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Abnormal Responses to Local Esophageal Food Allergen Injections in Adult Patients with Eosinophilic Esophagitis

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ABSTRACT

Skin tests and measurement of serum levels of immunoglobulin E do not accurately identify foods for elimination from the diets of patients with eosinophilic esophagitis (EoE). We investigated whether an esophageal prick test (EPT), in which the esophageal mucosa is challenged by local injection of allergen extracts, could identify individuals with esophageal sensitization. During endoscopy 6 allergens were injected in the esophagus of 8 patients with EoE and 3 patients without EoE (controls). A second endoscopy was performed after 24 hrs to evaluate delayed responses. Five of the 8 patients with EoE had evidence for an acute response (luminal obstruction and mucosal blanching); 2 other patients had a delayed wheal or flare reaction. No responses were observed in controls. We conclude that esophageal mucosal food allergen injections induce acute and/or delayed responses in patients with EoE but not controls. The EPT deserves further exploration because it might guide elimination diets.

Eosinophilic esophagitis (EoE) is an antigen-mediated disorder of the esophagus. Food allergens are presumed to trigger a Th2 inflammatory response, the exact mechanism by which foods can exacerbate EoE is unknown.[1][2] Dietary elimination can be an effective and drug-free solution. However, skin tests and specific IgE in serum perform poorly as tools to guide diets, possibly because the eosinophilic inflammation is restricted to the esophagus.[3] Therefore, our hypothesis was that an “Esophageal Prick Test” (EPT), in which the esophagus is challenged by local injection of allergen extracts, would yield more clinically relevant information about esophageal sensitizations. The aims of our study were to (1) investigate the esophageal response to food allergens in EoE, and (2) compare local esophageal sensitization patterns with those identified through skin prick tests (SPT) and serum IgE testing (sIgE).

A detailed description of study method is available in **supplementary materials and methods**. Briefly, in this prospective pilot study the esophageal mucosa was injected with food allergens. The EPT was performed in adult EoE patients, after a four-week course of dietary treatment because severe esophagitis could have hampered interpretation of EPT-responses, and in controls. All patients reported a list of clinically suspicious foods. During endoscopy 0.2ml of 6 diluted allergen extracts (wheat, milk, soy and 3 allergens based on patients’ history), a negative control (0.9% NaCl) and a positive control (3.4 µg/ml histamine diluted in 0.9% NaCl) were injected in a pre-specified order. Local responses were recorded up to 20 minutes. A second endoscopy was performed after 24 hours to evaluate delayed responses and to sample 4 biopsy specimens, proximal from the injection sites, to evaluate histologic disease activity. SPTs and sIgE tests were performed for the same allergens as the EPT.

We included 8 EoE patients, median age 49 (22 – 54) years, 75% male. Results were compared with 3 controls, median age 49 (22 – 54) years, 33% male. Six out of eight patients achieved remission (<15 eos/HPF) after either an elemental (n=3) or an elimination (n=5) diet, peak eosinophil counts are presented in table 1. EoE-associated endoscopic sings were present in all patients, median

endoscopic reference score (EREFS) 6 (4.3 – 7.8), and 50% presented with dysphagia, median symptoms score 1 (0 – 5.3). No abnormalities were observed in controls.

In 6/8 patients SPTs were positive for one or more food allergens and in 5/8 patients increased sIgE antibodies against one or more food allergens were found. All tests were negative in controls.

During the first endoscopy, in 5/8 patients an intense acute response was observed within 2 minutes after injection with soy (n=2), banana, apple, oats or hazelnut. Responses were characterized by complete luminal obstruction and blanching of the mucosa at the injection site (**figure 1a**). Within 3-10 minutes the obstruction decreased, enabling passage of the endoscope. During the second endoscopy the obstructive swellings were found to have dissolved completely. In 2 patients obvious delayed responses were identified, characterized by a flare or an erythematous wheal at the injection site of peach, and walnut (**figure 1c**). Of the 48 allergens that were injected (6 allergens in 8 EoE patients), 8 induced a local esophageal response, regardless of patients' histologic disease activity. EPTs performed in controls and injection of control solutions never induced mucosal changes. No severe systemic reactions occurred. However, after the EPT 3/5 patients with a positive acute EPT response experienced chest pain of a type that was recognized as similar to pain occurring after ingestion of these foods.

