

# A Novel Allergen-Specific Immune Signature-Directed Approach to Dietary Elimination in Eosinophilic Esophagitis

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- OBJECTIVES:** Dietary elimination for treatment of eosinophilic esophagitis (EoE) is limited by lack of accuracy in current allergy tests. We aimed to develop an immunologic approach to identify dietary triggers and prospectively test allergen-specific immune signature-guided dietary elimination therapy.
- METHODS:** In the first phase, we developed and assessed 2 methods for determining selected food triggers using samples from 24 adults with EoE: a CD4<sup>+</sup> T-cell proliferation assay in peripheral blood and food-specific tissue IgG4 levels in esophageal biopsies. In the second phase, we clinically tested elimination diets created from these methods in a prospective cohort treated for 6 weeks (NCT02722148). Outcomes included peak eosinophil counts (eos/hpf), endoscopic findings (measured by the EoE Endoscopic Reference Score), and symptoms (measured by the EoE Symptom Activity Index).
- RESULTS:** Parameters were optimized with a positive test on either assay, yielding agreements of 60%, 75%, 53%, 58%, and 53% between predicted and known triggers of peanut, egg, soy, wheat, and milk, respectively. In clinical testing, the mean number of foods eliminated based on the assays was 3.4, and 19 of 22 subjects were compliant with treatment. After treatment, median peak eosinophil counts decreased from 75 to 35 ( $P = 0.007$ ); there were 4 histologic responders (21%). The EoE Endoscopic Reference Score and EoE Symptom Activity Index score also decreased after treatment (4.6 vs 3.0;  $P = 0.002$ ; and 32.5 vs 25.0;  $P = 0.06$ , respectively).
- DISCUSSION:** We successfully developed a new testing approach using CD4<sup>+</sup> T-cell proliferation and esophageal food-specific IgG4 levels, with promising accuracy rates. In clinical testing, this led to improvement in eosinophil counts, endoscopic severity, and symptoms of dysphagia, but a smaller than expected number of patients achieved histologic remission.

**SUPPLEMENTARY MATERIAL** accompanies this paper at <http://links.lww.com/CTG/A113>

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## INTRODUCTION

Over the past decade, eosinophilic esophagitis (EoE) has become a major cause of chronic gastrointestinal morbidity (1,2). Dietary elimination is the nonpharmacologic mainstay of treatment in EoE patients of all ages, with efficacy shown in a number of studies and response rates varying by the degree of dietary restriction (3–8). There are several issues, however, with dietary elimination for EoE. Adherence to the most restrictive diets can be difficult. For example, more than 50% of adults were unable to tolerate an

elemental formula diet for more than a few days (4), and children may require placement of feeding tubes to obtain adequate nutrition (3,9). Although the six-food elimination diet (SFED), which removes dairy, wheat, egg, soy, nuts, and seafood, was conceived in part to improve adherence, response rates are variable (8,10), multiple endoscopies are required to determine food triggers (11), and despite elimination of 6 foods, most patients have only 1–3 triggers ultimately identified (6,10,11). Perhaps, the most vexing problem is that no currently available allergy test can

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accurately identify specific food triggers leading to EoE. Skin prick testing correctly identifies food triggers in  $\leq 13\%$  (6,11,12), and this is the likely explanation for low response rates in allergy test-directed diets. Reliable methods to determine food allergy triggers in EoE would be of enormous clinical utility.

The current model of EoE pathogenesis holds that when food antigens are presented to the GI tract, previously sensitized T cells are triggered to produce a cascade of Th2 cytokines (13). This model is neither an immediate IgE- nor a classic delayed IgG-mediated response, and a recent study has identified food-specific IgG4 as important to the pathogenesis (14). A new approach to “allergy testing” in EoE would be to create a food trigger-specific immunological signature using both sensitized T cells and food-specific IgG4 as the basis for elimination diet therapy. Our previous work has shown that T cells sensitized to specific foods could be readily expanded and characterized in response to antigen in non-EoE patients with IgE-mediated peanut or egg allergies (15–18), and that food-specific IgG4 levels measured in esophageal biopsies might correlate with clinically identified food triggers (19). However, these measures have never been applied to creating individual elimination diets for EoE patients.

Therefore, the aims of this study were (i) to develop an immunologic approach to identifying dietary triggers and (ii) to prospectively test allergen-specific immune signature-guided dietary elimination therapy. We hypothesized that T cells obtained from the blood of a subject with active EoE, when cultured and stimulated with food allergens, and when coupled with allergen-specific IgG4 measured in an esophageal biopsy, would result in a food trigger-specific immunological signature that could be used to tailor an effective dietary treatment specific to that individual.

## METHODS

This study was performed in 2 phases. In the first phase, we developed and assessed 2 methods for determining food triggers using samples from adults with EoE. We also optimized the laboratory techniques to be able to provide results within 2 weeks, a time frame felt to be clinically actionable. In the second phase, we clinically tested elimination diets created from the methods developed in the first phase (clinicaltrials.gov; NCT02722148). The study was approved by the University of North Carolina (UNC) Institutional Review Board, and all patients provided informed consent for participating and for use of biosamples.

### Phase 1: Development of an allergen-specific immune signature

**Patients and clinical data.** We enrolled adults ( $\geq 18$  years) with EoE as diagnosed per consensus guidelines, with a focus on patients who had undergone dietary elimination during the course of routine clinical care. The dietary elimination was performed on a clinical basis by the treating provider using SFED. In patients who were histologic responders (defined as  $< 15$  eosinophils per high-power field [eos/hpf; hpf size =  $0.24 \text{ mm}^2$ ] after 6 weeks of dietary elimination), foods were individually added back every 6 weeks, and endoscopy was repeated for each food, as per standard clinical algorithms (6,9–11). A food trigger was defined by an eosinophil count that increased to above 15 eos/hpf after food reintroduction. These patients were the source of the biosamples used in the laboratory assays for the first phase of this study. None of these patients had an immediate IgE-type food allergy reaction to any of the foods tested in this study.

