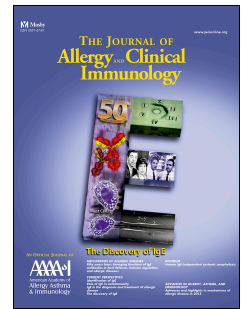


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Step-up empiric elimination diet for pediatric and adult eosinophilic esophagitis: the 2-4-6 Study

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Step-up empiric elimination diet for pediatric and adult eosinophilic esophagitis: the 2-4-6 Study

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Short title: Step-up empiric elimination diet (2-4-6) for EoE

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ABSTRACT

Background Numerous dietary restrictions and endoscopies limit the implementation of empiric elimination diets in eosinophilic esophagitis (EoE). Milk and wheat/gluten are the most common food triggers.

Objective To assess the effectiveness of a step-up dietary strategy for EoE.

Methods Prospective study conducted in 14 centers. Patients underwent a 6-week two-food group elimination diet (TFGED) (milk and gluten-containing cereals). Remission was defined by symptom improvement and <15 eos/HPF. Non-responders were gradually offered a four-food group elimination diet (FFGED: TFGED + egg and legumes) and a six-food group elimination diet (SFGED: FFGED + nuts and fish/seafood). In responders, eliminated food groups were individually reintroduced, followed by endoscopy.

Results 130 patients (25 pediatric) were enrolled with 97 completing all phases of the study. A TFGED achieved EoE remission in 56 patients (43%), with no differences between ages. Food triggers in TFGED responders were milk (52%), gluten-containing grains (16%) and both (28%). EoE induced only by milk was present in 18% and 33% of adults and children, respectively. Remission rates with FFGED and SFGED were 60% and 79%, with increasing food triggers, especially after SFGED. Overall, 55/60 (91.6%) of responders to TFGED/FFGED had one or two food triggers. Compared to initial SFGED, a step-up strategy reduced endoscopic procedures and diagnostic process time by 20%.

Conclusions A TFGED diet achieves EoE remission in 43% of children and adults. A step-up approach identifies early a majority of responders to empiric diet with few food triggers, avoiding unnecessary dietary restrictions, saving endoscopies and shortening the diagnostic process.

CLINICAL IMPLICATIONS

A step-up empiric elimination diet (2-4-6) identifies early a majority of EoE patients that respond to empiric diets with few food triggers, saving endoscopic procedures, shortening the diagnostic process, and avoiding unnecessary dietary restrictions.

CAPSULE SUMMARY

* A two-food group elimination diet achieves EoE remission in 43% of patients, identifying early 2/3 of potential responders to a SFGED.

* A step-up strategy saves endoscopic procedures, shortens the diagnostic process, and avoids unnecessary dietary restrictions.

KEY WORDS: eosinophilic esophagitis; diet; milk; wheat; six-food elimination.

Abbreviations: DSS: Dysphagia Symptom Score; EoE: eosinophilic esophagitis; eos/HPF: eosinophils per high power field; FFGED: four-food group elimination diet; PPI: proton pump inhibitor; SFGED: six-food group elimination diet; TFGED: two-food group elimination diet.

INTRODUCTION

Eosinophilic esophagitis (EoE) is a chronic, immune/antigen-mediated disease, isolated to the esophagus, characterized clinically by symptoms related to esophageal dysfunction and histologically by eosinophil-predominant inflammation¹. Since its initial description in the early 1990s^{2,3}, EoE has lately become the leading cause of dysphagia in children and young adults living in westernized countries⁴. The first study published in 1995 showed complete reversal of refractory EoE attributed to gastroesophageal reflux disease after exclusive feeding with an amino acid-based formula (elemental diet) for at least 6 weeks⁵. This seminal report first established the etiologic role of food in EoE, which is currently known to be an allergic condition predominantly triggered by food antigens. Unlike conventional IgE-mediated food allergy, EoE has been demonstrated to be a distinct form of food allergy, largely dependent on non-IgE delayed, cell-mediated hypersensitivity⁶.