In only 3/8 of the patients, the sensitizations identified through EPT corresponded with sensitizations identified through SPT and sIgE. According to patients' clinical anamnesis, six patients experienced symptoms of food impaction within minutes after ingestion of one (n=3), two (n=2) or four (n=1) culprit food(s). Of these 11 suspicious foods, 7 (63%) corresponded with sensitizations identified through EPT, 2 (18%) with SPT sensitizations and 4 (36%) with sIgE sensitizations.

This study was the first to report on local allergen injections into the esophageal mucosa. We demonstrated that the EPT is safe, feasible and can identify abnormal responses to food allergens in EoE patients. Compared with SPT and sIgE testing, the sensitization patterns identified through EPT correlated more accurately with patients' clinical suspicion of culprit foods. The convincing visible mucosal changes to esophageal allergen injections support that EoE is an abnormal response to food

allergens. The results of our study (**table 1**) provide new insights in the complex allergic pathophysiology of EoE but also raise new questions. We can only speculate on the pathway responsible for the obstruction and blanching of the mucosa after allergen injection. The endoscopic view looks similar to the patterns of distal esophageal spasms and type III achalasia, suggesting it is a spastic contraction. Activated mast cells and derived mediators can indeed increase smooth muscle contractions and perhaps initiate this response.[4] Clinically, these spastic episodes may coincide simultaneously with acute impaction after ingestion of culprit foods. Alternatively, the narrowing and blanching may be the result of esophageal edema, caused by the release of mast cell-derived mediators that increase vascular permeability.[5] Either way, both assumptions and the acute onset of the responses suggest a potential role for antigen-specific mast cell degranulation and is worth further exploration.

Based on our findings, we suspect that the pathogenesis of EoE is an interplay between an IgE-mediated reaction, responsible for esophageal dysmotility and acute food impactions, and a delayed non-IgE mediated reaction, inducing the Th2 cell-driven chronic eosinophil-predominant inflammation. This mixed model may also address how acute EPT-responses occurred in patients with limited numbers of intra-epithelial eosinophils. However, the chronic inflammation could also be a delayed consequence of acute mast cell degranulation caused by allergen exposure. This study lays the groundwork for future mechanistic studies, especially designed to elucidate the responsible pathway.

This study has limitations. First, our study was conducted in a small cohort, although the number of subjects was adequate to generate convincing data to proceed with further developments. Second, the mechanism by which we injected allergens is different from natural esophageal allergen exposure. However, the mucosal barrier of EoE patients is impaired, possibly facilitating epithelial permeation of food allergens.[6][7] Hence, by mucosal allergen injections we may have accelerated allergen permeation but the activation of the immune system through presentation of the allergens is comparable. Third, our positive control solutions might have been too diluted, for safety reasons, since they failed to induce a visible mucosal reaction in all subjects.

In conclusion, we demonstrated that esophageal mucosal food allergen injections induce impressive acute and/or delayed responses in EoE patients and not in controls. The esophageal response to food in EoE may be restricted to the esophagus which provides an explanation why skin tests and specific IgE in serum perform poorly as tools to guide diets. The EPT is safe and feasible and deserves further exploration as it can potentially guide elimination diets.

