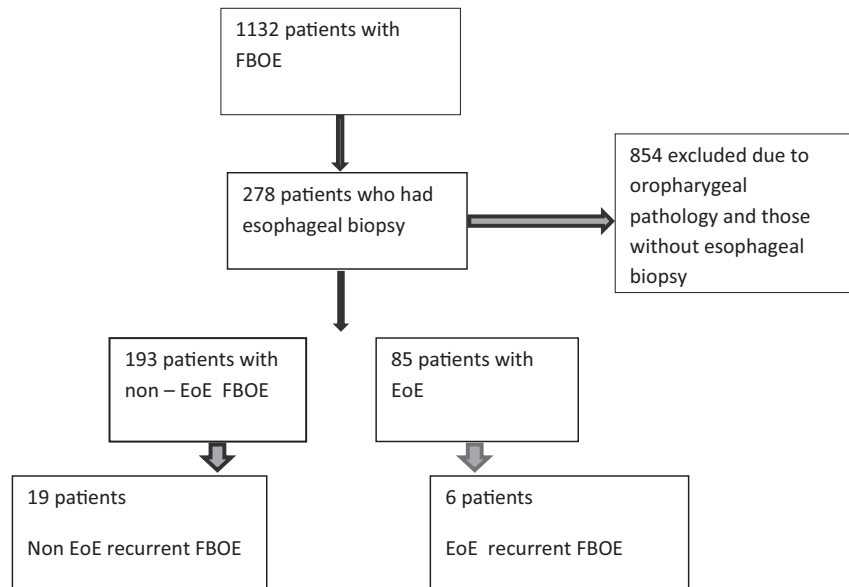
The proportion having esophageal biopsies had not significantly increased, but the proportion who were diagnosed with EoE had increased by 56% ($P = 0.029$).

Seasonality was not demonstrated in cases of EoE, nor the control group with an even number of cases occurring across the seasons (Fig. 2). Furthermore, equal proportions of patients residing in low population density regions of Melbourne occurred in both groups.

Recurrent FBOE in subsequent years were identified in six of those with EoE and 19 with alternative diagnosis (Table 3). Recurrent FBOE within the same calendar year (usually within months) were noted in some patients, mainly those with a diagnosis of esophageal neoplasia and are not shown in the table. The demographics of those with recurrent FBOE did not differ from those with single episodes. It is notable that the pattern of recurrence did differ between groups – that is when the recurrence occurred in subsequent years those with EoE were more likely to represent in the same season and even calendar month, and usually in the grass pollen season.

Discussion

A primary aim of the current study was to find evidence for the importance or otherwise of aeroallergens in the pathogenesis of EoE. Previous studies lacked controls and had heterogeneity of presentations. The use of FBOE as a measure of seasonal variation was novel as it provided a ready control subgroup with non-EoE causes. The results clearly show that the presentation of EoE with FBOE is equally distributed throughout the year, as were non-EoE causes. Since FBOE is one of the most common presenting features of EoE, we contend that this indicates that factors unrelated to seasonal variation in pollen count are most important for the *majority* of patients. However, the other novel feature of the present study was the examination of recurrent FBOE in terms of seasonality, which has not been previously addressed despite the description of the phenomenon for recurrent FBOE in patients with hiatus hernia and in those with EoE.[13,14] The observation that recurrent FBOE demonstrates a seasonal trend that correlates with the appearance of

Table 1 Characteristics of the patients presenting with foreign body obstruction of the esophagus and having oesophageal biopsies performed

	Eosinophilic esophagitis $n = 85$	Other diagnosis† $n = 193$	*P*-value
Mean age (range) (years)	38 (18–72)	64 (22–92)	0.001
Proportion male (male : female ratio)	81% (4:1)	63% (1.68:1)	0.002
Mean peripheral blood eosinophil count (reference range 0–0.2 × 10⁹/L)	0.237 (0.0–1.0) (available on 42)	0.06 (0.0 to 0.4) (available on 84)	0.235
Proportion living in area of low population density	22%	19%	0.6394

†Gastro-esophageal reflux disease (104), neoplasia (19), candidiasis (12), non-food foreign body (13), Barrett's esophagus (13), Schatzki ring (13), motility disorder (3), normal esophagus (17).

Table 2 Patients presenting with foreign body obstruction of the esophagus, the frequency of esophageal biopsy and diagnosis of eosinophilic esophagitis according to the time period

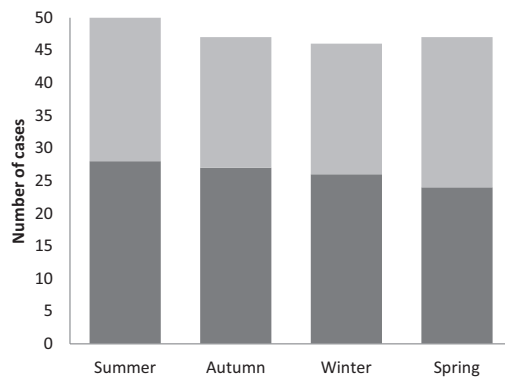|	2002–2006	2007–2012
Total cases of foreign body obstruction	571	707
Biopsies performed (percentage of cases)	110 (19%)	168 (24%)
Eosinophilic esophagitis		
• Number of diagnoses	25	60
• EoE as percentage of biopsies	23%*	36%*

*P = 0.029; Chi-squared = 5.2819.

the major allergenic pollen, ryegrass, suggests that, for a *subgroup* of patients with EoE, aeroallergens are important and that seasonal variations in disease activity could be expected. It is proposed that these dichotomous observations may assist in further characterisation of the relevance of food versus aeroallergens in driving disease activity. It could be inferred from the success of dietary therapy in the majority of patients that food allergens are important for most, but that aeroallergens are important for some. Future prospective longitudinal studies monitoring patients maintained on food allergen exclusion diets throughout the calendar year are indicated to further refine this hypothesis.

The characteristics of the patient population in our study are typical of those reported in previous literature relating to EoE and FBOE *per se*. Patients with EoE were more likely to be male and were younger that those with alternative diagnoses.[15] The causes of FBOE were also similar to previous studies. The apparent increase in EoE as proportion of total FBOE also supports previous research.[2] Patients with EoE were no more likely to reside in low density regions where exposure to aeroallergens is greater, although it is acknowledged that some of the population may be mobile, thus minimising the effect of local variations in pollen count. Also, the referral base of the hospitals involved may not fairly reflect the overall prevalence of the condition in the state of Victoria.

A supplementary aim for the study was to examine the current clinical practice around the diagnosis of patients presenting with FBOE. Critical to the diagnosis of EoE is

Figure 2 Number of cases presenting with foreign body obstruction of the esophagus according to the diagnosis and season. No significant differences were observed for the proportion of those with eosinophilic esophagitis (EoE) (Chi-squared test). (▨), EoE; (▦), non-EoE.

	Summer	Autumn	Winter	Spring
*[i]Total	50	47	46	47
*[ii]EoE	22	20	20	23

Table 3 Characteristics of the patients having recurrent foreign body obstruction of the esophagus†

	Eosinophilic esophagitis	Other diagnosis	*P*-value
Number	6	19‡	
Biopsy performed at first foreign body obstructive event	1/6 (16%)	4/19 (21%)	NS
Mean age (range) (years)	39.1 (21–54)	62 (range 22–90)	NS
Proportion male	83%	73%	NS
Peripheral blood eosinophil count	0.4 (range 0.2–0.7; 95% CI) (4 available)	0.1 (range 0.5-0.2) (95% CI) (11 available)	NS
Recurrence same month in subsequent years	67%	10%	0.005
Occurrence in peak pollen season for rye grass (1 October to 1 January)	67%	5%	0.005

†Only recurrences of FBOE that occurred in subsequent years are recorded. ‡Two patients had more than one recurrence, again in different months of the year and were incorporated in this data. NS, not significant.

the taking of esophageal biopsies for histopathological assessment. As the diagnosis of EoE has longer term implications and specific therapies are efficacious in preventing further problems, it would seem good clinical practice to pursue the reason why patients are presenting with an obstructed esophagus. Despite this, biopsies were on average taken in only one in four cases presenting with FBOE. While biopsy at the time of clearing the esophagus of the foreign body is often regarded as not of priority, the fact that few are coming back from repeat endoscopy in this case series and a diagnosis is not being reached in 75% is not acceptable, given the efficacy of dietary and topical therapies. Furthermore, among patients with recurrent FBOE, an esophageal biopsy was performed in less than 20% on the first presentation, suggesting that expensive and potentially risky hospital admission with endoscopic intervention could have been avoided.

Several limitations are present in the study design and are acknowledged. The hypothesis the FBOE occurs when EoE is most active, when an inflammatory infiltrate is florid, underlies the design of our study. It is plausible that several other factors could contribute, including the more chronic pathological change of subepithelial fibrosis, as well as external factors such as the intake of dense foods such as steak or chicken that conceivably may be ingested according to the season (e.g. barbeques) and in the setting where chewing may be suboptimal. Nonetheless, the use

of a control group (all other causes of FBOE) should mitigate this issue. The retrospective nature of data acquisition relies heavily on correct coding, and the number of identified cases may have been underestimated. One potential modifying factor, medication use, particularly of PPI and corticosteroids, was not reliably recorded and hence not analysed. Finally but unavoidably, the fact that few patients underwent histological assessment of the esophagus greatly reduced the sample size and reliability of the observations.

Conclusion

The present study has shown that adult patients with EoE presenting with FBOE do so evenly throughout the year, suggesting that for most patients non seasonal factors are more important. Of importance, however, was the pattern of seasonal recurrence in patients with EoE corresponding with the grass pollen exposure. Hence, the results have suggested that aeroallergens may be of great relevance to some patients with EoE. Of perhaps more alarming significance was the relative rarity of the practice of biopsying the esophagus at endoscopy, representing a significant departure from ideal management, and contributing to recurrent FBOE. There is a need for prioritisation of performing biopsies at the index endoscopy.

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APPENDIX 4

Letters to the Editors

Letter: seasonality in eosinophilic oesophagitis and food bolus obstruction — what about recurrent episodes?

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SIRS, We read the paper by Sengupta et al.[1] with interest, and have the following observations and queries stemming from our own similar study.

Firstly, the rate of previous oesophageal food bolus impaction was recorded as high (45%) among those with eosinophilic esophagitis (EoE), yet data regarding repeated episodes are not forthcoming, despite a 10-year analysis. We encourage explanation and if possible provision of such data, given our findings that suggest a subgroup of patients with EoE (and not other aetiologies) have seasonal oesophageal food bolus impaction.[2]

Secondly, oesophageal food bolus impactions are more common in spring/summer, a finding that the authors attribute potentially to elevated pollen counts. Our pollen season (Australia) for the pathogenic species (rye grass) is regularly between 9 and 11 weeks duration. Have the authors considered their region-specific pollen counts in this time-dependent manner in relation to oesophageal food bolus impaction?

Thirdly, the predominance of EoE as the established cause of oesophageal food bolus impaction is notable, while few cases of erosive reflux (four in total) are diagnosed. Is it the supposition that erosive reflux is a rare cause of oesophageal food bolus impaction, or rather that few biopsies are taken for cases other than suspected EoE?

Finally, the documentation of adverse events associated with the management of oesophageal food bolus impaction is admirable. The high rate of complications including aspiration coupled with the majority of procedures being performed using conscious sedation is of note. We suggest there is thus a case for mandatory general anaesthesia (hence protecting the airway).

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AP&T invited editorial and correspondence columns are restricted to letters discussing papers that have been published in the journal. A letter must have a maximum of 300 words, may contain one table or figure, and should have no more than 10 references. It should be submitted electronically to the Editors via http://mc.manuscriptcentral.com/apt.

APPENDIX 5

GASTROENTEROLOGY

Ultrathin unsedated transnasal gastroscopy in monitoring eosinophilic esophagitis

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Conflict of Interest: No conflicts of interest.

Abstract

Background: Ultrathin unsedated transnasal gastroscopy (UTEG) has a number of advantages applicable to eosinophilic esophagitis (EoE) and has not been evaluated for this condition.

Aim The aim of the study is to determine the feasibility of UTEG in patients with EoE and the acceptability of histological specimens obtained at biopsy.

Method: All patients with a diagnosis of EoE presenting to the outpatients department of two hospitals (Box Hill Hospital and The Alfred Hospital, Melbourne Australia) were asked to participate in the study. UTEG was performed on consenting individuals. Feasibility was determined by the success of nasal intubaton, patient perception according to post procedural survey, and adequacy of esophageal biopsies was assessed.