Despite dietary therapy being the only treatment targeting the cause of the disease instead of its inflammatory consequences, pharmacological therapy (proton pump inhibitor (PPI) or topical steroid therapy), has become more popular. Among the three major modalities of dietary therapy for EoE, elemental diet remains the most effective intervention in children and adults⁷, but it is hampered by multiple disadvantages, including complete avoidance of food, poor palatability, socialization impairment and lack of reimbursement. Results for food allergy testing-guided elimination have been consistently low in adults⁸⁻¹¹ and variable in children⁶. An empiric elimination diet was first tested in 2006¹². This diet, termed six-food group elimination diet (SFGED), consisted of eliminating the six food groups most commonly associated with food allergy in the pediatric population in Chicago (cow's milk protein, wheat, egg, soy, peanut/tree nuts, fish and seafood) for six weeks. A SFGED led to clinicohistologic remission in three quarters of children¹² and consistent results have been further obtained in adults from the US^{11,13}, Spain^{14,15} and Australia¹⁶. The effectiveness and wide reproducibility of a SFGED are counteracted by the large number of endoscopies

required after individual food reintroduction. Currently, food groups to be avoided in the long term for each responder to a SFGED can only be identified through individual food reintroduction followed by histologic reevaluation.

It is of note that up to three quarters of responders to a SFGED have been found to have just 1 or 2 food triggers after individual food reintroduction¹⁷. The most common causative foods identified after a response to a SFGED were cow's milk, wheat, egg and, to a lesser extent, soy/legumes. In light of these data, a four-food elimination diet (FFGED) was developed. A first prospective multicenter study in adult Spanish patients showed a 54% remission, with half of responders having cow's milk, gluten-containing grains or both as food triggers¹⁸. Therefore, the aim of this study was to determine the effectiveness and resource saving of a step-up strategy for empiric elimination diet in pediatric and adult EoE patients, by means of starting with elimination of the two most common food triggers (milk and gluten) and then stepping up to a FFGED and eventually to a SFGED in non-responders.

METHODS

Patient selection and eligibility

This was a multicenter prospective quasi-experimental study conducted in 13 Spanish and 1 Italian center between September 2014 and November 2016. Informed consent was obtained from all patients included in the study. Ethical approval was granted by the Institutional Review Board in all participating centers. Consecutive children > 2 yrs-old and adults with a diagnosis of EoE, defined by consensus guidelines [symptoms of esophageal dysfunction, ≥ 15 eosinophils per high power field (eos/HPF) and lack of histologic response (≥ 15 eos/HPF) after an 8-week trial of PPI therapy] were eligible for enrolment¹. Patients were recruited from outpatient gastroenterology clinics or endoscopy units. All eligible patients were naïve for topic steroid or dietary therapy. Exclusion criteria included previous diagnosis of eosinophilic gastrointestinal disorder, any potential cause for esophageal eosinophilia different from EoE (achalasia, caustic or radiation esophagitis, parasites, inflammatory bowel disease, neoplasm, drugs), food-associated anaphylaxis to milk or wheat, inability to adhere to an elimination diet, or inability to take biopsies because of the presence of esophageal varices or active anticoagulant therapy. Patients with severe fibrostricturing EoE, either with strictures or narrow caliber esophagus, were also excluded.

Assessment of clinic, endoscopic and histologic data

Physical examinations, clinical data records, and baseline endoscopies with esophageal biopsies on PPI therapy, at both distal and proximal esophagus, were recorded. Dysphagia in older children and adults were assessed by means of the Dysphagia Symptom Score (DSS), a non-validated instrument used in previous adult studies on EoE^{13,18}. This score assigns points for frequency, intensity, duration of symptoms, and presence of lifestyle changes, with a range from 1 to 18, with greater intensity of dysphagia reflected by higher scores. All patients or their parents were

asked whether they felt symptoms had been resolved or not after each dietary intervention.