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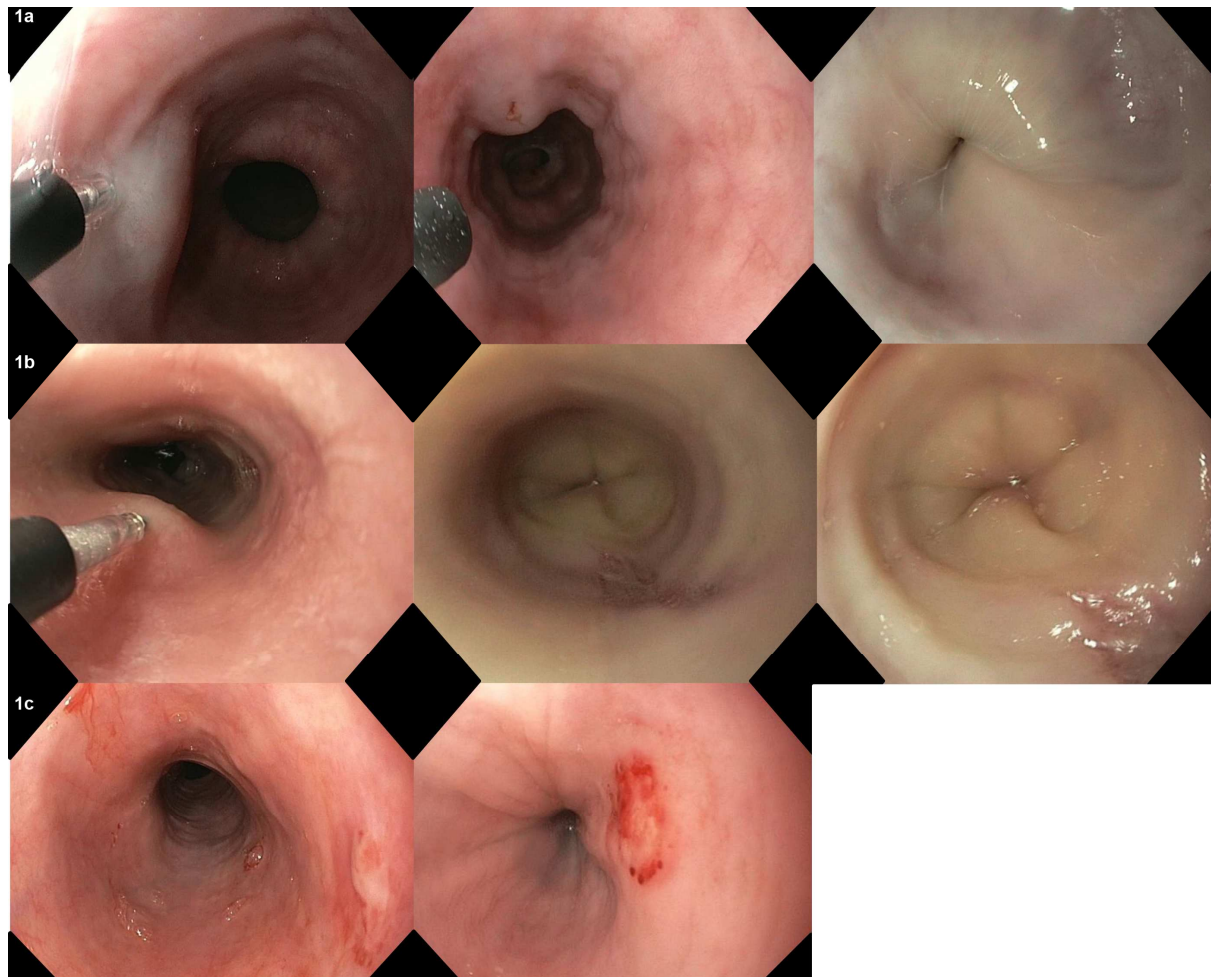
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Figure 1. Acute responses to banana (1a) and soy (1b) in two EoE patients. From left to right, within 2 minutes after injection of 0,2 ml allergen extract the esophageal mucosa blanches and the lumen occludes. 1c. Delayed responses to peach (left) and walnut (right) in two EoE patients during the next-day endoscopy, characterized by a wheal (left) or a flare (right)