Results: Ninety-six consecutive patients with EoE were offered UTEG, and 24 agreed to participate in the study. Seventy-four UTEGs were performed over a period of 26 months (September 2012 to December 2014). Nineteen patients had repeat procedures. Successful nasal intubation occurred in 97% (72 of 74 procedures), and 21 of 24 (86%) described high satisfaction with the procedure and minimal discomfort, and would choose UTEG for future procedures. Mean duration was 5 min. Adverse events of epistaxis (three cases) and vomiting of liquid contents during the procedure (two cases) were recorded, cardiorespiratory parameters remaining normal in all patients. All completed procedures produced adequate histological samples.

Conclusion: In those who decide to undergo UTEG, it is a safe and well-tolerated procedure

Introduction

Eosinophilic esophagitis (EoE) is a chronic inflammatory condition of the esophagus that is diagnosed and monitored on the basis of histopathological assessment of multiple biopsies of the esophagus obtained via video endoscopy. More than 15 eosinophils per high power field is considered diagnostic of EoE or indicates a lack of response to treatment if administered.[1] In Western countries such as Australia, video endoscopy is most frequently performed via the transoral route using a standard caliber video endoscope and requires intravenous sedation.[2] The costs and inconvenience associated with intravenous sedation (e.g. employment of a specialist anesthetist, inability to work, or drive a car on the day of the procedure) are considerable.[2] Furthermore, the success or otherwise of therapy for EoE also depends upon esophageal histopathology as there are no reliable non-invasive indices of response. When complicated treatment regimens such as the six-food elimination diet are applied, repeated endoscopic assessment is required to assess response. Indeed, eight or more gastroscopies are required for the successful implementation of dietary therapy for EoE.[3] Thus, there is the need for a less cumbersome method of obtaining esophageal biopsies.[4]

Improvements in technology have enabled the development of fine bore video endoscopes.[5] These devices have the advantage of greater patient comfort, and hence, the need for sedation is reduced. The application of local anesthetic spray alone is feasible.[5] The use of ultrathin unsedated transnasal gastroscopy (UTEG) for the performance of esophageal biopsies has been trialed successfully for the assessment of Barrett's esophagus.[6] In a comparative study of trans-oral *versus* trans-nasal video endoscopy in 32 patients with Barrett's esophagus, no differences in the quality of endoscopic vision or histological specimens were observe in either group.[6]

We hypothesized that the use of UTEG specifically for the assessment of EoE would likewise be safe and accurate. The current study aimed first to assess the endoscopic success of UTEG in patients with EoE; secondly, to define the experience of patients having UTEG; and thirdly, to compare the quality of esophageal biopsies obtained from patients undergoing UTEG without sedation with those obtained in a different cohort having standard transoral gastroscopy with sedation (STOG).

Materials and methods

Participants. Patients with an established diagnosis of EoE attending the outpatients department of two hospitals (The Alfred Hospital and Box Hill Hospital, Melbourne Australia) were invited to participate. Patients 18 years or older who were scheduled for

outpatient gastroscopy were eligible for inclusion. Excluded were those with significant cardiovascular or respiratory illness; coagulopathy; ear, nose, and throat conditions; or previous surgery or those unable to give informed consent.

Protocol. Demographic data that included gender, age, country of birth, and educational attainment were recorded for those electing to undergo either UTEG or STOG after giving written, informed consent. The endoscopic procedure was performed and biopsies prepared and examined as described in the succeeding texts. Adverse events were documented by the responsible endoscopist in association with the procedure. A questionnaire was administered to the participants just prior to discharge or within 1 week just prior to being reviewed in clinic. The study protocol was approved by the Eastern Health Research and Ethics Committee and Monash University (approval number E19/1213)

Endoscopy. The procedures took place in the day procedure unit of Box Hill Hospital. Topical pharyngeal anesthetic spray (lignocaine 100 mg/mL) was applied to the patients preferred nostril and to the posterior oral cavity 10 min prior to nasal intubation. Supplemental oxygen (2 L/min) was administered for 5 min prior to the commencement of the procedure. Oxygen saturations were recorded continuously throughout the procedure via a finger pulse oximeter.

UTEG was performed with the patient at 45°, with the head turned towards the endoscopist. Two endoscopists (HP and SN) performed all of the UTEGs, both previously having experience with the device. The Pentax EG-870 K gastroscope with an insertion tube diameter of 6 mm was inserted through the nostril following the application of a water-based lubricant. On reaching the piriform fossa, the patient was instructed to swallow to aid advancement of the gastroscope. Following visualization of the stomach, the gastroscope was withdrawn to 5 cm above the gastroesophageal junction (Z-line) and four biopsies were taken at this location ("lower esophagus"). Biopsies were repeated at two further locations, 5 and 10 cm proximal to the initial site (that is "middle" and "upper" esophagus). Two-millimeter diameter "pediatric" biopsy forceps were used.

STOG was performed by a number of different experienced and credentialed gastroenterologists. The study did not examine STOG and patient perception thereof. Biopsies were taken in a standardized manner (as per UTEG), and the histological specimens were analyzed. Sedation was administered intravenously using propofol.

Outcome measurements. Three groups of outcomes were measured:

- *Patient-reported outcomes*: These were obtained via a questionnaire that contained three questions that assessed patient satisfaction in comparison with previous transoral gastroscopy, procedural discomfort, and willingness to repeat UTEG. Responses were recorded using a Likert-type scale.
- *Endoscopic outcomes*: The successes of nasal and pharyngeal intubation and of the taking of esophageal biopsies as planned were recorded. Adverse events such as epistaxis and vomiting at the time of the procedure and, when applicable, following subsequent outpatient review were noted.

- *Histopathologist-reported outcomes*: Expert histopathologists (Eastern Health department of Pathology) estimated and recorded the size of biopsies taken in patients undergoing UTEG or STOG. The quality of the biopsies after sectioning and staining with H&E was qualitatively assessed as adequate or inadequate for diagnostic assessment. Sampling of lamina propria was assessed following additional staining of the tissue (Masson's Trichome) and reported as present or absent. Eosinophil counts per high power field were documented. This analysis was performed blinded to the type of gastroscopy performed.

Statistical analysis. Data were arranged and analyzed using Microsoft Access. For continuous variables, distribution is described by mean. For comparison of non-parametric data, the Fisher's exact test is used

Results

Participants. Enrollment took place between September 2012 and December 2014. Of the 96 patients invited to participate, 24 consented to undergo UTEG and the remainder consented to STOG. Seventy-four UTEGs were performed in total. The characteristics of the patients are displayed in Table 1. The mean age, gender, and education status do not differ between those who elected for UTEG and the conventional procedure. Those born overseas (UK and South Africa) were more likely to choose UTEG than Australian or New Zealand born individuals ($P = 0.0233$ Fisher's exact test)

Endoscopic outcomes. Successful nasal intubation occurred in 72 of 74 procedures (97%); enlarged turbinates were described as impeding scope passage in the two failed cases. Successful esophageal intubation was possible in 71 of 72 procedures (98%), with an excessive gag reflex preventing scope passage in one patient. Once successful esophageal intubation was achieved; 70 of 71 (98%) procedures were completed including the taking of all planned esophageal biopsies, with one procedure being terminated prematurely to recurrent gagging. Those patients who were unable to proceed with UTEG all successfully completed STOG on the same day.

No major adverse events were recorded. Three episodes of minor epistaxis that did not require treatment occurred on the day of the procedure. Two patients had one episode each of gagging and then vomiting a small volume of clear liquid at the time of the procedure. One patient reported sneezing small volumes of clotted blood in the week after the procedure, and two other patients reported soreness of the intubated nostril for a few days following the procedure.

Patient-reported outcomes. A post-procedural survey was completed by all patients and was available for 63 of 74 procedures. Of the 22 patients having repeated procedures, 17 (77%) preferred UTEG compared with previous STOG following their initial UTEG (Table 2). All patients who initially successfully completed the procedure elected to undergo subsequent repeat UTEG. Patient satisfaction and levels of discomfort recorded according to the Likert score are shown in Tables 2 and 3. Minor

Table 1 Demographic and other features of the study participants

		Unsedated transnasal gastroscopy (UTEG) n = 24	Standard transoral gastroscopy (STOG) n = 72
Mean age (range)		38 (19–62) y	39 (18–59) y
Gender		21 male (88%) 3 female	33 male (87%), 5 female
Educational attainment	Tertiary	13	45
	High school	8	19
	Primary School	3	8
Country of birth	Europe	8 (46%)	13 (33%)
	• *UK*	*5*	*11*
	• *Continental Europe*	*3*	*2*
	Non-Europe	16 (54%)	59 (77%)
	• *Australia/New Zealand*	*13*	*56*
	• *South Africa*	*3*	*1*
	• *North America*	*-*	*2*
Previous transoral gastroscopy		24	72
Previous transnasal gastroscopy		2	0
Duration of diagnosis of eosinophilic esophagitis		4 (0–9) years	3.5 (0–7) years
Food bolus obstruction events		17 (70%)	56 (80%)
Treatment			
• Successful elimination diet		9 (37.5%)	16 (22%)
• Unsuccessful elimination diet - budesonide		3	7
• Corticosteroids as only therapy		12 (50%)	47 (65%)
• Esophageal dilatation		0	2

Table 2 Post procedural questionnaire (initial UTEG, n = 24 patients)

Question	VAS[†] 0–25	VAS 25–50	VAS 50–75	VAS 75–100
1. "I am in general satisfied with the procedure"	Very satisfied n = 14	Satisfied n = 7	Dissatisfied n = 1	Very dissatisfied n = 2
2. "Please indicate the level of discomfort you experienced with the procedure"	Minor discomfort n = 16	Moderate discomfort n = 6	Severe discomfort n = 2	Very Severe discomfort n = 2
3. "I would prefer to have unsedated transnasal gastroscopy instead of standard transoral gastroscopy with sedation"	Strongly agree n = 18	Agree n = 3	disagree n = 1	Strongly disagree n = 2

[†]VAS = visual analogue score out of 100.

Table 3 Post procedural questionnaire (final UTEG, n = 22)

Question	VAS 0–25	VAS 25–50	VAS 50–75	VAS 75–100
"I am in general satisfied with the procedure"	Very satisfied 18	Satisfied 4	Dissatisfied 0	Very dissatisfied 0
"Please indicate the level of discomfort you experienced with the procedure"	Minor discomfort 16	Moderate discomfort 6	Severe discomfort 0	Very severe discomfort 0
"I would prefer to have unsedated transnasal gastroscopy instead of standard transoral gastroscopy with sedation"	Strongly agree 17	Agree 5	disagree 0	Strongly disagree 0

discomfort was experienced by most patients, severe in a minority. At first attempt, the majority of patients were very satisfied or satisfied with the procedure (88%). Levels of discomfort with the procedure tended to lessen with subsequent gastroscopies, and levels of satisfaction in turn rose in those with repeat procedures (Tables 2 and 3).

Histological sampling. The biopsy specimens taken at UTEG and STOG were judged adequate for histological assessment in all cases and in all locations. The sizes of the specimens taken at each location (upper, middle, and lower esophagus), using 2.0 mm forceps via UTEG, compared with large capacity 2.3 mm forceps at STOG, are shown in Table 4. Lamina propria sampling was achieved in a minority of those who underwent UTEG (22%) compared with those having STOG (68%; $P = 0.0070$, Fisher's exact test).

Discussion

UTEG has potential clinical and cost advantages particularly applicable to EoE, and the current study was undertaken to determine the feasibility, efficacy, and tolerance in this formerly unstudied patient group requiring repeated sampling over time. Previous data indicate that UTEG is safe and effective as a screening method for upper gastrointestinal malignancy when used in Japan and that histological sampling is adequate and well tolerated in older North American men with Barrett's esophagus.[6,7] The present study shows that UTEG is a safe and well-tolerated procedure in young predominantly white Caucasian male patients and that those electing to try the method are willing to undergo repeated procedures over time. Histological sampling is adequate for assessing the hallmark features of EoE. There was also interesting finding that an individual's country of birth was associated with the relative willingness to undergo the procedure.

The current study demonstrates that UTEG has a high degree of procedural success, with 71 of 74 procedures (96%) resulting in the requisite sampling of esophageal tissue. This is in keeping with previous studies on other patient groups.[2,6] Subsequent UTEGs required on these patients with EoE were all successful. The obstacles to completion of UTEG, being failure of nasal intubation because of enlarged turbinate's and excessive gagging, are similar to previous data. It is notable that the technique of application of local anesthetic varies between groups. In our study, local anesthetic was applied by spray to the nostril and posterior oropharynx, which is a common method, although others advocate the use of local anesthetic gel and application with a nasal catheter.[8] Procedures were performed at 45°, although some groups prefer an upright sitting position.[8] The safety of the procedure, with three episodes of minor epistaxis, some nasal soreness and stable respiratory observations is in keeping with previous other studies.[8]

Patient perception of the procedure in the present study was similar to earlier studies in other populations.[5,7] Minor nasal discomfort was the major side effect, and the majority of patients are satisfied with the procedure. The current study differs from others in that repeated procedures is performed over time. Of importance, all patients who underwent a successful transnasal gastroscopy were willing to have and did subsequently have at least one repeat procedure. A trend towards greater patient satisfaction and decreased procedural discomfort was observed with repeated procedures (Tables 3 and 4).