All endoscopic procedures were performed with either topic pharyngeal anesthesia or propofol-based sedation, according to patient preference, by board-certified gastroenterologists and pediatricians. Using conventional grasping forceps, at least 4 biopsy specimens were taken from both the distal and proximal esophagus. Endoscopic abnormalities suggestive of EoE were recorded following a standardized classification¹⁹. Mucosal biopsy specimens were fixed in formalin, embedded in paraffin, and stained with hematoxylin and eosin for histologic examination. They were reviewed by senior gastrointestinal pathologists with expertise in EoE at each center. Peak eosinophil count was determined in the area of highest density of eosinophils and esophageal eosinophilia was defined upon the presence of 15 or more eos/HPF in at least one field¹.

Definition of clinic and histologic remission

A decrease of more than 50% of baseline DSS score after dietary therapy was considered as clinical remission in older children and adults. Subjective symptom improvement reported by either children or parents was considered for younger children. Histologic remission was defined by an eosinophil peak count below 15 eos/HPF at both distal and proximal esophagus. Response to any dietary intervention was defined by a combination of clinic and histologic remission.

Two-food group elimination diet

A two-food group elimination diet (TFGED) with elimination of cow's milk and wheat was instituted in all patients for 6 weeks. Due to potential cross-reactivity between food allergens²⁰ and in accordance with our previous experience with a FFGED¹⁸, we decided to eliminate all dairy products (either goat's or sheep's milk can cross-react with cow's milk) and all gluten-containing grains (cross-reactive with wheat,

including barley, rye and oats). Concomitant PPI therapy was allowed if gastro-esophageal reflux disease symptoms were present. Treatment with oral, nasal, airway, or swallowed steroids was not allowed from 8 weeks before enrolment until the end of the study. In case of exacerbated rhinitis or asthma during the study period, anti-H1, inhaled beta2-agonists and anticholinergic bronchodilator drugs were allowed.

Food allergens known to cause oral allergy syndrome symptoms were already avoided by patients prior to enrolment. Over the study period with a TFGED, patients were allowed to eat rice and corn, eggs, all kind of legumes and vegetables, meat, fish/seafood, fruits and nuts. Gluten-free products were also permitted, provided they did not contain milk. They could also drink coffee, tea and herbal infusions, soy/rice/almond/hazelnut milk, soft drinks, and alcoholic beverages, although beer or whiskey consumption was not allowed because of gluten content. Written instructions for adequate reading of food labeling were given to patients in order to avoid foods containing potential hidden names for milk (casein & caseinates, lactalbumin, hydrolysates, whey, custard, animal protein, cream, flavouring) and wheat (farina, flour, starch, vegetable protein, glutamate, dextrin, maltodextrine, seitan, semolina, couscous, kamut, spelt, triticale, triticum) in food labels. Patients were also advised to avoid processed foods, due to the high likelihood of containing wheat- or milk-traces, including processed meats (e.g, sausages, hamburgers), soups, sauces, pizza, mashed potato, instant rice. A thorough list of foods and sample menus either allowed and to be avoided was also provided to patients (Supplementary material 1 in English, http://www.aegastro.es/sites/default/files/archivos/documento-grupo/esofagitis_eosinofilica_0.pdf in Spanish). No registered dietitian or nutrition specialist was involved in the study. A telephone number and e-mail address were also provided to patients in case of further doubts regarding the TFGED.