**CD4<sup>+</sup> T-cell proliferation assay.** We obtained 30 mL of whole blood (in sodium heparin-containing tubes) for the CD4<sup>+</sup> T-cell proliferation assays from the above EoE patients when their disease was active ( $> 15$  eos/hpf on biopsy). Samples were placed on ice and immediately transported to the laboratory. The assays were conducted using standard methods established in our previous studies (15–18). In brief, peripheral blood mononuclear cells were separated from whole blood by Ficoll separation, labeled with carboxyfluorescein succinimidyl ester (CellTrace kit; Thermo Fisher Scientific, Waltham, MA), and placed into coculture (RPMI media) with the following allergens and controls: media alone (negative control); casein (milk protein); wheat; egg; soy; peanut; and anti-CD3/CD28 beads (positive control; Gibco Dynabeads for Human T-Cell Activation, Thermo Fisher Scientific). All food allergens were added to cell culture at  $200 \mu\text{g/mL}$ , and the anti-CD3/CD28 beads were added at  $5 \mu\text{L}$  per  $0.5 \times 10^6$  peripheral blood mononuclear cells. Allergens were prepared as aqueous extracts from powdered food sources (wheat flour from Honeyville; egg white powder from Debel; soy flour from Honeyville; and peanut flour from Golden Peanut Company) following our previously published methods (20), except for casein, which was purchased from Sigma-Aldrich. After 7 days in culture with 5% CO<sub>2</sub> at 37 °C, cells were stained with anti-human CD4 (PE-Cy5; BD Biosciences, San Jose, CA), and flow cytometry was performed to quantify the proportion of CD4<sup>+</sup> carboxyfluorescein succinimidyl ester-low T cells, reflecting the proportion of antigen-specific CD4<sup>+</sup> T cells. Flow cytometry data were acquired on a CyAn ADP (Beckman Coulter, Brea, CA) and analyzed with FlowJo Software (FlowJo, LLC, Ashland, OR). We selected the 5 antigens of interest as these are among the most commonly reported food triggers for EoE (6,11,21,22) and also overlapped with the clinical data for food triggers in our patient population (10,23).

**IgG4 assay.** We used a single esophageal biopsy that was obtained from each patient during an endoscopy where the EoE was histologically active ( $\geq 15$  eos/hpf) before dietary elimination and flash frozen with liquid nitrogen and then stored at  $-80$  °C. Of note, patients were on a proton pump inhibitor (PPI) and had failed the PPI trials as required by diagnostic guidelines at the time of the study conduct (24,25); no patients with PPI-responsive esophageal eosinophilia were included. However, no patients were on other medications such as H<sub>2</sub> receptor blockers, topical/swallowed steroids, or systemic steroids. Using methods we have previously reported (19), biopsies were homogenized in protease inhibitor, and protein content was determined and normalized to  $100 \mu\text{g/mL}$ . ELISA was used to quantify total and food-specific IgG4. For total IgG4 measurements, Immulon 4HBX microtiter plates (Thermo Fisher Scientific) were coated with mouse anti-human Ig  $\kappa$  light chain (clone G20-193; BD Biosciences; diluted 1:250). For food-specific IgG4 measurements, plates were coated with  $20\text{-}\mu\text{g/mL}$  casein, wheat, egg, soy, or peanut extract (same source of allergens used in the CD4 T-cell assay above). A standard curve was created by coating wells with purified mouse anti-human IgG4 (clone G17-4; BD Biosciences; diluted 1:250) and using serial dilutions of native human IgG4 (Abcam, Cambridge, MA). Detection was performed using mouse anti-human IgG4-horseradish peroxidase antibody (clone HP6025; Southern Biotech [Birmingham, AL]; diluted 1:1,000) with tetramethylbenzidine substrate. Food-specific IgG4 was normalized as a percentage of total tissue IgG4.

**Statistical analysis for thresholds.** Analysis was performed using the known food triggers for each patient and the matched

blood lymphocyte proliferation results and tissue IgG4 levels. We used a multistep process to determine the optimal cutoff values for the 2 assays. First, we assessed the distribution of the results for each assay for each of the foods. Then, for each of the 5 potential food triggers, we compared the mean proportion of proliferating lymphocytes in patients with and without the food trigger and the mean food-specific IgG4 level (normalized for total tissue IgG4) in patients with and without the trigger. These 2 methods provided data regarding the range of the assay results and relation to the different food triggers. Next, using receiver operator characteristic curve analysis, we calculated the area under the curve and explored thresholds for lymphocyte proliferation and food-specific IgG4, both individually and then combined, that maximized sensitivity, specificity, and accuracy for each food trigger. Maximizing accuracy (the number of positive results in patients with a given food trigger, plus the number of negative results in patients without the given food trigger, divided by the total number of patients) was prioritized as this was felt to be the most clinically meaningful value. In this article, we use the terms accuracy and agreement interchangeably. Because this phase of the study was developmental, there was no formal sample size calculation.

## Phase 2: Clinical testing

**Study design, patients, and samples.** We conducted a prospective cohort pilot study at UNC Hospitals. Patients aged 16–80 years with active EoE, as diagnosed per consensus guidelines (24,25), who had never been on dietary elimination therapy, were eligible. Patients were excluded if they had concomitant eosinophilic gastroenteritis, any systemic or swallowed corticosteroid exposure in the 4 weeks before their baseline endoscopic examination, previous esophageal surgery, medical instability that precluded safely performing upper endoscopy, inability to read or understand English, and pregnancy. If patients were still taking a PPI at study enrollment, they were required to maintain the same dose unchanged until the end of the study. Before the baseline (predietary elimination) endoscopy, we obtained a blood sample (30 mL; sodium heparin-containing tubes, as above), and during the procedure, an esophageal biopsy was obtained for IgG4 determination, flash-frozen in liquid nitrogen, and kept at  $-80^{\circ}\text{C}$ . The samples were immediately transported to the laboratory for the lymphocyte proliferation assay and food-specific IgG4 level determination, using the same protocols as described above.

**Individualized diet creation and dietary elimination.** Using the thresholds that maximized predictive accuracy from both assays, we created an individualized diet eliminating between 1 and 5 foods (dairy, wheat, egg, soy, and/or peanuts). Patients then had a standard-of-care appointment with a clinical nutritionist to guide dietary elimination and provide information about avoidance of their specific food triggers. At this time, they also underwent skin prick testing for the 5 trigger foods of interest, using standard clinical techniques (26,27). After this appointment, the patients were treated with dietary elimination for 6 weeks, at which point repeat upper endoscopy was performed. Dietary compliance was assessed by patient interviewers before the post-treatment endoscopy reviewing their adherence to the recommended elimination regimen.

**Outcomes.** The primary outcome was histologic response, defined as a post-treatment esophageal eosinophil count of  $<15$  eos/hpf after 6 weeks of dietary elimination (28–30). The secondary

outcomes were (i) change in the absolute esophageal eosinophil count; (ii) improvement in the endoscopic appearance, as measured by the EoE Endoscopic Reference Score (EREFS), a validated endoscopy score (31); and (iii) improvement in symptoms, as measured by the EoE Symptom Activity Index (EEsAI), a validated dysphagia symptom score (32); patient-reported global symptom improvement was also assessed. All data were collected on standardized case report forms by the study coordinator and entered into a secure database.