The patients in our study were reluctant to have UTEG instead of STOG. Only about one quarter of patients elected for UTEG. This is similar to the experience elsewhere, although our population was younger than those with Barrett's esophagus.[9] This would not necessarily reflect the proportion who would accept such a method in routine clinical practice, because these patients were provided a choice in an open manner that reflects good research practice. In routine practice, if sedated endoscopy was not readily available, uptake may have been greater. However, the present study was not designed to examine factors related to this choice. Nonetheless, those electing to undergo UTEG as opposed to STOG were more likely to have been born in UK, South Africa, or Continental Europe as opposed to those born in Australia or New Zealand. This may reflect cultural expectations and current medical practice. In Japan, for example 50% of gastroscopies undertaken for gastric cancer screening are via the transnasal route.[7] Age, gender, and education status did not influence the choice of endoscopic procedure in our group.

The histological samples taken via UTEG using the 2.0 mm biopsy forceps provided adequate specimens in all cases and in all esophageal locations. Estimation of eosinophil counts was possible. Tissue samples were measured to be within 1 and 3 mm in length in all UTEGs. Sampling of the lamina propria was rare. Previous work has demonstrated that the depth and length of tissue specimens obtained using 2.0 mm biopsy forceps is less than the standard 2.3 mm forceps.[8] STOG (with 2.3 mm biopsy forceps) enabled larger specimens to be taken and lamina propria to be sampled in nearly three times as many patients. Current diagnostic guidelines related to EoE do not mention analysis of the lamina propria, with treatment response guided by epithelial eosinophil count only; thus, deeper biopsies seem unnecessary in patients with EoE (as opposed to Barrett's esophagus where this may be important). Importantly, the mean eosinophil count was the same regardless of whether biopsies were taken via STOG or UTEG,

Table 4 Comparison of esophageal biopsies when obtained via UTEG or STOG

Biopsy site		Standard Transoral Gastroscopy (STOG) $n = 188$	Unsedated transnasal gastroscopy (UTEG) $n = 74$
Lower esophagus	Mean length (range)	3.3 (1.8–4.2) mm	1.8 (1–2.4)mm
	Lamina propria sampled	124 (66%)	17 (23%)
	Mean eosinophil count	24.3 (0–80)	22.6 (0–80)
Middle esophagus	Mean length (range)	3 (2–4)mm	2.1 (1–3)mm
	Lamina propria sampled	126 (67%)	18/74 (24%)
	Mean eosinophil count	23.2 (0–80)	23.1 (0–80)
Upper esophagus	Mean length (range)	3.1 (2–4)mm	1.8 (1–4)mm
	Lamina propria sampled	126 (67%)	17 (23%)
	Mean eosinophil count	21.4 (0–80)	21.8 (0–80)

discounting concerns that the depth of biopsies may influence diagnostic yield.[10] While a larger representative sample with larger forceps may have theoretical advantages as EoE is a patchy disease, the benefit of marginally more sampling of an organ whose surface area is approximately 125 cm^2 is debatable.[11] Thus, it is apparent in routine diagnosis and in planning response to treatment; biopsies taken with 2.0 mm forceps are adequate.

The financial implications of using transnasal gastroscopy and the potential savings were not addressed in the present study as patients were treated in the same day procedure unit and on the same list as those with other conditions undergoing transoral gastroscopy for other reasons. Nonetheless, future potential cost savings appear sizeable, particularly if the length of hospital admission (and required staffing) and the lack of a requirement for an anesthetist, nursing staff, and medications such as fentanyl and propofol are considered. Previous work indicates a cost saving of approximately $180 per procedure (in terms of costs to the health sector) in favor of transnasal gastroscopy.[9] Cost savings to the patient are also substantial, for example the ability to return to work on the same day of the procedure and to drive a car. In a patient requiring eight procedures as a part of the six-food elimination diet, the cost savings are multiplicative.[3] The true benefit of transnasal gastroscopy has arguably not been realized until offered in the outpatient setting, thus abrogating the need for hospital admission. Outpatient transnasal fibreoptic laryngoscopy is routinely used by ear, nose, and throat surgeons, highlighting the potential for future implementation in managing patients with EoE.[12]

Several limitations with the current study are acknowledged. Patient perception and adverse effects were not directly compared with STOG, but extensive data already exist confirming the acceptance and adequate histological sampling achieved by STOG, and the safety of UTEG. STOG and UTEG were not performed "head to head" on the same patient to determine the quality of tissue sampling, but once again, the quality of sampling in STOG is established, and as patient were undergoing treatment tissue sampling may have altered with the disease state *per se*. No attempt was made to determine or describe the quality of views obtained at video endoscopy using UTEG. UTEG can, however, provide adequate visualization for conditions where this is important, especially in Barrett's esophagus. In contrast, in EoE, the histological sample rather than the endoscopic appearance has diagnostic utility.[13,14]

The role that UTEG may play in the future management of EoE will depend on the efficacy of alternative minimally invasive techniques that sample the esophagus, or ideally novel biomarkers in, for example the blood or sputum. The cytosponge is one such technique that obtains esophageal tissue (without endoscopic visualization) and is apparently well tolerated, despite requiring the patient to sleep with the device *in situ*. The patchy nature of esophageal disease, with a tendency for lower esophageal infiltration arguably suggests that visually guided sampling of all esophageal areas is an advantage, as recently described[15] .Obviously, the utility to such techniques will only be clarified with systematic study.

In conclusion, UTEG is a safe, effective, and well-tolerated procedure in patients who have been diagnosed with EoE and agree to it. The advantages are particularly evident in this condition where multiple endoscopies are often required to assess the response to treatment via histological sampling. The major impediment to widespread implementation of UTEG for patients with EoE is initial reluctance to try the procedure, which relates to sociocultural expectations. Patient education and gastroenterological confidence that the technique is not compromising diagnostic ability may facilitate future widespread implementation of UTEG.

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APPENDIX 6

A prospective open clinical trial of a proton pump inhibitor, elimination diet and/or budesonide for eosinophilic oesophagitis

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SUMMARY

Background
Elimination diets and high-dose proton pump inhibitors (PPI) are advocated as first-line treatments in patients with eosinophilic oesophagitis (EoE).

Aim
To record the treatment outcome for patients with EoE prospectively managed according to a clinical algorithm.

Methods
Patients with oesophageal eosinophilia commenced esomeprazole 40 mg twice daily for 8 weeks. Those in histological remission were re-classified as PPI-responsive oesophageal eosinophilia. Nonresponders were offered the 6-food elimination diet with a PPI, or topical budesonide monotherapy (1 mg orally twice daily as an aqueous gel). Once disease control was achieved remission was reassessed at 3 months (all modalities) and an additional 6 months (diet group).

Results
Of 107 patients who completed 8 weeks of PPI, 25 (23%) were PPI-responsive. 56 of 81 (69%) of patients with EoE chose the elimination diet with PPI. 29 (52%) had complete remission, 23 completed dietary reintroduction and food triggers were identified in 20 (36%). 25 chose budesonide with 23/25 (92%) responding. Remission was sustained in >85% of patients at 3 months with all treatment modalities. At 9 months, only 10/18 (55%) of patients who responded to the elimination diet with PPI remained complaint and sustained remission.

Conclusions
Many patients previously diagnosed with EoE will respond to PPI. Initial response >50% is possible with the elimination diet plus PPI, but many will fail to undergo food reintroduction, or will cease the diet and relapse, resulting in only one in four patient sustaining remission at 9 months. Budesonide is very effective short term, but longer term study is needed.

INTRODUCTION

Eosinophilic oesophagitis (EoE) is a recently recognised and defined condition, characterised clinically by symptoms of dysphagia and food-bolus obstruction events and pathologically by oesophageal findings of eosinophilic infiltration, epithelial and muscular hyperplasia and resultant luminal narrowing.[1] Recent studies have demonstrated a response in many patients to high dose, twice-daily proton pump inhibitors (PPI) and to elimination diets, targeting food antigens thought to drive the inflammatory process in the context of a failure of immune tolerance.[2–4] International organisations have been quick to integrate the findings of a number of therapeutic clinical trials into published management guidelines with high-dose PPI advocated for all patients with suspected EoE. Those responding to twice-daily PPI are labelled as PPI-responsive oesophageal eosinophilia rather than EoE. For those with EoE, dietary therapy is recommended as a first-line therapeutic option.[3] It is apparent, however, that the 'external validity' of this recommendation – the efficacy of such treatment outside of the closely supervised clinical trial setting, in alternative patient groups and followed over long-time periods – has yet to be established.[5] This would seem particularly important given the awkward nature of dietary therapy, the potential for region-specific allergens to alter sensitisation and thus therapeutic efficacy, and the unknown mechanism whereby PPIs exert their therapeutic effect.[6, 7]

The aims of the present study were, therefore, to record the clinical outcome of patients enrolled prospectively and managed according to a structured clinical algorithm.

## MATERIALS AND METHODS

Patients

This study prospectively examined all patients aged 18 years or older referred to the gastroenterology outpatient clinic of two tertiary hospitals (Box Hill Hospital and The Alfred Hospital, Melbourne, Australia) between September 2013 and January 2015 with oesophageal eosinophilia. This finding must have been made by previous upper gastrointestinal endoscopy and biopsy demonstrating an oesophageal eosinophil count of ≥15 eosinophils per HPF in at least one section. Excluded were patients with gastric or duodenal eosinophilia, and those taking medications or with medical conditions likely to produce eosinophilia or alter results (e.g. antiepileptics, inhaled corticosteroids or oral corticosteroids for asthma, lymphoproliferative conditions). All participants provided written informed consent before commencement of the study. The study protocol was approved by the Eastern Health and Monash University Human Research and Ethics Committees.

Study protocol

This was a prospective observational study. The design was quasi-experimental and involved a removed-treatment method in that patients entering the study on corticosteroids were required to cease these medications for 8 weeks prior to commencing the treatment algorithm, as shown in Figure 1. All patients were required to take esomeprazole 40 mg orally twice-daily for 8 weeks followed by gastroscopy and biopsy of the oesophagus, stomach and duodenum. If oesophageal eosinophil density was <15/HPF then PPI-responsive eosinophilia was diagnosed and PPI therapy continued. After 3 months, these patients underwent repeat gastroscopy and biopsy. They then left the study protocol irrespective of the results. Patients who were not PPI-responsive were offered the choice of one of two therapies:

