

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.

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 respectively.
- *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 – formylmethionyl – leucyl – phenylalanine (fMLP) and anti-Fcε R1mAb. 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,

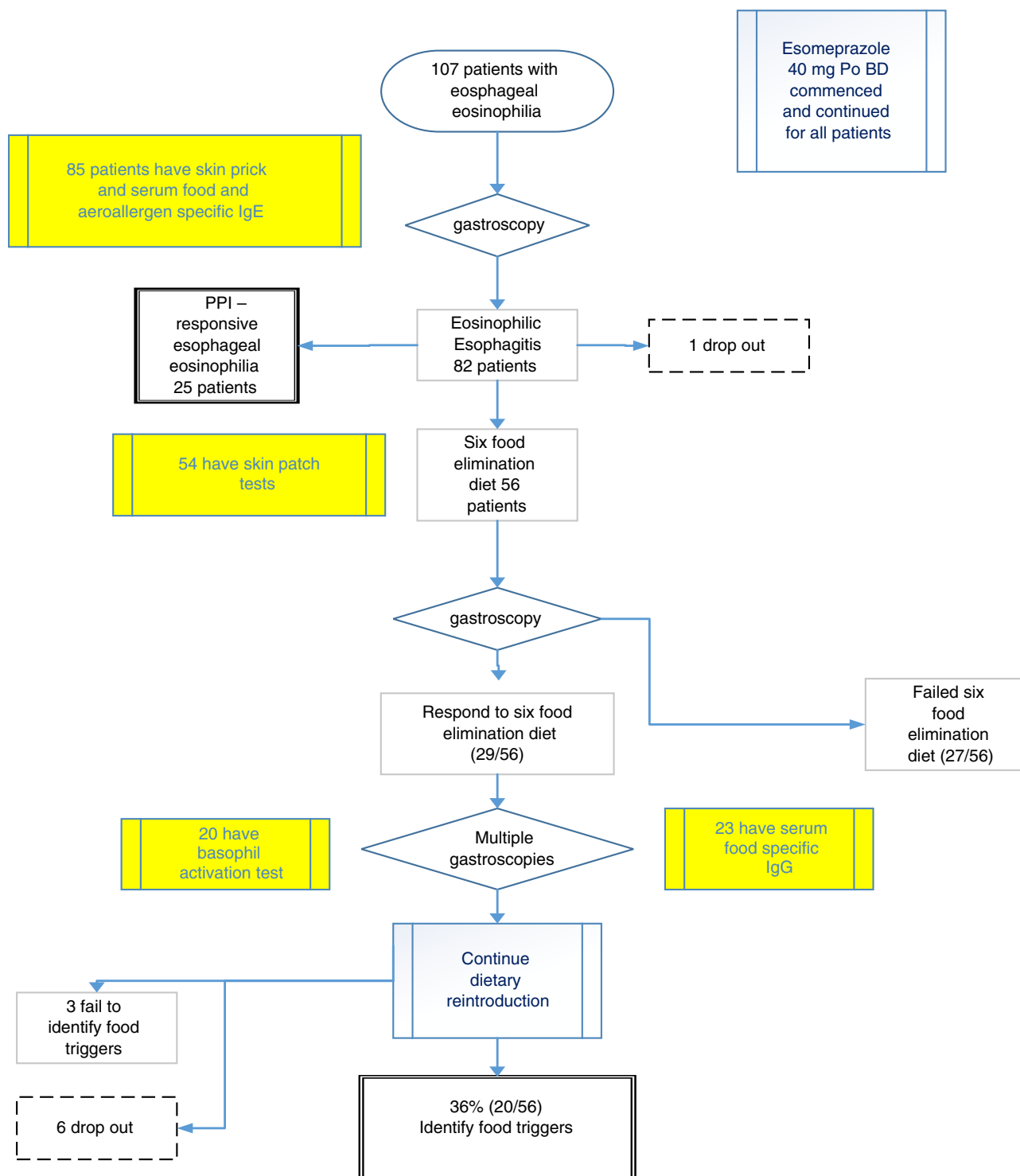


Figure 1 | Allergy tests performed on patients with eosinophilic oesophagitis (EoE) who chose the six-food elimination diet (6FED).

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 gastro-oesophageal 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%)
Celiac disease	2 (2%)	0
<i>Helicobacter pylori</i> 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 cohort⁴ 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 addition 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 dietitian 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.

AUTHORSHIP

Guarantor of the article: None.

Author contributions: Hamish Philpott: research and writing; Peter Gibson: writing; Sanjay Nandurkar: concept and research/edits; Simon Royce: research; Francis Thien: concept and edits.

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