**Statistical analysis.** To determine the response rate for the primary outcome, the number of subjects with  $<15$  eos/hpf on follow-up endoscopy was calculated. For secondary outcomes, eosinophil counts, endoscopy scores, and symptoms scores were compared before and after treatment with either paired *t* tests or the Wilcoxon signed-rank test, depending on data distributions. We also performed exploratory analyses examining the median change in CD4<sup>+</sup> cell proliferation and food-specific tissue IgG4 levels between baseline and after treatment, both overall and stratified by histologic response. As this was a proof-of-principle pilot study, we focused only on patients who were compliant with the dietary elimination regimen. For the same reason, and because this study did not have a comparison group, there was not a formal sample size calculation. However, we planned to enroll at least 20 subjects and hypothesized that 75% would have a histologic response to the allergen-specific immune signature dietary elimination.

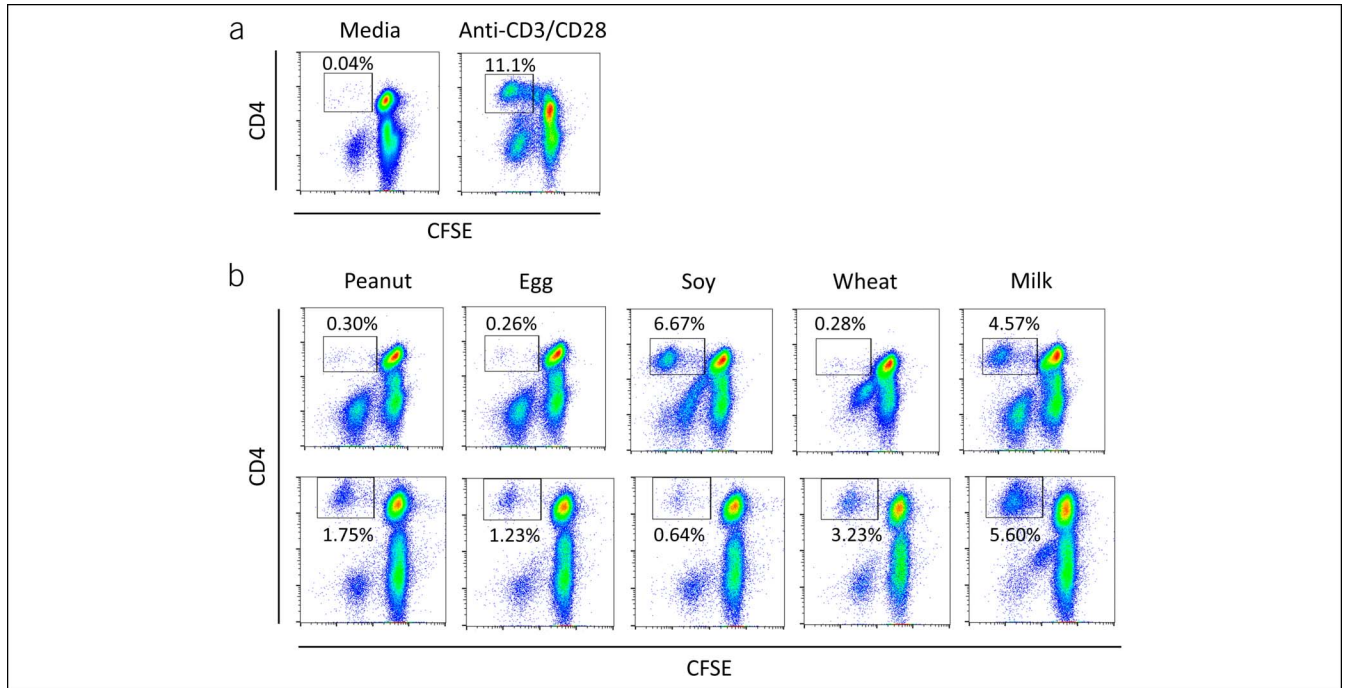
## RESULTS

### Phase 1: Performance of allergen-specific thresholds for identifying food triggers

Samples from 24 EoE subjects who met inclusion criteria were analyzed for this phase of the study. The mean age was 38 years, 25% were men, 96% were white, 75% had at least 1 atopic condition, and dysphagia was the predominant symptom in 92% (Supplemental Table 1, Supplementary Digital Content 1, <http://links.lww.com/CTG/A113>). From the clinically conducted dietary elimination, 15 patients were histologic responders (63%) with subsequent determination of known triggers, and 9 were nonresponders. All these subjects were included in the analyses, as the nonresponders were required to assess false-positive rates. Dairy was the most commonly identified trigger, seen in 11 (73%), followed by egg (53%), wheat (33%), soy (27%), and peanut (20%) (Supplemental Table 1, Supplementary Digital Content 1, <http://links.lww.com/CTG/A113>).

The CD4<sup>+</sup> T-cell stimulation assays were performed, and flow data were acquired and gated as shown (Figure 1) The proportion of proliferating CD4<sup>+</sup> T cells was numerically higher in patients with wheat, egg, and peanut as a known trigger, compared to those without these foods as a trigger (Figure 2a). For example,  $7.0\% \pm 5.4\%$  of lymphocytes were stimulated by egg in patients with egg as a known trigger, compared with  $2.8\% \pm 3.5\%$  where egg was not a trigger ( $P = 0.07$ ). Food-specific IgG4 levels were numerically higher in patients with milk, wheat, and egg as a known trigger, compared to those without these foods as a trigger (Figure 2b). For example, the proportion of wheat-specific IgG4 normalized for total tissue IgG4 was  $15.2\% \pm 15.2\%$  in patients with wheat as a known trigger, compared with  $2.7\% \pm 2.5\%$  where wheat was not a trigger ( $P = 0.04$ ).

For the CD4<sup>+</sup> T-cell proliferation assay, agreement between the proliferation threshold and the known food trigger was 75%, 71%, 50%, 42%, and 47% for peanut, egg, soy, wheat, and milk,



**Figure 1.** Representative flow data from peripheral blood mononuclear cells cultured with indicated antigens. Lymphocytes are gated, then CD4+ CFSE-low cells are selected. (a) Negative control (media) with 0.04% stimulated CD4+ CFSE-low cells and positive control (anti-CD3/CD28) with 11.1% stimulated cells. (b) Two representative subjects. The subject in the top line has elevated responses to soy and milk at 6.67% and 4.57% stimulated, respectively. The subject in the bottom line has elevated responses to wheat and milk, at 3.23% and 5.60% stimulated, respectively. CFSE, carboxyfluorescein succinimidyl ester.