(i) *Dietary therapy with the six-food elimination diet*: PPI therapy was continued at the same dose. Written
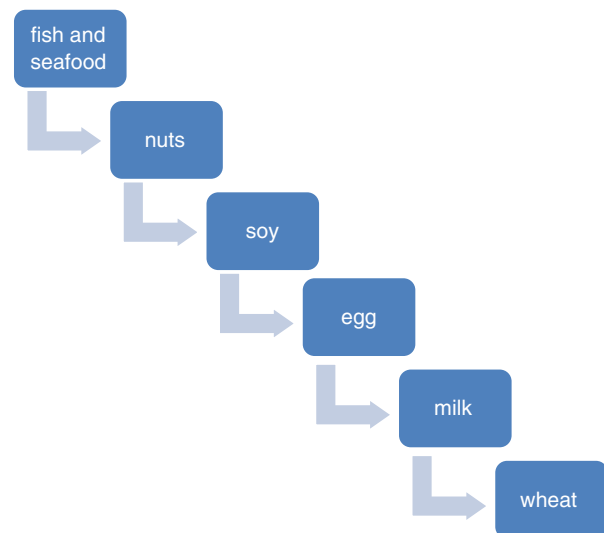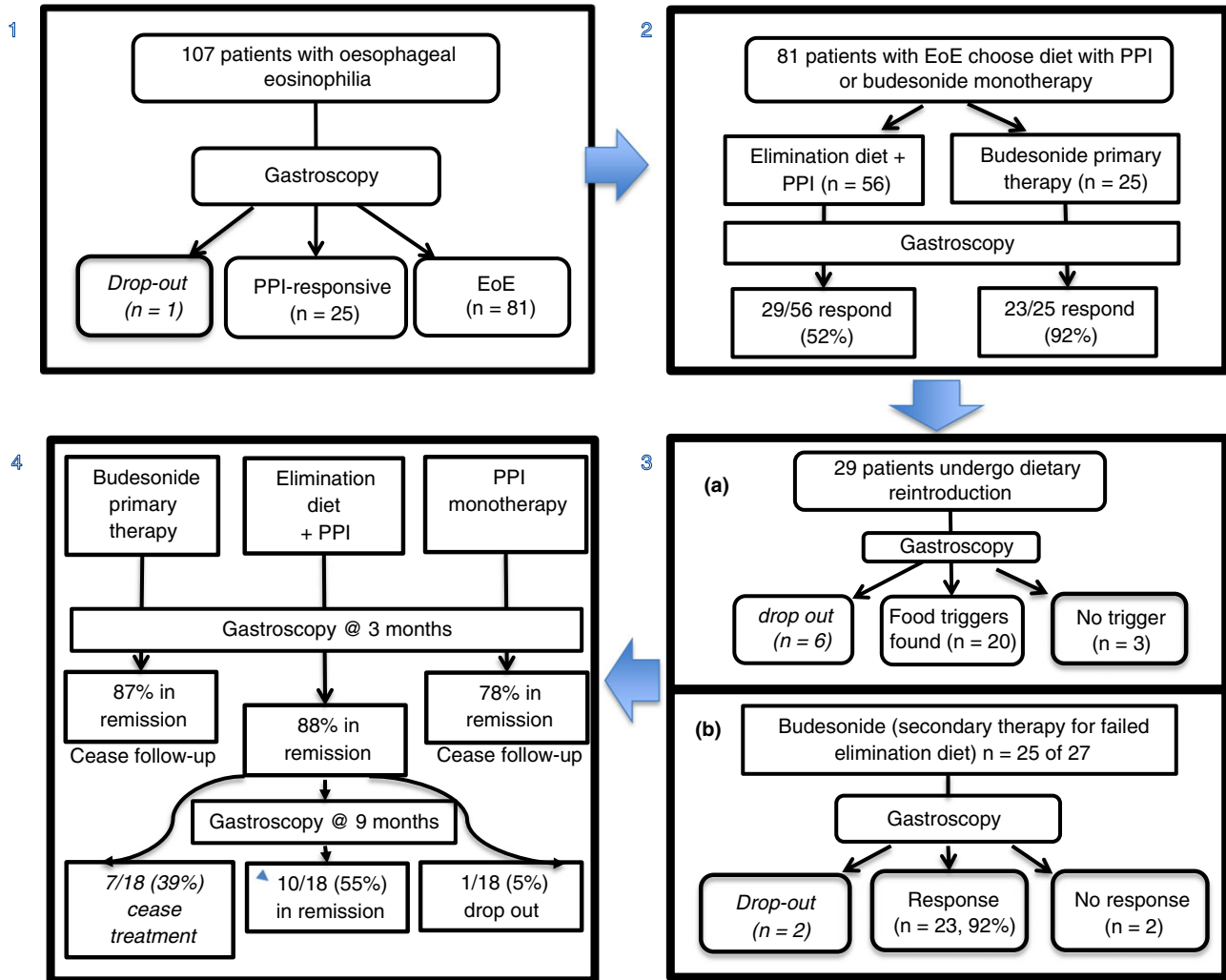

Figure 1 | Process and order of dietary reintroduction in patients initially responding to the six-food elimination diet. Gastroscopy is performed 2 weeks following food introduction. If recurrence of eosinophilia is detected, the culprit food is removed and a 4 week (wash-out) interval occurs before repeat gastroscopy.

information about the diet was provided to all patients and consultation with a dietitian was offered. Patients who responded to dietary therapy had sequential reintroduction of foods according to the algorithm in Figure 2. Briefly, this involved food challenges (where the participant was instructed to consume at least one serve of the additional, 'culprit' food at least twice per day (e.g. one slice of bread per twice-daily, one glass of milk or one egg twice-daily) followed by gastroscopy in 2 weeks. Absence of oesophageal eosinophilia led to a challenge with the next food type, whereas recurrence of eosinophilia led to exclusion of that food type.

Figure 2 | Treatment outcome of patients presenting with oesophageal eosinophilia and diagnosed with proton pump inhibitor-responsive oesophageal eosinophilia or eosinophilic oesophagitis (EoE) after induction therapy following a structure treatment algorithm using combinations of proton pump inhibitors, 6-food elimination diet and to topical budesonide 1 mg twice-daily). Treatment outcome is divided into four phases:

(i) Determine if EoE or PPI-responsive oesophageal eosinophilia is correct diagnosis with esomeprazole 40 mg twice-daily and gastroscopy after 8 weeks

(ii) Patients choose if they wish to have budesonide monotherapy or elimination diet and PPI, with the results determined by gastroscopy after 6 weeks

(iii) (a) Patients responding to elimination diet undergo process of food reintroduction and repeat gastroscopy. (b) Patients failing elimination diet choose budesonide monotherapy and have repeat gastroscopy.

(iv) Determine durability of treatment. Outcomes for patients with PPI-responsive oesophageal eosinophilia (on esomeprazole 40 mg twice-daily) and EoE (budesonide monotherapy or diet with PPI) as determined by gastroscopy and biopsy were assessed at 3 months. The durability of diet with PPI therapy also determined at 9 months.

Subsequent food challenge was given immediately when a putative food trigger failed to cause eosinophilia, while a positive response lead to food removal and a 4 week 'washout' period. Partial responders continued the culprit food for a further 2 weeks and repeated the gastroscopy, and if this persisted dietary failure was defined. Those who failed the elimination diet were offered budesonide.

(ii) *Oral topically acting corticosteroids*: PPIs were ceased on commencement of budesonide that was made up from 1 mg/2 mL ampoule mixed with sucralose to make a gel and administered twice-daily. Gastroscopy and oesophageal biopsy were performed 6 weeks following commencement.

Patients in remission at the completion of the elimination diet (and subsequent reintroduction) or on budesonide had a repeat gastroscopy after 3 months. Patient on dietary therapy were offered repeat gastroscopy every 3 months for 12 months post-remission.

Endoscopy, biopsy and histological assessment

All gastroscopies were performed by gastroenterologists from the respective departments of both hospitals, the majority by HP and SN. Biopsies were taken from the lower oesophagus (defined as 5 cm proximal to the gastro-oesophageal junction) and from the middle and upper oesophagus at 5 cm intervals. Four specimens of tissue were taken at each location. Transoral and transnasal gastroscopes were used, sedation use as previously described.[8] Specimens were transported in 4% neutral-buffered formalin, then imbedded in paraffin and stained with haematoxylin and eosin. Standard histopathological analysis of gastric and duodenal biopsies occurred. The peak eosinophil count was recorded in all three areas of the oesophagus in the most densely infiltrated areas, where 10 respective areas analysed at HPF (400 times magnification, area measured in each case 0.212 mm^2) were averaged to give the mean eosinophil count. All specimens were reviewed by consultant pathologists blinded to the treatment method. The same pathologist reported the results of each individual patient over time. The accuracy of eosinophil counts independently was cross-checked by HP and SR using digital technology (Aperio imagescope), the results were almost identical and led to no change in categorisation.

Demographic data

All patients were assessed by a gastroenterologist (HP) and the following data were recorded: date of birth, country of birth, migration and date of migration from overseas, coexistent allergic conditions, previous food bolus obstruction events, date diagnosed, previous treatment and current symptoms.

Outcome measures

The success of any treatment modality was defined according to histopathology. A *complete response* was defined as <5 eosinophils per HPF in all oesophageal locations, a *partial response* as 5−14 eosinophils is one or more location, and *no response* as 15 or more eosinophils in one or more location.

Statistical analysis

The demographic data were expressed as mean and standard deviation or as a percentage of the total individuals. Only patients who had endoscopic evaluation after 8 weeks' PPI therapy were included in the analysis. For categorical data, inter-group comparisons were made using Chi-square analysis, while maintenance of disease remission among treatment groups was assessed using the one-way ANOVA. The paired *t*-test was used to compare mean eosinophil counts post-treatment. A $P \leq 0.05$ was considered significant. Microsoft Excel was used for statistical analysis.

RESULTS

Patient characteristics and clinical features

A total of 156 patients were invited to participate, 115 patients were enrolled to study, seven failed to return for gastroscopy following 8 weeks of treatment with esomeprazole. Thus, 107 were included in the analysis. Demographic details are listed in Table 1. The male predominance, white Caucasian racial background and mean age of 37 years are evident. All patients had been previously treated with either a single therapy or combination therapies including PPI and topically acting corticosteroids. None had received systemically acting corticosteroids.

Treatment outcome

Treatment outcomes are shown in Figure 2. For clarity, patient management is considered in four phases;

Diagnosis of EoE or PPI-responsive oesophageal eosinophilia. The initial therapy with twice-daily PPI induced a complete response in 25 (23%), of whom 12 (48%) had previously received a daily PPI and 2 (8%) twice-daily PPI. Thus, 25 of the 107 patient with oesophageal eosinophilia were labelled as PPI-responsive oesophageal eosinophilia and 82 patients had a diagnosis of EoE.

Table 1 | Demographic and clinical data on the 107 evaluable patients with oesophageal eosinophilia and according to a diagnosis of proton pump inhibitor-responsive oesophageal eosinophilia (PPI-REE) or eosinophilic oesophagitis (EoE)

Index	PPI-responsive oesophageal eosinophilia (n = 25)	Eosinophilic oesophagitis (n = 82)	P value
Mean age (s.d.)	44 (14)	34 (11)	<0.01
Male gender	18 (72%)	69 (84%)	N.S.
Mean age (s.d.) at diagnosis	42 (11)	32 (10)	<0.05
Ethnicity			
White Caucasian	23 (92%)	80 (98%)	N.S.
Asian	1 (4%)	1 (1%)	N.S.
Middle Eastern	1 (4%)	1 (1%)	N.S.
Presence of atopic illness			
Seasonal rhinitis	16 (58%)	36 (44%)	<0.05
Asthma	5 (20%)	16 (19%)	N.S.
Food allergy or oral-food allergy syndrome	3 (12%)	6 (7%)	N.S.
Coeliac disease	2 (2%)	2 (2%)	N.S.
Helicobacter pylori positive (at initial endoscopy post 8 weeks of BD esomeprazole)	1 (1%)	2 (2%)	N.S.
Presenting symptom			
Food bolus obstruction	16 (64%)	31 (38%)	<0.05
FBOE and dysphagia	5 (20%)	25 (30%)	N.S.
Dysphagia alone	3 (8%)	19 (23%)	<0.05
Heartburn	1 (1%)	4 (5%)	N.S.
Other	0	4 (5%)	N.S.
Previous treatment of oesophageal eosinophilia			
PPI – daily	12 (48%)	66 (80%)	<0.05
PPI – BD	4 (16%)	8 (10%)	N.S.
Swallowed topically acting corticosteroid	8 (32%)	35 (43%)	N.S.
Diet	0	0	–
No treatment	0	0	–

Patient-directed choice of first-line therapy for EoE: trial of diet with PPI or budesonide monotherapy. Characteristics of patients choosing budesonide monotherapy or diet with budesonide are shown in Table 2. Of the 56 patients who elected to follow the elimination diet with PPI, 29 (52%) responded completely while 27 (48%) recorded persistent eosinophilia. In patients who did not respond, however, the eosinophil count did fall in all three regions of the oesophagus from a pre-diet eosinophil count in the upper, middle and lower oesophagus respectively of 36, 39 and 35 per HPF to a post-diet eosinophil count of 19, 19 and 24 per HPF respectively ($P \leq 0.005$; paired t-test). A dietitian was consulted by 14/29 (26%) of responders and 14/27 (52%) of non-responders. Two were initially defined as partial responders, but both had relapsed at the repeat biopsies after 4 weeks.

Budesonide monotherapy induced complete resolution in 23/25 (92%) of patients.

Dietary reintroduction or trial of budesonide according to response to diet. Of the 29 patients who responded

Table 2 | Comparison of patients who chose either dietary therapy or budesonide after failing to respond to twice-daily PPI

Index	Elimination diet (n = 56)	Budesonide (n = 25)
Mean age (s.d.)	36 (10.4) years	39 (12) years
Male gender	40/56 (71%)	19/25 (76%)
Previous treatment		
Budesonide	27/56 (48%)	7/25 (28%)
Diet	0	0
Mean eosinophil count prior to treatment (s.d.)		
Upper	24 (9)	28 (8)
Middle	32 (9)	29 (9)
Lower	29 (7)	31 (8)
Mean eosinophil count after treatment		
Upper	0	0
Middle	0	0
Lower	0	0

to the elimination diet, 23 had food triggers defined and again achieved resolution of eosinophilia following removal of the culprit food or foods. Six patients

dropped out before completing the process of reintroduction and three reintroduced all foods, but still failed to define a food trigger. A preponderance of lower oesophageal eosinophilia was observed in response to culprit foods. Thus, participants who were in remission following the elimination diet and who had a disease recurrence following food introduction demonstrated isolated lower oesophageal eosinophilia in 12 of 34 such flares, lower and middle oesophageal eosinophilia in a further 14 of 34, and isolated upper oesophageal eosinophilia in only one case ($P = 0.005$; Chi-square). The focal eosinophilia was deemed secondary to food exposure as this resolved during the wash-out phase in 32 of 34 such flares (one lost to follow-up, the other failed to resolve and abandoned the diet). As outlined in Table 3, common food triggers included gluten, dairy and eggs, alone or in combination.