Step-up dietary therapy

Non-responders to a TFGED were offered to step up to a FFGED (TFGED + elimination of egg and legumes, including soy, lentils, chickpeas, peas, beans and peanuts) during 6 weeks. Patients were instructed to read carefully gluten-free product labels, since milk, egg and legume flour were not allowed. Non-responders to a FFGED were offered rescue dietary therapy with a SFGED (FFGED + additional exclusion of all kind of nuts, fish, and seafood) for an additional 6-week period. Response to a FFGED or SFGED was also defined by clinico-histologic remission. Non-responders to a step-up dietary approach or those unwilling to increase dietary restrictions after TFGED or FFGED were offered treatment with swallowed topical steroids (viscous oral budesonide 2 mg bid adults and 1 mg bid in children, or swallowed fluticasone 800 mcg bid) for eight weeks, with further histologic re-evaluation.

Food reintroduction in responders to empirical diets

Patients achieving clinicopathologic remission on any empiric diet underwent individual food reintroduction of eliminated food groups to identify food triggering EoE. Gluten, especially white bread, was suggested to be the first food to be reintroduced, but the order of food reintroduction was set according to patient preferences. A daily consumption of foods from each food group was encouraged for a 6-week period, with endoscopic reevaluation after each reintroduced food group. If peak eosinophil count was below 15 eos/HPF after a single-food challenge, this food was considered to be well tolerated and maintained in the diet. In contrast, if inflammation (≥ 15 eos/HPF) recurred, that food was considered an EoE trigger and removed from the diet indefinitely. There was no wash-out period after inflammation recurrence with a food challenge.

Study endpoints

The primary study endpoints were clinico-histologic remission rates after a first-line TFGED in patients with EoE, as well as after stepping up to a FFGED and SFGED

rescue therapies. Secondary endpoints included identifying the frequency and number of food triggers through systematic reintroduction of individual food groups and determining savings regarding endoscopic procedures and diagnostic process time when starting with a TFGED compared to beginning dietary therapy with a FFGED or SFGED.

Statistical analysis

The SPSS (version 21.0; SPSS, Inc, Chicago, Illinois) statistical analysis package was used. Categorical variables were described with frequencies and percentages, and continuous variables were described with mean (standard deviation) or median (IQR) as appropriate. Associations between categorical variables were tested with the χ^2 test (with Fisher correction when necessary), and continuous data were assessed using the 2 sample t-test or the Mann Whitney U test for parametric and nonparametric data, respectively. A signed Wilcoxon rank test was used to assess differences in eosinophil counts and symptom scores before and after empiric diet treatment and after reintroduction of the trigger food. Effectiveness of each of the dietary approaches was measured “per protocol” as the number of patients who responded to a particular diet divided by the total number of patients who effective underwent to that diet. Missing data from patients who abandoned the step-up protocol before starting a FFGED or a SFGED were managed with data imputation techniques (by imputing response rates assuming patients who discontinued from the step-up protocol had the same response rate as those who completed the step-up protocol). A p value lower than 0.05 was considered statistically significant. In line with previous studies and meta-analyses^{7,14,18}, a pre-specified sample size of 100 patients was considered, with 1 child for every 4 adult patients included.

RESULTS

Baseline characteristics of patients

Over the recruitment period, a total of 227 consecutive patients with EoE and documented lack of histological response to PPI therapy were eligible for enrolment. Seven patients were excluded due to severe fibrostenotic EoE and 90 refused dietary interventions. Finally, 130 patients (25 pediatric patients < 14 yrs-old) were included. Among children, 13 patients were between 5 and 9 yrs-old and 12 between 10 and 13 yrs-old. Sixty two patients (48%) remained on concomitant PPI therapy during the TFGED. The flow of patients during the study is exhibited in **Figure 1**. Before enrolment, all patients consumed regularly foods within the two food groups excluded in the TFGED. Additional food avoidance at baseline were observed in eleven patients suffering from IgE-mediated egg, legumes, nuts and fish allergy, and fifteen patients suffered from oral allergy syndrome to some nuts and fruits. Baseline characteristics of patients included in the study are presented in **Table 1**. Children had a higher rate of food allergy and symptoms such as epigastric pain, abdominal pain, and nausea or vomiting, whereas adult patients were more likely to suffer from dysphagia/food impaction and show rings or reflux esophagitis on endoscopy.