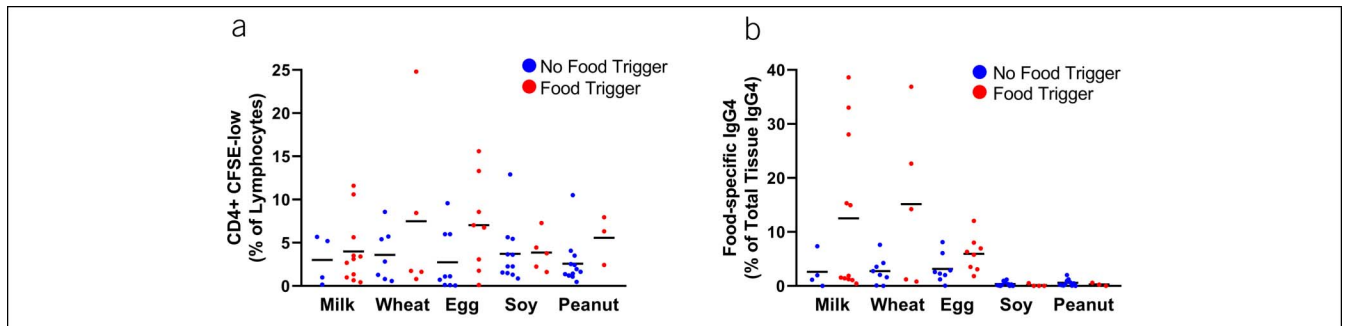
respectively (Table 1). For food-specific tissue IgG4, agreements were 67%, 80%, 67%, 77%, and 53%, respectively. Sensitivity, specificity, and agreement were optimized when a positive test on either assay was used, with agreements of 60%, 75%, 53%, 58%, and 53% for peanut, egg, soy, wheat, and milk, respectively (Table 1).

### Phase 2: Clinical testing and outcomes

Of 27 patients screened for this phase, 24 were enrolled (the 3 patients who did not qualify were responsive to PPI therapy and were not considered to have EoE at the time this study was conducted (24, 25)), 2 were lost to follow-up and never received their dietary treatment, and 22 completed the study protocol. The mean age was 44 years, 45% were men, 95% were white, at 55%

had at least 1 atopic condition (Table 2). Dysphagia was the predominant symptom, and while the median symptom length before EoE diagnosis was more than 7 years, the median length of time between diagnosis and study entry was <1 year. Of note, 16 of the patients (73%) had previously been treated with topical steroids, and 8 were steroid nonresponders; 15 patients (68%) had previously required esophageal dilation (Table 2).

After the CD4+ T-cell proliferation and IgG4 assays were complete, a total of 12 individualized diets were created spanning the 22 patients who received treatment (Supplemental Figure 1, Supplementary Digital Content 1, <http://links.lww.com/CTG/A113>). The mean number of foods eliminated was  $3.4 \pm 1.2$ , with 27% of subjects eliminating 3 foods and 23% eliminating 2 foods.



**Figure 2.** Comparison of assay results by presence or absence of food triggers. (a) Results of the CD4+ T-cell proliferation assay show the mean proportion of T cells stimulated by those who have a food trigger present (gray bars) compared with those who do not (black bars). None of the comparisons are statistically significant, although there was a trend with egg ( $P = 0.07$ ) and peanut ( $P = 0.10$ ). (b) Results for food-specific IgG4 (normalized to total tissue IgG4). The comparison for wheat is significant ( $P = 0.04$ ), and there is a trend for egg ( $P = 0.08$ ). CFSE, carboxyfluorescein succinimidyl ester.



**Table 1.** Sensitivity, specificity, and accuracy of the immune signature thresholds for predicting known eosinophilic esophagitis food triggers

	Peanut	Egg	Soy	Wheat	Dairy
CD4 <sup>+</sup> T-cell proliferation testing parameters (%)					
Sensitivity	100	63	60	20	45
Specificity	82	75	78	50	75
Agreement	75	71	50	42	47
Tissue IgG4 testing parameters (%)					
Sensitivity	0	88	0	60	55
Specificity	80	75	100	100	100
Agreement	67	80	67	77	53
Combined parameters with a positive on either test (%)					
Sensitivity	100	100	75	80	64
Specificity	50	50	45	43	25
Agreement	60	75	53	58	53

In the 19 subjects compliant with the diet, 14 (74%) had a decrease in eosinophil count after treatment, and there were 4 histologic responders (21%). The foods eliminated for the diets for these responders were dairy/wheat/soy/peanut, dairy/wheat/egg/peanut, and dairy/wheat/egg/soy/peanut for 2 patients. The median peak eosinophil count decreased from 75 (interquartile range [IQR] 40–100) to 35 (IQR 20–63) ( $P = 0.007$ ) (Table 3; Figure 3a), and the median percent decrease was 65% (IQR –77 to 0). The proportion of subjects with any atopy present was similar in nonresponders and responders (60% vs 75%;  $P = 0.58$ ). In the subgroup of patients previously treated with topical steroids (13 of 19 compliant patients), the proportion of subjects with previous steroid response was lower in diet nonresponders (4 of 11; 36%) than in responders (2 of 2; 100%), but this was not significant ( $P = 0.10$ ).

The mean total EREFS significantly decreased after treatment ( $4.6 \pm 3.0$  vs  $3.0 \pm 1.9$ ;  $P = 0.002$ ), and 10 patients (53%) had a  $\geq 50\%$  decrease in the EREFS (Figure 3b). All components of the EREFS system improved with the exception of strictures (Table 3). The mean EEAI score decreased after treatment ( $32.5 \pm 32.5$  vs  $25.0 \pm 23.1$ ;  $P = 0.06$ ) in 17 subjects with this data available (Figure 3c), and 7 (41%) were in symptomatic remission defined by an EEAI of  $< 20$ . Thirteen patients (68%) had a global symptom improvement in dysphagia.