Of the 27 patients who failed to respond to the elimination diet, 25 elected to commence budesonide monotherapy and 23 (92%) responded.

Maintenance and comparison of treatment durability. The durability of responses after the 'induction' ther-

apy was assessed at 3 months in the majority of patients. Fourteen of 18 patients (78%) maintained remission after continuing twice-daily esomeprazole for a further 3 months. Twenty of 23 patients (87%) had continuing remission at 3 months while taking budesonide. Sixteen of 18 patients assessed at 3 months on a maintenance diet (88%) were in remission. Patients on the elimination diet were in addition followed up over 9 months, where 10/18 (55%) continued the diet and were in remission, 7/18 (39%) had ceased the diet and one was lost to follow-up.

DISCUSSION

This prospective study evaluated the external validity, or real-world application, of the various contemporary treatment strategies available for adult patients with oesophageal eosinophilia. Twice-daily PPIs are advocated as the initial treatment of oesophageal eosinophilia, and for the differentiation of EoE and PPI-responsive oesophageal eosinophilia.[3] Our cohort referred with suspected EoE included many previously under the care of specialist gastroenterologists, and, despite this, only a minority had received twice-daily PPIs highlighting a deviation from currently recommended practice. Subse-

Table 3 | Longer term follow-up of individuals with eosinophilic oesophagitis who responded to the six-food elimination diet

Patient	Age (years), sex	Foods avoided	Follow-up oesophageal histology		
			3 months	6 months	9 months
1	55, female	Gluten*	Remission	Remission	Remission
2	58, female	Gluten	Remission	Not done	Remission
3	21, male	Gluten	Not done	Remission	Remission
4	44, female	Gluten	Remission	Not done	Remission
5	54, female	Gluten	Remission	Remission	Remission
6	29, male	Gluten	Not done	Remission	Ceased diet/flare
7	62, male	Dairy	Remission	Remission	Remission
8	19, male	Dairy	Remission	Not done	Remission
9	61, male	Dairy	Remission	Not done	Remission
10	19, male	Egg	Remission	Remission	Pending
11	19, male	Egg	Remission	Ceased diet	Ceased diet
12	64, male	Soy	Remission	Remission	Ceased diet
13	49, female	Dairy, egg, gluten, nut	Remission	Remission	Not done
14	27, male	Nut, fish	Remission	Remission	Remission
15	33, female	Dairy, egg	Remission	Ceased diet	Ceased diet
16	52, male	Dairy, egg	Ceased diet/flare	Ceased diet	Ceased diet
17	48, male	Gluten, egg, dairy	Remission	Remission	Ceased diet
18	19, female	Egg, soy	Remission	Remission	Remission
19	35, male	Gluten, egg, dairy	Ceased diet/flare	Ceased diet	Ceased diet
20	34, male	Gluten, dairy	Remission	Remission	Not done

* Gluten-containing food was avoided in according with guidelines issue for coeliac disease.

quently, one quarter of all patients previously diagnosed with EoE were re-categorised as being PPI-responsive. Interestingly, 12 of 25 patients (48%) responding to twice-daily PPIs had a documented history of daily PPI for at least 4 weeks *before* a gastroscopy and initial diagnosis of EoE. This suggests a dose–response relationship, although other variables including the individual responsiveness to different PPIs and adherence to the prescribed regimen, as well as the number and location of oesophageal biopsies may have played roles. This is an important issue because the obvious corollary is whether or not patients with PPI-REE must be maintained on twice-daily PPI indefinitely or transitioned to daily therapy. The related issue of durability of PPI response is questioned by the finding of relapse at 3 months in 22% of patients despite apparent adherence. Both the durability of PPI and the possible dose–response relationship was addressed in a very recent study, where patients were transitioned from twice to once daily PPI and a 18% relapse rate demonstrated.[9] PPI-responsive oesophageal eosinophilia had previously been defined using varying doses and durations of PPIs, a systematic review identifying eight studies all with different doses and variable use of pH studies (see below).[10] Relapse of eosinophilia on PPI was identified in only six of 258 patients in a pooled analysis.[10] Closer supervision of a larger number of patients over a longer time period in further dedicated studies may better answer if durability and/or dose related effects are important considerations.[5, 11]

The response to the elimination diet in the current study was numerically inferior to previous dedicated controlled trials of elimination diet (<55% compared to >65%).[2, 12] This may relate to the relative lack of structured resources available to our patients (such as a clinical trial nurse), and the variable use of a dietitian, which was dictated by patient-choice in line with routine practice. However, the current study was not adequately powered to examine such issues. The continued surveillance of patient on dietary therapy over 9 months demonstrated that only 10/18 (55%) remained compliant and in remission. This has major implications as to the real-world application of the diet suggesting that efficacy is compromised presumably due to factors such as palatability. Our study differed from previous work in that twice-daily PPIs were continued for all patients, oesophageal pH studies were not performed, gastroscopes occurred at 2 weeks (instead of four or more weeks) after each food was introduced and biopsies were taken from the lower, middle and upper oesophagus (in-

stead of two locations).[2, 12] However, it is unlikely that continuation of high-dose PPIs had influenced the initial success of dietary therapy and this may have in fact increased the response rate (see below). The duration of food reintroduction appears adequate as previous work has demonstrated recurrence of eosinophilia within 72 h of rechallenge.[13] Taking more biopsies may have decreased the number of patients declared as responsive to the elimination diet, although previous work indicates five samples taken from two locations should diagnose all cases of EoE.[14] Indeed eight of 27 patients (30%) who were determined to have initially failed dietary therapy had eosinophilia in the lower oesophagus only. Intriguingly, the combination of diet and PPIs did decrease the mean eosinophil count and in all oesophageal locations in patients deemed unresponsive to the elimination diet. This is a novel observation that we speculate may have relevance in patient's refractory to monotherapies, and arguably suggests polysensitisation with some reduction of antigenic load with the elimination diet (see Figure 2). Three of the 29 patients who responded to the elimination diet continued in remission despite reintroduction of all foods. This also has not been previously reported. It is speculated that these patients have become tolerant to the putative food antigens in concert with continuation of the PPIs. Close follow-up with repeated gastroscopies over time may answer this question.

The response to budesonide therapy in our cohort is higher than attained in other adults studies and more akin to the response commonly demonstrable in paediatric centers.[15–18] Potential reasons to explain this difference might include better adherence to the therapy, though this was not formally assessed, the careful explanation of the timing of budesonide therapy (before bed, after brushing teeth and after breakfast after brushing teeth) and instructions to avoid eating or drinking for at least 2 h after the budesonide, and the use of a budesonide slurry rather than powder. Our patient cohort is very similar in terms of demographics and risk factors to previous studies.[19]

The sparing of the upper oesophagus, and predominance of lower and middle oesophageal eosinophilia in our cohort of patients maintained on twice-daily PPIs and who received the elimination diet is worthy of special consideration. Our findings, which differ from previous work where generalised eosinophilia has been noted following food reintroduction,[2, 12, 13] could potentially be related to the continuation of twice-daily PPIs in all patients in our study, the shorter duration between

food exposures and biopsies (2 weeks compare to greater than 4 weeks) and the supposition that PPIs reduce eotaxin-3 predominantly in the upper oesophagus.[2, 6, 12] From a clinical standpoint, we assert that patients undergoing elimination diets must have lower oesophageal biopsies taken. We also speculate that the lower oesophagus is most important in initiating a recurrence of eosinophilia to trigger foods, possibly due to increased exposure to food antigens due to reflux of gastric contents, in a time-dependent manner. The effect of gastric refluxate in causing lower oesophageal eosinophilia, possibly by influencing barrier integrity, has been proposed previously and warrants further study.[20]

Several weaknesses are acknowledged in the design of the current study. First, treatments were not randomised or placebo-controlled. Patients who did not respond to PPI were thus able to choose if they received dietary therapy or budesonide, and this limits the validity of comparative data, particularly as many patients had received budesonide (albeit in dry powder form) previously. However, our aim was to evaluate the external validity of contemporary management outside of a clinical trial setting. A related point is that the efficacy of previous treatments for oesophageal eosinophilia (prior to enrolment) was not available and thus comparable, preventing analysis of secondary treatment success. Indeed the response to PPI therapy in defining PPI – REE is considerably lower than reported elsewhere.[21] This may be explained by the recruitment method and treatment setting, whereby patients were often referred by gastroenterologists in private practise to our specialist academic centre. Second, pH studies were not performed, potentially falsely ascribing cases as EoE that were in fact GERD. However, clinical guidelines advocate empirical use of twice-daily PPI as described, citing a failure of pH studies as a discriminative tool.[3, 10] Third, the use of frequent gastroscopies to evaluate oesophageal eosinophilia may be considered cumbersome, hazardous and expensive. Certainly, we acknowledge the cost of this approach, although the potential for lifelong remission may offset the initial outlay. We utilised transnasal gastroscopy in some patients, and previous work by our group indicates the safety of either nonsedated transnasal or standard transoral gastroscopy with sedation.[8] Fourth, PPIs were continued for patients who responded to the elimination diet. Currently, clinical guidelines do not specify the use of PPI with dietary therapy. The notion of combination therapy is proposed and subsequent follow-up by our group may help answer this question. Patients commenced on budesonide therapy on the other hand had their PPI ceased, as described in the majority of previous studies. In contrast, PPI use has varied in association with dietary treatment.[2, 12, 15, 16, 18] Thus, our trial design we felt better reflected current reasonable clinical practice in this 'real world' study. A fifth point is that symptoms (e.g. dysphagia), endoscopic features and blood tests were not recorded or analysed. Previous data however have demonstrated a poor correlation between these features and eosinophil count at biopsy.[22] Admittedly, ongoing work in refining these relationships shows promise.[23, 24] Finally, ideally all patient groups would have been followed – up for a longer interval (at least for 9 months as for patients receiving the elimination diet). Given the invasive nature of surveillance requiring gastroscopy, and the relative abundance of data pertaining to PPI and budesonide use in EoE, this was not undertaken.[18]

In conclusion, the current real-world experience suggests that re-evaluation of PPI therapy in those patients presenting with oesophageal eosinophilia may be required both in the durability of resolution of that finding in those deemed to have PPI-responsive oesophageal eosinophilia, and in its continued use in patients responding to the six-food elimination diet. The current approach as indicated by clinical practice guidelines warrant further evaluation given the limited initial response to the elimination diet, the cumbersome nature of the diagnostic process and the tendency of patients to cease the diet as determined at 9 months follow-up. In contrast, budesonide will successfully treat more the 90% of patients and few gastroscopies are required, suggesting that the latter is a more viable first-line therapy. If dietary therapy is considered, biopsy of the lower oesophagus should be performed given the predilection for disease recurrence during food reintroduction in this area.

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APPENDIX 7

Letter to the Editor

Letter: avoiding misconceptions about elimination diet for eosinophilic oesophagitis – authors' reply

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Sirs, We thank Molina-Infante et al. for their interest and comments[1] illustrating controversies regarding treatment allocation and histological sampling of adult patients with eosinophilic oesophagitis (EoE), and our alternative emphasis on real world clinically acceptable investigation, treatment and outcome measures.

First, regarding combined use of diet with proton pump inhibitor (PPI), we agree that this methodology may not be employed by future researchers, but equally attest that previous work was marred by a lack of consistency with regard to pre-diet PPI trial and/or the use of pH studies.[2] The initial published study in this field arguably arose prior to the current understanding of PPI-responsive oesophageal eosinophilia (REE), and many patients may have been re-categorised had a PPI trial occurred prior to dietary therapy.[2] Continuing PPI therapy during the dietary therapy in our study, not only saved precious time (that would have been required for wash-out) but also the need for a repeat gastroscopy prior to commencing dietary therapy.

A second and related issue is that we did not compare diet and PPI with budesonide and PPI, but rather used budesonide monotherapy. Budesonide with PPI in our view would be foolish, given the already significant risk of oropharyngeal candidiasis with monotherapy, and because budesonide has demonstrable efficacy as a sole agent.[3]

Molina-Infante et al. suggest longer periods of food challenge may be required to determine individual culprit foods (e.g. 6 weeks compared to the 2-week period in our study).[4,5] This may be so, although Pedersen et al. demonstrated recurrent eosinophilia within 3–7 days in patients controlled on elemental diet and PPI. The notion that EoE is an IgG mediated disease, and thus of delayed onset, is controversial.[6]

In terms of 4-food or 6-food elimination diets, only two groups apart from ourselves have published data, namely that of Molina-Infante et al. themselves, and a North American group.[7] The only way to answer this question would be to do repeated gastroscopies at set time intervals following the introduction of each food, e.g. at 3 days, 2 weeks and 6 weeks. In reality, the need to deliver acceptable and safe treatment regimens dictated our study algorithm. That is, we deemed an extremely restrictive diet that should be used for the minimum possible period of time.