Effectiveness of TFGED

Regarding dysphagia symptoms, median baseline DSS (IQR) score in the whole series was 10 (4) and significantly decreased after a TFGED to 4 (7). Overall 75.4% of patients achieved clinical response criteria (reduction $\geq$ 50% of baseline DSS). The decrease in DSS was higher among patients who achieved histological response (3 [4] $p < 0.001$) than in those who did not (6 [9] $p = 0.02$) (**Figure 2**). Symptom improvement after a TFGED was reported in a similar proportion of older children and adults (73% vs. 91%, $p = 0.28$).

As for histopathologic remission, 56 patients (43%) achieved histological remission on a TFGED (all of them having achieved clinical remission). There were no differences in histologic remission rates for a TFGED when comparing adult and pediatric patients (46/105 (44%) vs. 10/25 (40%), $p=0.6$), concomitant use of PPI therapy (PPIs 28/62 (45%) vs. no PPIs 28/68 (41%), $p=0.5$) or implementation of the diet within the pollen season, which lasts from March to August in Spain (during pollen season 22/51 (43%) vs. out of pollen season 34/79 (43%), $p=0.8$). Demographic and clinical characteristics of patients, as well as personal or family atopic background, did not predict response to any dietary therapy (data not shown). The effectiveness of the different dietary interventions evaluated in the study are summarized in **Figure 3**.

Effectiveness of step-up FFGED and SFGED

Dysphagia regarding the baseline DSS remained unchanged when increasing the level of dietary restriction to either a FFGED (5 [8.75] $p=0.01$) or a SFGED (6.5 [8.25] $p=0.011$). Of note, no differences were observed in DSS scores among responders and non-responders to a FFGED. However, symptom scores were significantly reduced after a SFGED among those patients who experienced histologic remission (3 [3] in responders vs 10 [8] in non-responders; $p=0.009$) (**Figure 2**).

Among 74 non-responders to a TFGED, 54 patients (72%) accepted stepping up to a FFGED. Ten of 54 (18%) achieved histologic remission on a FFGED. Remission rates in adults and children were 9/45 (20%) and 1/9 (11%), $p=0.2$, respectively. Among 44 patients who achieved no histologic remission on TFGED and FFGED, 27 patients (61%) decided to step-up to a SFGED. Eight out of 27 (29%) achieved histologic remission on a SFGED [6/21 pediatric patients (28%) vs. 2/6 adult patients (33%)]. Cumulative “per protocol” clinico-histological remission rates after TFGED, FFGED and SFGED were 43%, 60% and 79%, respectively and are summarized in **Figure 3**. For the whole recruited series and having assumed no patient dropout, data imputation

analysis provided remission rates after TFGED, FFGED and SFGED of 43%, 54% and 68%, respectively (**Supplementary Table 1**).

Identification of food triggers through individual food group reintroduction

Individual food reintroduction was completed in 50/56 (89%) TFGED responders, 10/10 (100%) of FFGED responders and 4/8 (50%) of SFGED responders. The most common food triggers were milk [52/64 (81%)], gluten [28/64 (43%)], egg [10/64 (15%)] and legumes [6/64 (9%)]. Food triggers identified in responders to each dietary intervention is displayed in **Table 2**. Twenty six patients (19 adults, 7 children) were found to have milk as the only food trigger. Therefore, milk-induced EoE was present in 19/103 (18.4%) and 7/21 (33.3%) of adult and pediatric patients in our study, respectively. It is of note that 55/60 (91.6%) of responders to TFGED/FFGED were found to have one or two food triggers identified after individual food reintroduction. The number of food triggers notably increased with increasing dietary restrictions, especially with SFGED, as shown in **Figure 4**.