#### Post hoc analyses and patient follow-up

Of the 4 histologic responders, 3 underwent food reintroduction on a clinical basis, and food triggers were confirmed to be dairy, wheat, and soy (in the subject eliminating these 3 foods and peanut), dairy, wheat, and egg (in a subject eliminating all 5 foods), and dairy (in a subject eliminating dairy, wheat, egg, and peanut). Of note, there were 2 patients who had a symptom and endoscopic response, but no histologic response, who were maintained on the same diet off protocol for an additional 3 months, and these patients subsequently had a histologic

**Table 2.** Characteristics of the clinical study cohort

	EoE cases (n = 22)
Mean age (yr $\pm$ SD)	43.9 $\pm$ 15.6
% Male (n, %)	10 (45)
% White (n, %)	21 (95)
BMI (mean kg/m <sup>2</sup> $\pm$ SD)	27.4 $\pm$ 5.8
Symptoms (n, %)	
Dysphagia	20 (91)
Symptom length before EoE diagnosis (median years, IQR)	7.4 (4.9–18.8)
Heartburn	12 (54)
Chest pain	4 (18)
Abdominal pain	6 (27)
Nausea/vomiting	6 (27)
Any atopy (n, %)	
Asthma	5 (23)
Eczema	2 (9)
Seasonal allergies/allergic rhinitis	11 (50)
Food allergies	1 (5)
Length of time since EoE diagnosis (median years, IQR)	0.8 (0–3.5)
Previous EoE treatments (n, %)	
PPI (all nonresponsive) <sup>a</sup>	22 (100)
Topical steroids	16 (73)
Previous steroid nonresponse	8 (50)
Esophageal dilation	15 (68)

<sup>a</sup>At the time of entry into the phase 2 study, 13 of 22 subjects (59%) were taking a PPI and continued this through the study time frame. BMI, body mass index; EoE, eosinophilic esophagitis; IQR, interquartile range; PPI, proton pump inhibitor.

response. The diets for these subjects were dairy/wheat/egg/peanut elimination (subsequent triggers identified were dairy, wheat, and egg) and dairy/wheat/egg/soy/peanut elimination (subsequent triggers identified as dairy, wheat, egg, and soy).

For this set of patients with an initial and eventual histologic response, the agreement between their individualized testing on the lymphocyte proliferation and IgG4 assays and the clinically confirmed food triggers was 100% for dairy, 80% for wheat, 75% for egg, 66%, for soy, and 0% for peanut. For these patients, the agreement between their baseline skin prick testing and the clinically confirmed food triggers was 0% for dairy, 20% for wheat, 50% for egg, 33% for soy, and 60% for peanut.

We also compared the assay results at baseline and after treatment, and overall, there were mild decreases in the median levels for both assays in the total study population (Supplemental Table 2, Supplementary Digital Content 1, <http://links.lww.com/CTG/A113>). When stratified by histologic response, there were numerically greater median decreases in CD4<sup>+</sup> T-cell stimulation levels for all 5 foods in the histologic responders (initial and eventual responders) compared with nonresponders, but these decreases were not statistically significant. Trends were not as clear for the

**Table 3.** Baseline and post-treatment outcomes data—per-protocol in compliant patients (n = 19)

	Baseline	After treatment	P <sup>a</sup>
Peak median eos/hpf (IQR)	75 (40–100)	35 (20–62)	0.007
Proximal counts	33 (8–75)	20 (4–40)	0.09
Distal counts	65 (37–100)	30 (20–45)	0.007
Symptom data			
DSQ (mean ± SD)	8.1 ± 11.2 (n = 11)	10.2 ± 10.7 (n = 11)	0.73 (n = 6)
EEsAI (mean ± SD)	32.5 ± 23.5 (n = 17)	25.0 ± 23.1 (n = 17)	0.06 (n = 15)
Total EREFS (mean ± SD)	4.6 ± 1.7	3.0 ± 1.9	0.002
Exudates	1.0 ± 0.8	0.4 ± 0.6	0.02
Rings	1.2 ± 0.6	0.7 ± 0.7	<0.001
Edema	0.8 ± 0.4	0.5 ± 0.5	0.06
Furrows	1.1 ± 0.3	0.7 ± 0.6	0.02
Stricture	0.6 ± 0.5	0.6 ± 0.5	1.0
Dilation (n, %)	10 (53)	12 (63)	0.53

<sup>a</sup>Means compared with the paired *t* test; medians compared with the Wilcoxon signed-rank test; proportions compared with McNemar's test.

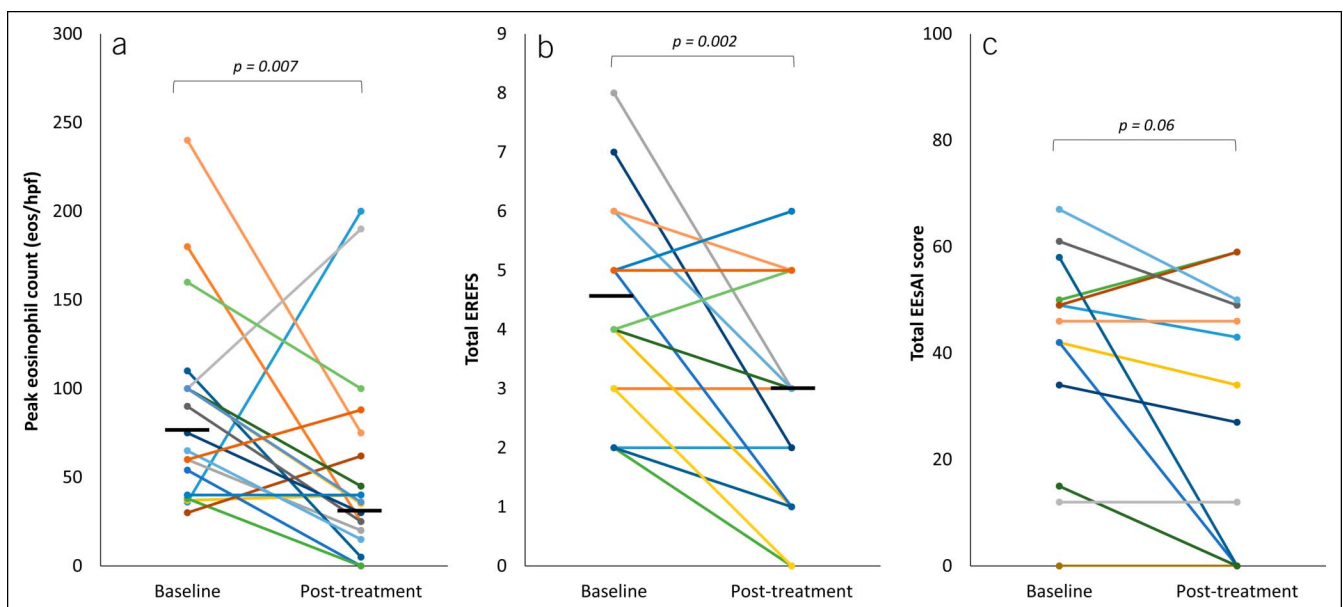
DSQ, Dysphagia Symptom Questionnaire; EEsAI, EoE Symptom Activity Index; EoE, eosinophilic esophagitis; EREFS, EoE Endoscopic Reference Score; IQR, interquartile range.

food-specific tissue IgG4 levels. In addition, baseline proportions of both CD4+T cells and food-specific tissue IgG4 levels were numerically higher in histologic responders than nonresponders, but these differences also were not significant (data not shown).