Finally, our observation that only 55% of patients remained compliant with their diet at 9 months, the rest relapsing with eosinophilia is, we believe, a fair assessment.[8] Figure 2 clearly demonstrates that 7/18 patients representing for gastroscopy had ceased treatment and relapsed, one patient dropped out and 10/18 (all compliant patients) sustained remission. The question 'can dietary therapy cause remission of EoE' has already been comprehensively answered. Our contribution is, that outside of expert centres, the response to dietary therapy and the long-term compliance, and thus success, is modest.

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APPENDIX 8

Invited Editorial

Editorial: management of eosinophilic esophagitis – efficacy vs. effectiveness. Authors' reply

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We thank Frazier *et al.* for their insightful commentary concerning our paper concerning the treatment outcome of adult patients with eosinophilic oesophagitis (EoE).[1, 2] We agree with much of the analysis, although a few clarifications appear justified.

The use of twice daily proton pump inhibitors (PPIs) for 8 weeks prior to the diagnosis of PPI-responsive oesophageal eosinophilia is (as eloquently summarised by Frazier *et al.*) an arbitrary intervention based upon the GERD treatment paradigm. Indeed, so called 'step – down' therapy, where the dose of PPI is reduced (and remission sustained in most patients) suggests that lower doses may be effective in treating the oesophageal eosinophilia.[2] The mechanism whereby PPI's exert their therapeutic effect is of ongoing debate, and effects distinct from the ability to decrease gastric pH appear possible, including the ability to downregulate eotaxin-3 expression.[3]

It may however be premature to conclude that the ability of PPIs to increase gastric pH plays no part in treatment response. It is not unreasonable to propose that decreasing refluxate of acidic, and thus erosive, gastric contents will improve barrier integrity and lessen food antigen interaction with the immune system.[4] Evolving research appears aimed at determining the significance of proposed impairments in barrier function in this patient group.[4]

Frazier *et al.* point out a number of potential limitations in study design including that perceived as a lack of treatment standardisation, no symptom report measures, and an intensive endoscopy protocol. It is our belief that treatment was standardised both in terms of dose and duration of medication (esomeprazole 40 mg PO BD or budesonide 1 mg PO b.d.), duration and sequence of dietary reintroduction, that endoscopic surveillance was the minimum necessary, and not dissimilar to previous studies, and that symptom report correlates poorly with disease relapse in any case.[5] Furthermore, we utilised minimally invasive transnasal endoscopy without sedation in some patients.[6] In the future, alternatives such as cytosponge may decrease the need for endoscopy.[7]

The difference between effectiveness and efficacy appears to hold particular significance when considering the steps required to institute successful dietary therapy in EoE. Previous studies had conclusively demonstrated that resolution of oesophageal eosinophilia can be achieved with dietary 'restriction'.[8, 9] What had been overlooked was the need to 'reintroduce' foods (requiring multiple gastroscopies) and then 'maintain' a diet. A clearer perspective of the difficulties likely to be encountered is thus achievable if this process is considered to entail multiple steps. The results of our study are testament to the real world difficulties of such a regimen.

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APPENDIX 9

Allergy tests do not predict food triggers in adult patients with eosinophilic oesophagitis. A comprehensive prospective study using five modalities

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SUMMARY

Background
The use of allergy tests to guide dietary treatment for eosinophilic oesophagitis (EoE) is controversial and data are limited. Aeroallergen sensitisation patterns and food triggers have been defined in Northern Hemisphere cohorts only.

Aims
To determine if allergy tests that are routinely available can predict food triggers in adult patients with EoE. To define the food triggers and aeroallergen sensitisation patterns in a novel Southern Hemisphere (Australian) cohort of patients.

Methods
Consecutive patients with EoE who elected to undergo dietary therapy were prospectively assessed, demographic details and atopic characteristics recorded, and allergy tests, comprising skin-prick and skin-patch tests, serum allergen-specific IgE, basophil activation test and serum food-specific IgG, were performed. Patients underwent a six-food elimination diet with a structured algorithm that included endoscopic and histological examination of the oesophagus a minimum of 2 weeks after each challenge. Response was defined as <15 eosinophils per HPF. Foods defined as triggers were considered as gold standard and were compared with those identified by allergy testing.

Results
No allergy test could accurately predict actual food triggers. Concordance among skin-prick and serum allergen-specific IgE was high for aeroallergens only. Among seasonal aeroallergens, rye-grass sensitisation was predominant. Food triggers were commonly wheat, milk and egg, alone or in combination.

Conclusions
None of the currently-available allergy tests predicts food triggers for EoE. Exclusion-rechallenge methodology with oesophageal histological assessment remains the only effective investigation. The same food triggers were identified in this southern hemisphere cohort as previously described.

Aliment Pharmacol Ther

INTRODUCTION

Eosinophilic oesophagitis (EoE) may be successfully treated by removing from the diet food antigens that are responsible for inciting the immune reaction and characteristic pathological changes demonstrable at gastroscopy and tissue biopsy.[1] While elimination diets are successful, the requirement for multiple gastroscopies – one after each food is reintroduced requiring eight or more in the case of the six-food elimination diet – makes this management untenable to many.[2] The need for non-invasive, once-off investigations that can accurately predict food triggers in EoE is hence obvious. To date, skin-prick and skin-patch testing combined have shown marginal benefit in guiding dietary treatment in paediatric patients with EoE, while skin-prick alone or in combination with specific serum IgE for adult patients was of no benefit in two prospective studies.[3–5] The allergy tests so far utilised for EoE have been borrowed from experience gained with other disease states. Thus, for conditions characterised by the development of symptoms within minutes (oral-food allergy syndrome, food allergy with anaphylaxis, atopic rhinitis or atopic asthma), skin-prick or specific serum IgE are validated, clinically useful and conceptualised to measure immediate hypersensitivity (Gell and Coombe type 1).[6, 7] EoE, a condition that has slow onset and is slow to resolve, is more akin to contact dermatitis, and thus skin-patch testing was used and validated for the latter as a measure of type 4 (cell-mediated) immunity has also been adopted.[5, 8] However, for unknown reasons, all of these investigations have so far failed to deliver acceptable accuracy such that empirical dietary therapy followed by repeat gastroscopy is advocated.[9]

The basophil activation test measures acute IgE-mediated (type 1) immune responses, as well as non-IgE-mediated responses, and has a role in detecting drug allergy and anaphylaxis to food and aeroallergens, particularly when skin-prick tests are unavailable or contraindicated.[10, 11] The basophil activation test is of unknown value in EoE. We hypothesised that the basophil activation test may have utility in EoE, particularly given the central role that non-IgE-mediated basophil activation may play in controlling the inflammatory response. This has been demonstrated by the so-called 'basophil-TLSP axis' (basophil – thymic stromal lymphopoietin axis) in animal models.[12, 13] Briefly, TLSP, a cytokine produced by epithelial and stromal cells, can activate basophils, which express TSLP receptors. In a murine model, EoE will only develop in the presence of TSLP and basophils.[13]

Patients with EoE may be atopic, with coexistent allergic conditions and elevated IgE levels to common aeroallergens. Recently, dense oesophageal deposits of IgG and elevated serum IgG to common food allergens have been demonstrated in EoE, raising the hypothesis that IgG and IgE may mediate the disease.[3] Serum IgG levels have been used by alternative medical practitioners to guide elimination diets targeting a range of gastrointestinal conditions, although the validity and rationale of this approach is questioned.[14] We hypothesised that serum IgG levels to common food antigens will reflect immune tolerance, and will have no predictive value in guiding dietary elimination.

Food and aeroallergens may be important in the pathogenesis of eosinophilic esophagitis (EoE). To date, studies performed in the Northern Hemisphere have concluded that seasonal exacerbations of EoE may be related to tree pollinosis, with birch-pollen sensitisation (and potential cross-sensitisation with food allergens) being particularly common.[15, 16] Lacking are data relating to regions where grass pollinosis (as opposed to tree pollinosis) is predominant, with the corollary that food allergen sensitisations may in turn be influenced and be different.

In view of the apparently strong relationship between immune responses to specific antigens and the pathogenesis of EoE, and of its association with atopy in at least a northern hemisphere population, this study had two aims. First, we determined if skin-patch and skin-prick testing, the basophil activation test, specific serum IgE and serum IgG levels to food antigens, as available in routine clinical practice, predicts proven food allergens in patients with EoE undergoing an elimination diet. Second, we sought to characterise the demographic details and atopic characteristics of a novel 'southern hemisphere' cohort of patients.

METHODS

Recruitment

This study prospectively examined patients aged 18 years or older presenting to the gastroenterology outpatient clinic of two tertiary hospitals (Box Hill Hospital and The Alfred Hospital, Melbourne, Australia) between September 2013 and January 2015 with oesophageal eosinophilia. This finding must have been made previously by upper gastrointestinal endoscopy and biopsy demonstrating an oesophageal eosinophil count of ≥15

eosinophils per HPF in at least one section. Excluded were patients with gastric or duodenal eosinophilia, those taking medications or with medical conditions likely to produce eosinophilia or alter results (e.g. antiepileptics, antihistamines, inhaled corticosteroids or oral corticosteroids for asthma, lymphoproliferative conditions). Subjects gave written, informed consent and the protocol was approved by the Ethics Committees of Eastern Health and of Monash University (E 119/1213 and E120/1213).

Study protocol

This was a prospective observational study designed to examine the real-world outcomes in patients presenting with histopathologically proven oesophageal eosinophilia. The design of the main study is presented elsewhere in detail.[17] Briefly, after withdrawal of all corticosteroid, therapy for 8 weeks for those previously treated with topical corticosteroids, patients were asked to take esomeprazole 40 mg orally twice daily for 8 weeks followed by histopathological assessment of the oesophagus. Those with oesophageal eosinophil density >15/HPF were then diagnosed with EoE. A gastroenterologist (HP) assessed all patients and the following data were recorded: date of birth, country of birth, migration and date of migration from overseas, coexistent allergic conditions, previous food-bolus obstruction events, date diagnosed, previous treatment and current symptoms.

Patients were then offered topical corticosteroids or dietary therapy with the six-food elimination diet. Esomeprazole was continued at the same dose. Written information about the diet was provided to all patients and consultation with a dietitian was offered. Patients who responded to dietary therapy had sequential reintroduction of foods according to an established algorithm as previously reported.[18] Briefly, this involved food challenges, where the participant was instructed to consume at least 1 serve of the additional, 'culprit' food at least twice per day, e.g. 1 slice of bread per twice daily, 1 glass of milk or 1 egg twice daily). This was followed by gastroscopy with oesophageal biopsies after a minimum of 2 weeks. Absence of oesophageal eosinophilia led to a challenge with the next food type, whereas recurrence of eosinophilia led to exclusion of that food type. Subsequent food challenge was given immediately when a putative food trigger failed to elucidate eosinophilia, while a positive response led to food removal and a 4-week 'washout' period. Those who failed the six-food elimination diet were offered topical budesonide.

Allergy tests

Allergy tests were performed at specific intervals, dependent on the response to dietary modification (Figure 1).

- *Skin-prick tests:* These were performed using commercially prepared antigens (Alloystal, Stallergenes France, allergen concentration approximately 5000 AU/mL) and standard methodology, where a positive result was recorded as a wheal 3 mm greater than the negative control at 15 min post skin prick. Antigens tested were the foods; egg, wheat, milk, soy, peanut, hazelnut, fish and shellfish and the aeroallergens ryegrass, dust mite and birch pollen. Histamine (10 mg/mL) and saline were used as positive and negative controls respectfully.

- *Skin-patch tests:* These utilised the same commercially prepared antigens with three drops of allergen placed on a filter paper disc, secured with petrolatum in 12 mm Finn chambers, and covered with adhesive tape, the reading performed at 72 ± 6 h. A result was considered positive if erythema and clear infiltration with papules (+) or vesicles (++) occurred, as previously described.[19]

- *Serum food- and aeroallergen-specific IgE levels:* These were assessed to the same antigens as above on peripheral blood using the Immunocap test according to the manufacturer's instructions (Pharmacia Diagnostics, Uppsala, Sweden) in a single hospital laboratory. Scores recorded between <0.35 (low or undetectable) to greater than 100 Ku/L. Moderate or strong positives were considered clinically significant and recorded.