Endoscopic and diagnostic process time with a step-up dietary strategy

Assuming histologic remission rates of 40% (TFGED), 60% (FFGED) and 70% (SFGED) and a 6-week period before endoscopy for either elimination diet or food reintroduction, we compared the number of endoscopies and time on dietary restriction necessary to complete the diagnostic process for different top-down and step-up strategies in 10 EoE patients unresponsive to PPI therapy. The results are shown in **Table 3**. Compared to initial SFGED, step-up 2-4-6 or 2-4 strategies may save 20% and 30% of endoscopic procedures and diagnostic process time, respectively.

Effectiveness of rescue topical steroid therapy

From 130 included patients, 73 patients were responsive to some dietary intervention. Thirty seven patients refused to further increase dietary restrictions on a step-up basis (20 after TFGED and 17 patients after FFGED), whereas 20 patients did not respond to TFGED, FFGED and SFGED. Among these 57 patients (13 children), 24 patients (9 children and 15 adults) accepted rescue therapy with swallowed topical steroids for 8 weeks, of whom 19 (79%) achieved histologic remission.

DISCUSSION

In the present study, a TFGED achieved a 43% efficacy, and obtained similar results to those previously reported¹³⁻¹⁸ when patients were stepped up to a FFGED (60%) and SFGED (79%). This step-up strategy, starting with a TFGED, exhibits a relevant number of advantages over beginning with highly restrictive diets (top-down dietary strategies). To begin with, it allows early identification of two thirds of responders to any empiric elimination diet with the performance of one single endoscopic procedure. Secondly, we showed that a single food trigger (either animal milk or gluten-containing cereals) was present in up to 70% of TFGED responders, with the remaining patients having both. Additionally, 70% of responders to a FFGED were also found to have one or two food triggers. As such, a step-up combination of TFGED and FFGED is capable of detecting the vast majority of those potential SFGED responders aforementioned who had just one or two food triggers after six food challenges, followed by six endoscopies¹⁷. Undoubtedly, these patients with one or two food triggers are the best candidates for maintenance dietary therapy through long-term avoidance of these few food triggers. Thirdly, a TFGED can be easily undertaken without the need of a dietician. Allergists and pediatric and adult gastroenterologists are familiar with gluten-free, lactose-free and/or cow's milk protein-free diets, usually prescribed for common conditions like celiac disease, wheat allergy, irritable bowel syndrome or food protein-induced enterocolitis. Fourthly, specific referral to an allergist for food allergy testing can be avoided with a TFGED, resulting in improved resources management and avoiding confusion from positive results on food allergy testing. Fifthly, a TFGED allows egg and legumes intake, which may be difficult to avoid, especially in gluten-free products. Sixthly, a TFGED or FFGED can save a relevant proportion of endoscopic procedures and time on unnecessary dietary restrictions. Last but not least, patient uptake for dietary therapy may notably increase with less restrictive diets and a step-up strategy may definitely boost patient willingness to give a try to dietary therapy.

Milk was found to be the only food triggering the disease in half of TFGED responders. Overall, milk-induced EoE was present in 18% and 33% of adult and pediatric patients, respectively. Our results are consistent with our previous sub-analysis from the first FFGED study¹⁸, but discrepant with previous pediatric studies reporting 65% and 61% cure rates with a cow's milk elimination diet^{22,23}. Concerns about methodological flaws hang over these studies, including selection bias (exclusive inclusion of patients with IgE-mediated cow's milk food allergy resolved after cow's milk oral desensitization)²² or concomitant use of PPI and dietary therapy²³. The effectiveness of a milk elimination diet should be evaluated in well-designed rigorous multicenter studies in children and adults. In line with previous studies on SFGED¹²⁻¹⁷, we obtained similar results in children and adults with every evaluated dietary intervention.