## DISCUSSION

Dietary elimination is the only nonpharmacologic treatment for EoE and is currently recommended as an option for first line therapy (9). Empiric elimination diets are commonly used primarily because targeted elimination diets using traditional IgE-based allergy testing have a poor performance predicting food

triggers for EoE (6,8,11,12). Inability to identify food triggers of EoE in a given patient before elimination is currently a major limitation of dietary therapy in EoE, and new methods to detect food triggers are sorely needed. In this study, we successfully developed a 2-pronged approach for identifying food triggers. First, based on the fact that EoE is a T-cell-driven process, we used peripheral blood samples to perform lymphocyte proliferation assays to quantify relative proportions of antigen-specific T cells. Second, based on the hypothesis that IgG4 may be involved in EoE pathogenesis, we measured food-specific IgG4 in esophageal biopsies. After assessing these assays in patients with



**Figure 3.** Outcomes before and after dietary elimination: (a) peak eosinophil counts; (b) total EREFS; and (c) EEsAI score. EEsAI, Eosinophilic Esophagitis Symptom Activity Index; EREFS, Eosinophilic Esophagitis Endoscopic Reference Score.

known food triggers, we were able to develop thresholds for combined results from the 2 test methods. Accuracy rates for this approach were 53%–75%, and although these rates were not perfect, they were substantially higher than previously reported for skin prick testing alone (6,11,12), suggesting there may be clinical utility in this approach. We were also able to optimize the assays to provide results in less than 2 weeks, a clinically actionable time frame (particularly since it can take up to a week for esophageal biopsies results to be returned from the clinical pathology laboratory). In the subsequent prospective pilot trial, an individualized allergen-specific immune signature-based diet led to improvement in eosinophil counts, endoscopic severity, and symptoms of dysphagia, but a smaller than expected number of patients achieved histologic remission after 6 weeks of treatment. However, the average number of foods eliminated was 3, fewer than either the empiric SFED or 4-food (4FED) approach, although we acknowledge that the responders were eliminating more than 3 foods.

The resource intensity of the SFED has been well described in the literature (9,11,33). However, this diet can be highly efficacious in motivated patients at expert centers with appropriate dietician/nutritionist support and a multidisciplinary approach, with histologic response rates of ~70% reported in a meta-analysis (8). It may be difficult and adds extra cost for patients to adhere to this diet (34), and the long-term feasibility of this approach is still not established (11,22,23). Because of this, less restrictive empiric elimination diets have been examined, including 4FED (dairy/wheat/egg/soy) (35–37), 2FED (dairy/wheat) (38), and 1FED (dairy) (39,40). In general, as the number of foods is decreased, the efficacy also drops. One study used results from all published dietary studies to model different empiric elimination approaches and found that a 1-4-8 approach (dairy elimination, followed by dairy/wheat/egg/soy, followed by dairy/wheat/egg/soy/corn/chicken/beef/pork) might be the most efficacious approach, but this has yet to be tested clinically (41). There have been other investigations into directing dietary elimination as well, but results with component resolved diagnostics and serum IgE testing have been disappointing (42,43). A combination of skin prick testing, atopy patch testing, and empiric elimination of dairy has shown excellent results in children (21), but the patch testing component has not been able to be reliably reproduced (26,42,44). One recent study found increased activated Th2 cells as measured by CD154 upregulation after antigen stimulation (a technique in peripheral blood similar to our approach) in pediatric patients with milk-induced EoE, but the results were not applied clinically during the study (45).

Our histologic response rate, using an eosinophil count threshold of <15 eos/hpf, was lower than expected and also lower than previously reported targeted elimination approaches using traditional allergy testing (8,44). There are several possible reasons for this. First, we enrolled a relatively severe EoE patient population. They had a long duration of symptoms before diagnosis, a high rate of strictures or narrowing requiring previous dilation (which has been shown to be a risk factor for nonresponse to topical steroid therapy (46,47), although it is not known whether this also predisposes to dietary nonresponse), and a high proportion of patients who were steroid refractory. In our previous work, steroid refractory patients have been less likely to respond to dietary therapy (23,47), and future studies of this technique could consider excluding steroid refractory patients. Although the numbers in our study were small, it is interesting to note that all the histologic responders in this study who had previously been treated with topical steroids were also

previous steroid responders. Second, we only tested 5 food allergens, and it is possible that there could have been other food triggers that were not eliminated in the nonresponders. Ideally, future studies will evaluate a larger group of food antigens. Third, it is possible that the thresholds that we set based on the first phase of this study were not optimal, and adjustments as more patients are treated with this modality could improve outcomes. However, despite the low response rate at the 15 eos/hpf level, we still noted that most patients had a decrease in the eosinophil count, and the post-treatment count was significantly lower than the baseline. With this, there was concomitant significant improvement in endoscopic severity as measured by the EREFS and a strong trend toward improvement in dysphagia symptoms measured by the EEsAI. In addition, in the post hoc analysis, there were an additional 2 patients who achieved histologic remission based on continuing the diet for a longer treatment period, a phenomenon that we had previously observed (48). The immune signature-based dietary elimination approach also has some important advantages. There were fewer foods eliminated on average, and if this holds during future studies, it would imply that fewer repeat endoscopies are needed during the food reintroduction phase. It is also a patient-centered and individualized approach, which is highly desirable as reflected in our high initial enrollment rate. Although the numbers are small and the results must be interpreted conservatively, we found better correlation with this technique than with the skin prick test in the patients who had histologic response.

It is important to acknowledge limitations of this study. As a proof-of-principle study, the sample size was small, it was conducted at a single referral center, and for the clinical testing phase, there was no comparator arm; so, symptom data should be interpreted with caution. In addition, the study included only EoE patients with nonresponse to PPI, and so, the results can not necessarily be applied to PPI responders. The set of foods tested was small (only 5 food antigens), and these were all nested within what would typically be performed with the SFED. This is an issue because results might be due to chance, and in the present iteration, this approach may not offer benefit above a 4FED or 6FED. However, as more food antigens are tested and thresholds developed in the future, we would expect the utility of this individualized approach to increase. Last, the results are presented only in the compliant patients in what is essentially a per-protocol analysis. However, because the study is proof of principle, the best way to determine whether there is a signal is to focus on the subjects in whom the treatment was optimized; noncompliant patients did not provide any actionable data for the purpose of this study. The study also has a number of strengths. The overall approach was comprehensive, with a development phase followed by initial clinical testing. The clinical phase was a prospective cohort, with rigorous conduct and use of validated outcome measures. It also merged novel laboratory techniques with actionable results in a clinically relevant time frame.