- *Basophil activation test:* This was performed on whole blood using the same antigens via the Flow-CAST assay (Bühlmann, Schoenberg, Switzerland) on the FACSverse (BD Biosciences, Melbourne, Vic., Australia) flow cytometer according to the manufacturer's instructions. Specifically, venous blood was collected in K-EDTA (potassium ethylenediaminetetraacetic acid) venepuncture tubes and samples stored at 6 °C before being processed within 2 h of collection in all cases. 3.5 mL polypropylene tubes were used for subsequent analysis. Stimulation controls were N – formlynethionyl – leucyl – phenylalanine (fMLP) and anti-Fcε RImAb. Stimulation buffer contained calcium, IL-3 and heparin. Staining reagent was a mix of anti-CD-63 and anti-CCR3-PE mAb. Additional cell surface receptor antibodies to CD 203c and TSLP were also utilised. FLOW-CAST antigens were used, namely egg white and egg yolk, wheat, milk, soy, peanut,
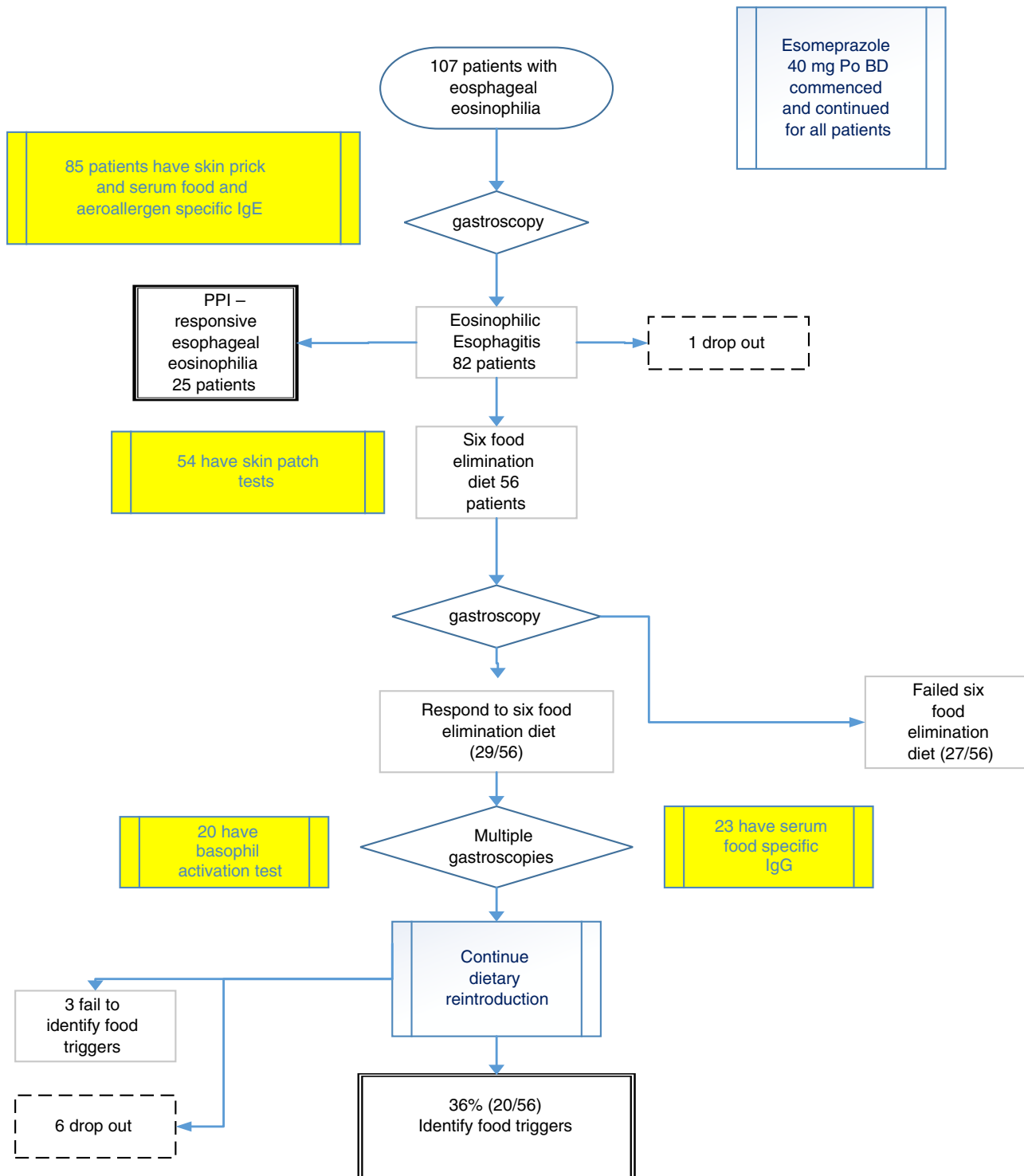
Figure 1 | Allergy tests performed on patients with eosinophilic oesophagitis (EoE) who chose the six-food elimination diet (6FED).

hazelnut, fish and shellfish and the aeroallergens rye-grass, dust mite and birch pollen. Samples were incubated in a water bath, and following use of lysing

reagent were centrifuged and then resuspended with wash buffer prior to flow cytometry. A positive result to a given antigen was defined as >15% CD 63 positive

basophils (CCR3 positive) in a subject, where one or both positive controls (fMLP or anti-FcRAb) were positive (>15%) in accordance with the manufacturer's protocol.

- *Serum food-specific IgG antibodies levels:* Healthscope Functional Pathology, Melbourne, Australia using the Genova Diagnostics Food IgG ELISA test kit (Asheville North Carolina), performed these. Specifically, the samples were stored at between 2 and 8 °C for <48 h. Trained laboratory staff performed the procedure using a manufactured (automated) standardised microplate coated with food antigens. A goat anti-human IgG conjugated to horseradish peroxidase was added prior to incubation, and a solution of 3,3,5,5-tetramethylbenzidine (TMB) is added to trace specific antibody binding before using the STOP solution (sulphuric acid) and optical densities are measured using a microplate reader at 450 nm. A positive control containing human serum is used. Food-specific IgG was reported as positive if greater than 12.5 units/mL (manufacturers own arbitrary reference range and units)

Endoscopy, biopsy and histological assessment

As previously outlined, gastroenterologists from the respective departments of both hospitals performed all gastroscopies. Four biopsies were taken each from the lower oesophagus (5 cm proximal to the gastroesophageal junction) and from the middle and upper oesophagus at 5 cm intervals. Transoral and transnasal gastroscopies were used, the latter with local anaesthetic spray and the former with propofol sedation. Histopathological analysis of gastric and duodenal biopsies was performed by consultant pathologists blinded to the treatment method in sections of tissue fixed in 4% neutral-buffered formalin and stained with H&E. The peak eosinophil count was recorded in all three areas of the oesophagus in the most densely infiltrated areas. The mean eosinophil count of 10 respective areas analysed at HPF were calculated.

Outcome measures and analysis

A response to specific food elimination was defined as <15 eosinophils per HPF in all three oesophageal locations. A positive reaction to foods during a dietary challenge was defined as recurrence of >15 eosinophils per HPF in one or more location following the reintroduction of a food. The performance characteristics of the results of the allergy tests were compared to the foods

identified by the elimination-rechallenge methodology, considered as gold standard.

RESULTS

Characteristics of patients with EoE

Eighty-two patients with EoE were identified following the course of twice-daily PPI therapy. The patient demographics are shown in Table 1. Most patients were male (84%), white Caucasian (98%) and presented with food-bolus obstruction events and/or dysphagia. Coexistent atopic illness was present in many, with seasonal rhinitis most common (42%).

The results of skin-prick and IgE testing are shown in Table 2. Aeroallergen sensitisation was frequent, with rye grass the predominant allergen (approximately 70% of patients), followed by dust mite (Table 2). Birch-pollen sensitisation was rare (2–7% skin prick or serum allergen specific IgE). Sensitisation to putative food allergens was more often demonstrated with serum food-specific IgE than by skin-prick tests. Wheat (45%), milk (32%) and egg (19%) were most frequent as defined by serum food-specific IgE.

Timing of gastroscopies and allergy tests

The gastroscopies to determine response to the six-food elimination diet were performed at a time interval closely matching the intended protocol of 42 days [mean 41.36 days (s.d. 2.82)], and the gastroscopies to determine food triggers were similarly well timed [mean 15.61 days (s.d. 0.71, protocol was 14 days]. Figure 1 shows the timing of allergy tests, with skin prick and serum food and aeroallergen-specific IgE being performed at the commencement of the study immediately prior to the introduction of PPI. Skin-patch testing was performed immediately before commencing the 6FED, while the basophil activation test and the serum IgG to food antigens were performed following a response to the 6FED [mean 43.2 days (s.d. 3.6)].

Performance of allergy tests in patients completing six-food elimination diet

Of 56 patients who commenced the six-food elimination diet, 29 initially responded to the diet and 23 of these completed the diet. The characteristics of these patients are shown in Table 1. Food triggers were identified in 20 patients as outlined in Table 3. A recurrence of EoE following food reintroduction was caused by a single food in 12 cases, and by two or more foods in the case of

Index	EoE (*n* = 82)	Treated with 6FED (*n* = 56)
Mean age (s.d.) in years	34 (11)	39 (12)
Male gender	69 (84%)	38 (67%)
Mean age (s.d.) at diagnosis in years	32 (10)	34 (11)
Country of birth		
Australia	52 (61%)	38 (67%)
New Zealand	3 (12%)	1 (3%)
UK	18 (21%)	12 (21%)
USA	1 (2%)	0
Continental Europe	2 (3.5%)	2 (4%)
South Africa	3 (3.5%)	1 (2%)
Other	3 (3.5%)	1 (2%)
Season of birth (adjusted according to hemisphere born)		
Summer	15 (18%)	11 (18%)
Autumn	19 (22%)	14 (25%)
Winter	31 (38%)	19 (34%)
Spring	15 (18%)	12 (21%)
Other equatorial	3 (4%)	0
Mean age (s.d.) at migration	26 (7)	24 (11)
Ethnicity		
White Caucasian	80 (98%)	56 (100%)
Asian	1 (1%)	0
Middle Eastern	1 (1%)	0
Presence of atopic illness		
Seasonal rhinitis	36 (42%)	28 (48%)
Asthma	16 (19%)	12 (21%)
Food allergy or oral-food allergy syndrome	6 (7%)	3 (5.3%)
Coeliac disease	2 (2%)	0
Helicobacter pylori positive (after 8 weeks of twice-daily esomeprazole)	2 (2%)	0
Presenting symptom		
Food-bolus obstruction	31 (37%)	20 (35%)
FBOE and dysphagia	25 (29%)	15 (26%)
Dysphagia alone	19 (23%)	12 (21%)
Heartburn	4 (5%)	2 (3.5%)
Other	4 (5%)	1 (2%)
Previous treatment of oesophageal eosinophilia		
Proton pump inhibitor – daily	66 (78%)	40 (71%)
Proton pump inhibitor – twice daily	8 (9.5%)	4 (7%)
Swallowed topically acting corticosteroid	35 (42%)	20 (35%)
Diet	0	0
No treatment	0	0

Table 1 | Demographic and clinical data of patients with eosinophilic esophagitis (EoE) and the sub-group who underwent six-food elimination diet (6FED) therapy

eight patients. The commonest food triggers were wheat (implicated alone or in combination in 10 cases), milk (alone or in combination in nine cases) and egg.

None of the five allergy testing modalities could accurately predict food triggers. Skin-patch testing was always negative with respect to food, and serum IgG levels to food antigens was positive to two or more foods in all cases, showing no correlation with actual triggers. Serum IgG levels to food antigens would accurately predict an individual food trigger in 13/20 patients, miss a food trigger in 11/20 and lead to an over-restrictive diet in

19/20 patients. Skin patch detected no food triggers. Specific serum IgE and, to a lesser extent, skin-prick tests were positive to a number of food allergens but were not accurate in correctly predicting dietary triggers of EoE, except in one case where childhood milk allergy (manifesting as classical anaphylaxis) was recalled by the patient and the individual had positive specific serum IgE, skin-prick test and the basophil activation test to milk. Interestingly, a patient with known classical food allergy had positive skin-patch test, specific serum IgE and the basophil activation test to the culprit antigen

Table 2 | Result of allergy testing in those with eosinophilic esophagitis (n = 82). Not all patients agreed to undergo allergy testing; skin-prick tests were not performed on individuals with a history of severe asthma; and those with known food-induced anaphylaxis to a given food did not have testing with that food

Putative allergen	Skin-prick test positive N = 72	Serum specific IgE positive N = 75
Cow's milk	20 (27%)	25 (33%)
Wheat	14 (19%)	32 (43%)
Egg white	3 (4%)	15 (20%)
Tuna fish	0	3 (4.0%)
Shell fish	0	2 (3%)
Peanut	3 (4%)	15 (20%)
Almond	0	9 (12%)
Soy	7 (10%)	13 (17%)
Rye grass	50 (70%)	53 (71%)
Dust mite	48 (67%)	47 (61%)
Birch pollen	3 (2%)	5 (7%)

(soy). The basophil activation test was otherwise negative to all food antigens. As both TSLP and CD 203c were universally positive in the first 10 patients, these assays were subsequently abandoned. Given the obvious lack of utility, calculations of sensitivity, specificity, positive and negative predictive values were not appropriate. The propensity for false-positive results is demonstrated by the three patients who had no food triggers identified.