Interestingly, egg has been reported as the third most common food trigger for EoE, closer to gluten as the second most common food trigger¹⁷. Milk, gluten-containing grains and eggs are staple foods worldwide. In contrast, legumes seem to be common food triggers only in Spanish studies^{14,15,18} (including the present one), with a minor role reported in studies conducted in the US^{12,13} and Australia¹⁶. In these countries, soy bean seems to be the only relevant legume related with EoE. These discrepant findings may merely reflect distinct patterns of food consumption among different geographical areas. Legumes like lentils, chickpeas, beans, and peas are regularly consumed in Mediterranean countries. On account of these data, the effectiveness of a three-food elimination diet (milk, gluten-containing grains and egg) warrants further research in specific settings where legumes, including soy and peanuts, are not elements of a regular diet. In our country, we firmly believe that a TFGED and FFGED should be tested in all EoE patients willing to undergo dietary therapy. Whereas stepping-up to a SFGED might be reserved for highly motivated patients who wish to identify potential causative foods and are willing to remove several food groups from their diets indefinitely.

In accordance with our previous research with a FFGED¹⁸, concomitant PPI therapy or implementing the diet out of the pollen season did not increase the efficacy of a TFGED. Likewise, we found two responders to a TFGED who achieved sustained remission despite reintroduction of both milk and wheat. The significance of this intriguing phenomenon, which has been described in other studies^{16,18,24}, remains unknown. Sampling error in esophageal biopsies, misdiagnosed responders to PPI therapy, cross reactivity with airborne allergens or disease activity fluctuations may be potential explanations. We also replicated typical clinico-histologic dissociation after therapeutic interventions in EoE, with higher clinical response over histologic remission after a TFGED, but no clinic improvement despite histologic remission with FFGED or SFGED. This finding might be related to a high degree of motivation at the first step of this multistage process, which may decline and then alter symptom perception when more restrictive diets are accomplished.

Our study has several strengths, such as being the first multicenter prospective study on a step-up dietary strategy, the first study coupling children and adults and the study with the largest sample size reported so far. No differences were observed between children and adults in the present study, confirming previous reports of similar remission rates for a SFGED, regardless of patient age⁷. On account of recent homogeneous cure rates also reported for PPI²⁵ and topic steroid²⁶ therapy between pediatric and adult patients, our findings confirm that EoE is likely a continuum of the same disease in children and adults. Several limitations, however, should be acknowledged to this study, namely the absence of a control group and use of a non-validated questionnaire to assess symptoms in EoE. The more recent EoE activity index (EEsAI) instrument, validated only in adult patients²⁷, has not been validated yet to be used in the Spanish language. Nevertheless, esophageal symptoms alone, measured through the EEsAI instrument, have recently showed a modest predictive capacity for estimating the presence of either histological and endoscopic remission in adult patients with EoE²⁸. No validated²⁹ or non-validated³⁰ symptom scores were used

for younger children with dysphagia. The Pediatric Eosinophilic Esophagitis Symptom Score has lately shown a good correlation with clinic and histologic outcomes after therapy³¹. Of note, a 28% drop-out rate of the total initially enrolled patients should be acknowledged, which may mistakenly lead to a sense of overestimation of the success rate of the diet. However, the design of the present study only allows a “per protocol” analysis, in patients who were assessed before and after the dietary intervention. All previous studies and meta-analyses published on dietary therapy have also followed this methodology. Indeed, efficacy rates calculated by data imputation were slightly lower for a FFGED and SFGED. In either case, the observed “per protocol” response rates paralleled all previously published data, so the effect of a possible selection bias would have been quite limited. Compliance with dietary recommendations was not structurally assessed and the lack of a dietitian might have underrated weight loss or growth failure. Though the need of a wash-out period between endoscopies after disease relapse during food reintroduction remains unproven, its lack in the present study may have potentially led to misleading results. However, our findings are quite similar to those described in previous literature^{14-18,24}. Finally, our results may be transferable to settings with similar staple diets and food consumption habits. Therefore, generalization should not be made until further validation is made in other geographical areas with different food consumption habits.