In conclusion, an allergen-specific immune signature testing approach was successfully developed using CD4<sup>+</sup> T-cell proliferation and esophageal food-specific IgG4 levels. Initial accuracy rates for this approach were substantially higher than previously reported for skin prick testing alone, indicating a potential clinical application. In the subsequent prospective pilot trial, the individualized dietary elimination led to improvement in eosinophil counts, endoscopic severity, and symptoms of dysphagia, but a smaller than expected number of patients achieved histologic remission. Because fewer foods than traditional elimination diets

were required, further investigation of this individualized approach is warranted with testing of a wider set of food antigens.

### CONFLICTS OF INTEREST

**Guarantor of the article:** Evan S. Dellon, MD, MPH.

**Specific author contributions:** E.S.D.: project conception, study design, data collection, data analysis/interpretation, manuscript drafting, critical revision, and obtained funding. R.G.: data collection, data analysis/interpretation, and critical revision. S.J.M., J.C., S.E.M., and A.A.: data collection and management and critical revision. D.K.H.: data collection, data interpretation, and critical revision. B.L.W.: data interpretation and critical revision. A.W.B.: data interpretation and critical revision. B.P.V.: project conception, data interpretation, critical revision, and obtained funding. M.K.: project conception, data collection, data analysis/interpretation, critical revision, and obtained funding.

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**Potential competing interests:** None to report.

## Study Highlights

### WHAT IS KNOWN

- ✓ Dietary elimination is a first-line nonpharmacologic treatment for EoE.
- ✓ Current allergy tests do not have sufficient accuracy to guide dietary elimination, and so, empiric diets are typically used.

### WHAT IS NEW HERE

- ✓ An allergen-specific testing approach was successfully developed using CD4<sup>+</sup> T-cell proliferation and esophageal food-specific IgG4 levels.
- ✓ Initial accuracy rates for this approach were substantially higher than previously reported for skin prick testing alone.
- ✓ In a prospective pilot trial, this approach led to improvement in eosinophil counts, endoscopic severity, and symptoms of dysphagia.
- ✓ A smaller than expected number of patients achieved histologic remission.

### TRANSLATIONAL IMPACT

- ✓ Laboratory assays have the potential ability to identify food triggers for EoE.
- ✓ Dietary therapy can potentially be personalized and more efficiently targeted to an individual patient's food triggers.

## REFERENCES

1. Dellon ES, Hirano I. Epidemiology and natural history of eosinophilic esophagitis. *Gastroenterology* 2018;154:319–22.e3.
2. Jensen ET, Kappelman MD, Martin CF, et al. Health-care utilization, costs, and the burden of disease related to eosinophilic esophagitis in the United States. *Am J Gastroenterol* 2015;110:626–32.
3. Markowitz JE, Spergel JM, Ruchelli E, et al. Elemental diet is an effective treatment for eosinophilic esophagitis in children and adolescents. *Am J Gastroenterol* 2003;98:777–82.
4. Peterson KA, Byrne KR, Vinson LA, et al. Elemental diet induces histologic response in adult eosinophilic esophagitis. *Am J Gastroenterol* 2013;108:759–66.
5. Kagalwalla AF, Sentongo TA, Ritz S, et al. Effect of six-food elimination diet on clinical and histologic outcomes in eosinophilic esophagitis. *Clin Gastroenterol Hepatol* 2006;4:1097–102.
6. Gonsalves N, Yang GY, Doerfler B, et al. Elimination diet effectively treats eosinophilic esophagitis in adults; food reintroduction identifies causative factors. *Gastroenterology* 2012;142:1451–9.e1.
7. Spergel JM, Brown-Whitehorn TF, Beausoleil JL, et al. 14 years of eosinophilic esophagitis: Clinical features and prognosis. *J Pediatr Gastroenterol Nutr* 2009;48:30–6.
8. Arias A, González-Cervera J, Tenias JM, et al. Efficacy of dietary interventions for inducing histologic remission in patients with eosinophilic esophagitis: A systematic review and meta-analysis. *Gastroenterology* 2014;146:1639–48.
9. Dellon ES, Liacouras CA. Advances in clinical management of eosinophilic esophagitis. *Gastroenterology* 2014;147:1238–54.
10. Wolf WA, Jerath MR, Sperry SL, et al. Dietary elimination therapy is an effective option for adults with eosinophilic esophagitis. *Clin Gastroenterol Hepatol* 2014;12:1272–9.
11. Lucendo AJ, Arias A, Gonzalez-Cervera J, et al. Empiric 6-food elimination diet induced and maintained prolonged remission in patients with adult eosinophilic esophagitis: A prospective study on the food cause of the disease. *J Allergy Clin Immunol* 2013;131:797–804.
12. Philpott H, Nandurkar S, Royce SG, et al. Allergy tests do not predict food triggers in adult patients with eosinophilic esophagitis. A comprehensive prospective study using five modalities. *Aliment Pharmacol Ther* 2016;44:223–33.
13. O'Shea KM, Aceves SS, Dellon ES, et al. Pathophysiology of eosinophilic esophagitis. *Gastroenterology* 2018;154:333–45.
14. Clayton F, Fang JC, Gleich GJ, et al. Eosinophilic esophagitis in adults is associated with IgG4 and not mediated by IgE. *Gastroenterology* 2014;147:602–9.
15. Jones SM, Pons L, Roberts JL, et al. Clinical efficacy and immune regulation with peanut oral immunotherapy. *J Allergy Clin Immunol* 2009;124:292–300, 300.e1–97.
16. Varshney P, Jones SM, Scurlock AM, et al. A randomized controlled study of peanut oral immunotherapy: Clinical desensitization and modulation of the allergic response. *J Allergy Clin Immunol* 2011;127:654–60.
17. Vickery BP, Pons L, Kulis M, et al. Individualized IgE-based dosing of egg oral immunotherapy and the development of tolerance. *Ann Allergy Asthma Immunol* 2010;105:444–50.
18. Kulis M, Yue X, Guo R, et al. High- and low-dose oral immunotherapy similarly suppress pro-allergic cytokines and basophil activation in young children. *Clin Exp Allergy* 2019;49:180–9.
19. Wright BL, Kulis M, Guo R, et al. Food-specific IgG4 is associated with eosinophilic esophagitis. *J Allergy Clin Immunol* 2016;138:1190–2.e3.
20. Berglund JP, Szczepanski N, Penumarti A, et al. Preparation and analysis of peanut flour used in oral immunotherapy clinical trials. *J Allergy Clin Immunol Pract* 2017;5:1098–104.
21. Spergel JM, Brown-Whitehorn TF, Cianferoni A, et al. Identification of causative foods in children with eosinophilic esophagitis treated with an elimination diet. *J Allergy Clin Immunol* 2012;130:461–7.e5.
22. Philpott H, Nandurkar S, Royce SG, et al. A prospective open clinical trial of a proton pump inhibitor, elimination diet and/or budesonide for eosinophilic esophagitis. *Aliment Pharmacol Ther* 2016;43:985–93.
23. Reed CC, Fan C, Koutlas NT, et al. Food elimination diets are effective for long-term treatment of adults with eosinophilic esophagitis. *Aliment Pharmacol Ther* 2017;46:836–44.
24. Dellon ES, Gonsalves N, Hirano I, et al. ACG Clinical Guideline: Evidence based approach to the diagnosis and management of esophageal eosinophilia and eosinophilic esophagitis. *Am J Gastroenterol* 2013;108:679–92.
25. Liacouras CA, Furuta GT, Hirano I, et al. Eosinophilic esophagitis: Updated consensus recommendations for children and adults. *J Allergy Clin Immunol* 2011;128:3–20.e6.
26. Henderson CJ, Abonia JP, King EC, et al. Comparative dietary therapy effectiveness in remission of pediatric eosinophilic esophagitis. *J Allergy Clin Immunol* 2012;129:1570–8.
27. Sicherer SH, Wood RA, Stablein D, et al. Immunologic features of infants with milk or egg allergy enrolled in an observational study (Consortium of