Aeroallergen sensitisation was demonstrated in 14/20 patients (70%) using specific serum IgE, in 10/20 (50%) by skin-prick testing, and in 7/20 (35%) by the basophil activation test. Rye-grass sensitisation was predominant for all test modalities, and concordance was observed for this allergen between specific serum IgE and the skin-prick test in 10/14 (71.5%). Serum IgG levels to food antigens did not measure aeroallergen sensitisation.

DISCUSSION

Elimination diets have been successfully used to treat EoE in Northern Hemisphere patient cohorts.[20] To render such an approach practical, identification of trigger foods by relatively non-invasive means is desirable, but current techniques of empirical food reintroduction and frequent gastroscopy are cumbersome. Simple allergy testing is a much more attractive option but its role in EoE to guide dietary therapy has been debated.[2, 4, 5] Thus, we systematically investigated such testing using a panel of five available techniques prospectively in a consecutive cohort of patients presenting with EoE.

Crucially, none of the tests predicted the actual food sensitivity associated with EoE as defined by gold-standard elimination-re-challenge techniques.

The lack of utility of allergy tests in directing dietary therapy for EoE has been demonstrated previously in reference to skin-patch testing in one adult cohort[4] and to skin-patch testing and serum food antigen specific IgE combined in another group of adult patients.[2] Our study differed in that additional modalities of allergy test were applied prospectively to a patient cohort that was systematically followed up and subject to ongoing treatment with high-dose PPI. Notably, the skin-patch test was negative to food allergens in all cases and the basophil activation test was similarly negative, except for two cases where classical food allergy to milk and soy were correctly predicted. Skin-patch testing and serum food antigen specific IgE were positive in five patients (25%, a similar percentage to previous studies) but did not predict food triggers.[4]

Skin-patch testing has previously demonstrated poor sensitivity in determining food triggers for EoE in a paediatric cohort, as well as an adult cohort that were treated with an elemental diet, although the absolute inability to react to any food in our study of adult patients was novel.[5, 21] The method of SPT differed from some studies in that commercially prepared as opposed to fresh foods were employed, skin taping and stripping was not used prior to patch placement, and petrolatum was added to assist disc adhesion.[5, 21] Nonetheless, little consensus exists as to whether skin-patch testing has a use in food-related allergic disease *per se*, and the literature in adults is scarce.[8] Our experience would suggest that, as well as being a cumbersome test disliked by patients and clinicians, skin-patch testing should not be used in EoE.

In an attempt to explore non-IgE-mediated mechanisms of food allergy, the basophil activation test was applied using standard markers of basophil activation in additional to cell surface markers CD 203c and TSLP.[10, 11] Activation of CD 203c is thought to predict non-IgE-mediated immune activation of unspecified type and TSLP is the relevant receptor. The presence of exogenous food- or aero-antigens did not influence expression of these cell surface receptors. While TSLP may be important in EoE, the use of TSLP receptors on basophils was not differentially expressed (i.e. it was not dependent on exogenously applied food antigens) and thus, the assay as described is of no use in predicting food triggers for EoE. This observation is in keeping with recent work that demonstrated a lack of utility of serum

Table 3 | Comparison of food triggers identified by oesophageal eosinophilia following empirical reintroduction after normal eosinophil count with the elimination diet and with the findings of multiple allergy tests

| Patient | Age (years), sex | Food triggers identified at gastroscopy | IgE mediated measures | | Other | | Food-related IgG |
			Skin-prick test	Specific serum IgE	Skin-patch test	Basophil activation test	
1	55, female	Wheat	Rye grass	Rye grass		Rye grass	Milk, egg, wheat
2	58, female	Wheat		Rye grass	House dust mite	Rye grass	Milk, egg, wheat
3	21, male	Wheat	Rye grass, house dust mite	Rye grass, house dust mite	House dust mite	Rye grass, house dust mite	Milk, egg, wheat
4	44, female	Wheat	Nil	Rye grass	Nil	Rye grass	Milk, egg, nut
5	54, female	Wheat	Rye grass	House dust mite	Nil	Nil	Milk, egg, wheat
6	29, male	Wheat	Wheat	Peanut, soy, wheat	Nil	Nil	Wheat, nut
7	62, male*	Milk	Milk	Milk	Nil	Milk	Milk, egg, wheat
8	19, male	Milk	Rye grass, house dust mite	Rye grass	House dust mite	Nil	Milk, egg, wheat
9	61, male	Milk	Nil	Nil	Nil	Not performed	Milk, egg, wheat, soy
10	19, male	Egg	Nil	Nil	Nil	Not available†	Wheat
11	19, male	Egg	Nil	Rye grass	Nil		Milk, egg, fish
12	64, male	Soy	Nil	Birch, house dust mite, hazelnut	Birch, rye grass	Birch, house dust mite	Milk, egg, wheat, soy
13	49, female	Milk, egg, wheat, nut	Rye grass, house dust mite, milk	House dust mite, rye, peanut	Nil	Not available†	Milk, egg, wheat, nut, soy
14	27, male	Nut, fish	Rye grass	Rye grass	Nil	Nil	Milk, egg, nut
15	33, female	Milk, egg	Rye grass	Rye grass	Nil	Rye grass	Milk, egg, wheat
16	52, male	Milk, egg	House dust mite	Milk, egg, house dust mite	Nil	House dust mite	Milk, egg, wheat, soy
17	48, male	Wheat, egg, milk	Milk	Milk, rye grass	Rye grass	Rye grass	
18	19, female‡	Egg‡	Rye grass, wheat, soy	Rye grass, peanut, wheat, soy	Nil	Soy	Milk, egg, wheat, soy, fish
19	35, male	Wheat, egg, milk	Rye grass	Rye grass, house dust mite	Nil	Not available†	Milk, egg, soy, nut
20	34, male	Wheat, milk	Rye grass	Rye grass	Nil	Not performed	Milk, egg, wheat, soy, fish
21	26, male	Nil	Nil	Nil	Nil	Nil	Milk, egg
22	33, male	Nil	House dust mite	Rye grass, house dust mite	Nil	Rye grass, house dust mite	Wheat, milk, egg
23	44, male	Nil	Rye grass	Rye grass	Nil	Not performed	Soy, milk, egg

* Childhood milk allergy.

† Results not interpretable due to laboratory error.

‡ Classical food allergy to soy and thus no reintroduction.

biomarkers (including TSLP) in determining disease activity in EoE.[22]

The serum IgG levels to food antigens was invariably positive to two or more foods, but did not correctly identify food triggers in EoE. The use of this assay stemmed from a recent study that demonstrated a preponderance of IgG rather IgE in oesophageal tissue from patients with EoE and from elevated serum IgG levels to food antigens to a number of foods, again in patients with EoE, but to a lesser extent in patients with alternative diagnoses.[3] We concur that patients with EoE have elevated serum IgG levels to food antigens to two or more foods, and add that serum IgG levels to food antigens was not a useful test in predicting food triggers for EoE in the current prospective study. Serum IgG levels to food antigens use is most common in the practice of alternative and complementary medicine, and we utilised the same laboratory and methodologies that are commercially available. The validity of the so-called 'diagnostic' levels of IgG to food antigens using apparently arbitrary units is questionable given the limited studies of variable quality relating to these assays.[14] It is possible that IgG levels represent food exposure rather than being reflective of food triggers. In the context of classical IgE-mediated food allergy, it has been suggested that food-specific IgG become elevated with disease resolution and may thus facilitate immune tolerance. It is hypothesised that this mechanism of immune tolerance exists in EoE and further studies seem indicated.[23]

The reasons why all of the allergy tests failed to correctly identify food triggers deserves consideration. First, it is possible that non-IgE-mediated mechanisms of immune activation are responsible for EoE, and thus skin-patch testing and serum food antigen specific IgE would be unhelpful. Nonetheless, the basophil activation test, serum IgG levels to food antigens and skin-patch test, which are considered measures of non-IgE-mediated immune activation, also lacked utility. It is also possible that the gastrointestinal immune compartment responds differently from the systemic immune compartment. Methods capable of directly exposing the gastrointestinal mucosa to putative antigens may in future prove useful. Certainly, testing for food allergy *per se*, even when considering classical food allergy is less established and more problematic compared to tests for aeroallergens.[24] Tests that directly interrogate the gastrointestinal immune compartment already have a template in studies of food antigens for patients with IBS and/or food allergies.[25, 26] Perhaps such techniques applied to the oesophagus might also be applicable to identifying food antigens in patients with EoE.

The characteristics of our patient group in reference to age, gender, race and aeroallergen as opposed to food-allergen sensitisation are similar to those of previous studies. The predominance of rye-grass sensitisation is novel and deserves comment. Rye-grass pollinosis is very high in our region and the results of the allergy tests not surprisingly reflect this. The fact that rye-grass pollen (rather than birch pollen) is predominant, yet the food allergen triggers of EoE are the same as Northern Hemisphere cohorts, arguably counters a previously held hypothesis that birch-pollen cross-sensitisation is a potential mechanism in driving food antigen exacerbation of EoE.[2, 4, 15, 27] It is also suggests that the inverse situation is also true – that rye grass cross-sensitisation with wheat is not a valid hypothesis as previously debated.[28] This may be better resolved by performing the so-called 'component-resolved diagnostics', where putative shared antigen epitopes of aeroallergen and vegetable/fruit allergens such as profilins are analysed, but this was beyond the scope of our research.[29]

Several limitations are evident with our study design. First, the reluctance of patients to undertake the programme in the first instance, along with the high rate of dropout limited the numbers and ultimately the power of our analysis. However, this is readily understood, as the burden of eight or more gastroscopies is considerable, and emphasises the need for less invasive measures for identifying food triggers in EoE. Similarly, the variable use of a dietitian (16 out of 29 responding to dietary therapy elected to utilise this service), may have decreased the efficacy of dietary therapy and thus indirectly the results of allergy tests (although the study was not designed or adequately powered to examine this, as discussed previously). Indeed, the response to diet was inferior to earlier studies (52% in our cohort compared to >65% elsewhere) and may reflect the 'real – world' nature of the study where the utilisation of structured resources including a dietician was limited.[2, 4] Secondly, the timing of the basophil activation test and serum IgG levels to food antigens may have influenced the results. Both were performed following the 6-week removal of food antigens from the diet. Ideally, they should have been performed at the commencement of the study with the other assays. Countering this assertion is that antibodies of the IgE and IgG subclasses are both traditionally thought to be formed as a result of immunological memory, and that this time interval is relatively short in

any case. Third, commercially prepared as opposed to fresh food antigens were used for skin-prick and patch testing, the latter being preferred and affording improved accuracy according to some authorities.[6, 30] Fourth, the serum IgG levels to food antigens was performed by an alternative or 'functional' laboratory, albeit overseen by a mainstream organisation and IgG subclasses were not specified. Our choices of assays were governed by a need for reproducibility, external validity and practicality. Ideally, IgG4 to putative food antigens should have been used in line with the finding that this subclass is deposited in oesophageal tissue.[3] Thus, the results of the assay we used which measured IgG antibodies to food antigens per se (as opposed to the IgG4 subclass) need to be viewed with caution. Finally, the timing of oesophageal biopsies post-food ingestion (2 weeks), and the acquisition of tissue (using either 2.0 mm transnasal forceps or standard 2.3 mm transoral forceps) deviates from previous practice where variable (2–4 weeks) or more prolonged intervals (6 weeks) were allowed and standard forceps used.[2, 4] Nonetheless, we have previously demonstrated that tissue acquisition was adequate with smaller forceps, and another group found disease recurrence at between 3 and 7 days post-food reintroduction.[21, 31] We took biopsies in the upper, middle and lower oesophagus in all patients, thus a minimum of 12 tissue fragments was obtained which is far in excess of the suggested approach (five biopsies with inclusion of the upper and lower oesophagus only) for optimum diagnosis of EoE.[9, 32] Of the 56 patients who underwent the six-food

elimination diet with PPI, 12 chose to use transnasal gastroscopy and 10 of these patients had a response to dietary therapy and made the decision to continue to use transnasal gastroscopy. All of these had a food trigger identified. It is thus unlikely but not impossible that putative food triggers would have been missed as a result of this approach.

In conclusion, none of the commercially available allergy tests that measure systemic immune responses can accurately predict food triggers for EoE and should not be applied for this indication. Such findings emphasise the need for less invasive methods of identifying food triggers if dietary manipulation is to establish itself as the first-line therapy for EoE. The food antigens found responsible for causing EoE are similar to previous research, despite the predominant aeroallergen sensitisation to rye grass (and absent tree-allergen sensitisation) consistent with the Australian location of our cohort.

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