Table 1. Patients characteristics of EoE patients and sensitization patterns

ID	Sex	Age	Atopy	Diet	PEC	CSF	SDI	EREFS	SPT	sIgE	Acute EPT	Delayed EPT
1	M	58	Asthma, HF, FA, AD	ELED	0	Peach	0	4	Wheat	-	Soy	Histamine, peach
2	F	49	-	ELED	5	Yogurt, walnut	0	6	-	Milk	-	Histamine, walnut
3	M	47	Asthma, HF, FA	SFED	10	Banana	6	8	Buckwheat, milk, wheat, hazelnut	-	Banana	-
4	M	34	HF, FA	SFED	75	Fish	0	7	Egg, wheat	Grapefruit, soy, wheat, egg, milk	Soy	-
5	M	33	Asthma, HF, FA	ELED	5	-	3	9	Sesame	Soy, wheat	-	-
6	M	54	HF, FA	SFED	50	Apple, oats, beer	7	5	Soy, apple	Soy, peanut, apple, beer	Apple, oats	-
7	F	49	-	SFED	3	-	1	4	-	-	-	-
8	M	53	Asthma, HF, FA	SFED	13	Hazelnut	0	6	Hazelnut, apple	Apple, hazelnut	Hazelnut	-

Abbreviations; M: male; F: female; FA: food allergy, AD: atopic dermatitis; HF: hay fever; PEC: peak eosinophil count per high power field after dietary treatment; ELED: elemental diet; SFED: six food elimination diet; CSF: clinical suspicious food; SDI: Straumann Dysphagia Index; EREFS: endoscopic reference score; SPT: Skin Prick Test; EPT: Esophageal Prick Test; sIgE: allergen specific IgE; -: none



ACCEPTED

SUPPLEMENTARY MATERIALS AND METHODS

Study patients

In this prospective pilot study patients were included from the outpatient clinic of the Academic Medical Centrum in Amsterdam between January 2016 and October 2016. Adults were eligible for enrollment if EoE was diagnosed according to current guidelines, defined as typical symptoms of EoE (dysphagia and/or food impaction) and the presence of more than 15 eosinophils per high-power field (HPF) during a course of proton pump inhibitors. In order to demonstrate that EPT responses were exclusively found in EoE patients, we included healthy controls as a reference. Exclusion criteria were: a history of anaphylactic reactions, other esophageal or gastrointestinal diseases, previous surgery of the digestive tract, severe comorbidity scored as ASA class III or higher, and the inability to stop anti-inflammatory drugs (i.e. topical or systemic steroids and leukotriene inhibitors). For safety reasons SPTs were performed prior to the EPT and subjects with a severe systemic reaction to SPTs were excluded from participation. Controls were recruited through advertisement and all subjects were screened to ensure they had no severe comorbidities classified as ASA II or higher. Each study subject gave written informed consent, and the study protocol was approved by the Medical Ethics Committee of our institution and prospectively registered in the Dutch trial registry (Trialregister.nl, NR6361).

Study protocol

Consented patients were pre-treated with an elemental or a four-food elimination diet in the four to six weeks preceding the EPT, because presence of severe esophagitis could have hampered interpretation of responses. The diet was guided by a dietician with special expertise in EoE. During the trial patients were not allowed to use any immunosuppressive drugs. If appropriate, beta blockers and ACE inhibitors were stopped 3 days in advance, because of their possible interfere with management of systemic anaphylactic reactions. Before the initial endoscopy, serum was collected for analysis of allergen specific IgE and SPTs were performed for the same allergens as that were tested with the EPT.

All subjects underwent two endoscopies. During the first endoscopy allergens were injected and acute responses were recorded up to 20 minutes, thereupon four biopsy specimens were sampled proximal to

the injection sites and subsequently stained with hematoxylin and eosin to evaluate histologic disease activity. Peak eosinophil counts (PEC) were determined per HPF, an area of 0.24 mm². Active disease was defined as a PEC of more than 15 eosinophils/HPF. These biopsies were obtained approximately 5 cm proximal to the allergen injection sites in order to prevent incorrect interpretation of the delayed EPT responses. These delayed EPT responses were evaluated during a second endoscopy that was performed 24 hours after the first endoscopy. Symptoms of dysphagia were measured per questionnaire recording the frequency and severity of dysphagia on a 5-point Likert-type scale, comparable to the Straumann Dysphagia Instrument (SDI). Endoscopic signs were scored blindly according to the Endoscopic Reference Score (EREFS).