- Food Allergy Research) of food allergy. *J Allergy Clin Immunol* 2010;125:1077–83.e8.
28. Wolf WA, Cotton CC, Green DJ, et al. Evaluation of histologic cutpoints for treatment response in eosinophilic esophagitis. *J Gastroenterol Hepatol Res* 2015;4:1780–7.
  29. Reed CC, Wolf WA, Cotton CC, et al. Optimal histologic cutpoints for treatment response in patients with eosinophilic esophagitis: Analysis of data from a prospective cohort study. *Clin Gastroenterol Hepatol* 2018;16:226–33.e2.
  30. Dellon ES, Fritchie KJ, Rubinas TC, et al. Inter- and intraobserver reliability and validation of a new method for determination of eosinophil counts in patients with esophageal eosinophilia. *Dig Dis Sci* 2010;55:1940–9.
  31. Hirano I, Moy N, Heckman MG, et al. Endoscopic assessment of the oesophageal features of eosinophilic oesophagitis: Validation of a novel classification and grading system. *Gut* 2013;62:489–95.
  32. Schoepfer AM, Straumann A, Panczak R, et al. Development and validation of a symptom-based activity index for adults with eosinophilic esophagitis. *Gastroenterology* 2014;147:1255–66.e21.
  33. Groetch M, Venter C, Skypala I, et al. Dietary therapy and nutrition management of eosinophilic esophagitis: A work group report of the American Academy of Allergy, Asthma, and Immunology. *J Allergy Clin Immunol Pract* 2017;5:312–24.e29.
  34. Wolf WA, Huang KZ, Durban R, et al. The six-food elimination diet for eosinophilic esophagitis increases grocery shopping cost and complexity. *Dysphagia* 2016;31:765–70.
  35. Molina-Infante J, Arias A, Barrio J, et al. Four-food group elimination diet for adult eosinophilic esophagitis: A prospective multicenter study. *J Allergy Clin Immunol* 2014;134:1093–9.e1.
  36. Kagalwalla AF, Wechsler JB, Amsden K, et al. Efficacy of a 4-food elimination diet for children with eosinophilic esophagitis. *Clin Gastroenterol Hepatol* 2017;15:1698–707.e7.
  37. Eluri S, Dellon ES. Toward more efficient dietary elimination therapy for eosinophilic esophagitis: The fantastic 4? *Clin Gastroenterol Hepatol* 2017;15:1668–70.
  38. Molina-Infante J, Arias A, Alcedo J, et al. Step-up empiric elimination diet for pediatric and adult eosinophilic esophagitis: The 2-4-6 study. *J Allergy Clin Immunol* 2018;41:1365–72.
  39. Kagalwalla AF, Amsden K, Shah A, et al. Cow's milk elimination: A novel dietary approach to treat eosinophilic esophagitis. *J Pediatr Gastroenterol Nutr* 2012;55:711–6.
  40. Kruszewski PG, Russo JM, Franciosi JP, et al. Prospective, comparative effectiveness trial of cow's milk elimination and swallowed fluticasone for pediatric eosinophilic esophagitis. *Dis Esophagus* 2016;29:337–84.
  41. Zhan T, Ali A, Choi JG, et al. Model to determine the optimal dietary elimination strategy for treatment of eosinophilic esophagitis. *Clin Gastroenterol Hepatol* 2018;16:1730–7.e2.
  42. Molina-Infante J, Martin-Noguerol E, Alvarado-Arenas M, et al. Selective elimination diet based on skin testing has suboptimal efficacy for adult eosinophilic esophagitis. *J Allergy Clin Immunol* 2012;130:1200–2.
  43. Erwin EA, Tripathi A, Ogbogu PU, et al. IgE antibody detection and component analysis in patients with eosinophilic esophagitis. *J Allergy Clin Immunol Pract* 2015;3:896–904.e3.
  44. Molina-Infante J, Gonzalez-Cordero PL, Arias A, et al. Update on dietary therapy for eosinophilic esophagitis in children and adults. *Expert Rev Gastroenterol Hepatol* 2017;11:115–23.
  45. Cianferoni A, Ruffner MA, Guzek R, et al. Elevated expression of activated TH2 cells and milk-specific TH2 cells in milk-induced eosinophilic esophagitis. *Ann Allergy Asthma Immunol* 2018;120:177–83.e2.
  46. Eluri S, Runge TM, Cotton CC, et al. The extremely narrow-caliber esophagus is a treatment-resistant subphenotype of eosinophilic esophagitis. *Gastrointest Endosc* 2016;83:1142–8.
  47. Wolf WA, Cotton CC, Green DJ, et al. Predictors of response to steroid therapy for eosinophilic esophagitis and treatment of steroid-refractory patients. *Clin Gastroenterol Hepatol* 2015;13:452–8.
  48. Philpott H, Dellon E. Histologic improvement after 6 weeks of dietary elimination for eosinophilic esophagitis may be insufficient to determine efficacy. *Asia Pac Allergy* 2018;8:e20.

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