Outcome parameters

The primary outcome parameters of our study were: (1) visible mucosal changes within 20 minutes after esophageal allergen injections, the acute response, and (2) visible mucosal changes after 24 hours, the delayed response. Secondly, we compared the local sensitization patterns identified through EPT with those identified through SPT and serum allergen-specific IgE testing. Finally, we evaluated whether the allergens that tested positive on EPT were in agreement with the reported implicating foods in patients' history.

Esophageal Prick Test

Endoscopies were performed under mild sedation with midazolam and during the entire procedure blood pressure, heart rates and oxygen saturations were monitored. The selection of injected allergens consisted of three empiric allergens (e.g. cow's milk, wheat, soy or egg) and 3 allergens based on patients' clinical suspicion. During endoscopy 0.2 ml of diluted allergen extracts, a negative control (0.9%NaCl) and a positive control (3.4 µg/ml diluted in 0.9% NaCl) were injected, using a through the scope injection needle with a volume of 0.2 ml. Fresh allergens were homogenized (for solids), extracted and diluted, as is described below. The solutions were injected in a pre-specified order at the 12 and 6 o'clock position at 5 cm and 11 cm above the squamocolumnar junction, and at the 3 and 9 o'clock position at 8 cm and 14 cm above the squamocolumnar junction. This guaranteed enough space between injections and allowed separate reading of responses to different allergens. Videos and images for scoring were taken at the injection sites 5, 10 and 20 minutes after injection.

Additional videos were obtained if acute responses occurred during the procedure. After each injection the through-the-scope needle was flushed in the stomach with 8 ml of saline solution and consequently with 8 ml of air. All procedures were performed by the same gastroenterologist and accompanied by an allergist with expertise in anaphylaxis and prepared for emergency situations. All subjects underwent a second endoscopy after 24 hours during which again videos and still images of the injection sites were obtained.

Preparation food extracts

All allergens were extracted from fresh foods, because the sensitivity of allergy tests decreases significantly when commercial food extracts are used. (24) Allergens were homogenized and diluted as follows: foods were ground in a mortar using liquid nitrogen. Food proteins were extracted for two hours at 4°C in sterile water in 10% (w/v) extracts. After centrifugation (12000 rpm, 15 min at 4°C), extracts were filtered using a 0.45 µm filter (Whatman, Life Sciences, UK). After a protein determination (Pierce BCA protein assay kit, ThermoScientific, Rockford, IL, USA), samples were diluted to a final concentration of 2 mg/ml and filtered again using a 0.45 µm filter. Aliquoted samples were immediately stored at -24°C.

Specific serum-IgE antibodies (sIgE)

Levels of specific serum-IgE antibodies (sIgE) were determined for the same allergens as the EPT with the ImmunoCAP 250 system (Phadia AB [at present Thermo Fisher Scientific], Uppsala, Sweden). Measurements were performed according to manufacturer's recommendations as is previously described.[3] The cut-off value for the assay was set at 0.35 kilounits of antibody per liter (kUA/L).

Skin prick test

Preceding the EPT all subjects underwent skin prick testing for the same food allergens as the EPT. SPTs were performed under the standard clinical routine protocol. Histamine and saline were used as positive and negative controls, respectively. Diameters of each wheal and flare were measured after 20 minutes. A positive result was defined as a wheal diameter of ≥ 3 mm.

Sample size

This pilot study is explorative in nature, since we are the first to investigate the feasibility of testing sensitizations on esophageal tissue by local allergen injections. The sample size of this pilot study was based on the assumption that test results of 8 patients would generate sufficient data to determine whether the test was promising enough to proceed with further developments or whether to abandon the concept. If injections with negative control solutions (saline) failed to induce responses in EoE patients and food allergens would not to induce visible mucosal reactions in the controls, then any responses to food injections in patients could be trusted as true abnormal responses.

Statistical analysis

Statistical analysis was performed in SPSS 22. For normally distributed or continuous variables, mean and standard deviation (SD) were reported. Medians with interquartile ranges were reported for non-normally distributed variables and percentages for categorical variables. The small sample size of this pilot study did not allow statistical comparison between EoE patients and controls.