In conclusion, the present study prospectively demonstrates that a TFGED achieves remission in 43% of EoE patients, with no differences between children and adults. After rescue FFGED, 92% of responders to either TFGED or FFGED were found to have one or two food triggers identified after individual food reintroduction. Compared to initial SFGED, initial TFGED recognizes early two thirds of responders to any empiric elimination diet, whereas stepping up to a FFGED identified all patients with one of two food triggers, best candidates for maintenance dietary therapy. Whether exclusive milk elimination diet is suitable as a first-line therapy for pediatric

populations, with remission rates close to 30% in our study, should be evaluated in large prospective well-conducted studies. We also replicated previous data on the effectiveness of FFGED and SFGED, albeit the number of identified food triggers notably increased, especially after a SFGED. These findings provide useful and realistic information for patients undertaking empiric elimination diets. We do believe that this multistage step-up approach may be recommended to simplify dietary management of EoE, avoid unnecessary dietary restrictions, reduce the number of endoscopic procedures and shorten the diagnostic process time. All these advantages may definitely help engage both EoE patients and physicians with dietary therapy.

Table 1. Baseline characteristics of patients included in the study. Comparisons were made between adult and pediatric patients.

	Overall n=130	Adults n=105	Children n=25	p
Age, mean (range)	29 (5-59)	32 (14-59)	11 (5-13)	< 0.001
Male gender, n (%)	94 (72%)	77 (73%)	17 (68%)	0.5
Smoking habit, n (%)	14 (11%)	14 (13%)	-	-
Family history of EoE, n (%)	6 (5%)	5 (5%)	1 (4%)	0.8
Family history of atopy, n (%)	66 (51%)	49 (47%)	17 (68%)	0.004
Atopic disorders, n (%)	115 (88%)	94 (89%)	21 (84%)	0.7
Rhinoconjunctivitis	80 (61%)	67 (64%)	13 (52%)	0.09
Asthma	59 (45%)	48 (46%)	11 (44%)	0.8
Food allergy/oral allergy syndrome	27 (21%)	17 (16%)	10 (40%)	0.006
Atopic dermatitis	23 (18%)	14 (13%)	9 (36%)	< 0.001
Angioedema	13 (10%)	9 (8%)	4 (16%)	0.4
Symptoms, n (%)				
Dysphagia	110 (85%)	101 (96%)	9 (36%)	< 0.001
Food bolus impaction	71 (55%)	69 (66%)	2 (8%)	< 0.001
Heartburn/regurgitation	58 (45%)	47 (45%)	11 (44%)	0.8
Epigastralgia/abdominal pain	19 (15%)	4 (4%)	15 (60%)	< 0.001
Chest pain	14 (11%)	14 (13%)	-	-
Nausea/vomiting	11 (8%)	1 (1%)	10 (40%)	< 0.001
Dysphagia Symptom Score, points	9.6	9.8	5.5	< 0.001
Endoscopic findings, n (%)				
Normal endoscopic appearance	16 (12%)	13 (12%)	3 (12%)	0.6
Rings	76 (58%)	74 (70%)	2 (8%)	< 0.001
Longitudinal furrows	88 (68%)	68 (65%)	20 (80%)	0.1
Edema	111 (85%)	89 (85%)	22 (88%)	0.7
Whitish exudates	45 (35%)	35 (33%)	10 (40%)	0.3
Reflux esophagitis	13 (10%)	12 (11%)	1 (4%)	0.008
Crepe paper esophagus	10 (8%)	8 (8%)	2 (8%)	0.8
Esophageal eosinophilia, eos/HPF				
Proximal esophagus	45 (0-300)	47 (0-300)	41 (0-60)	0.6
Distal esophagus	42 (0-157)	42 (0-157)	37 (16-90)	0.4

Table 2. Food triggers identified after individual food reintroduction followed by endoscopy in each dietary intervention

TFGED RESPONDERS (50/56)		
One food trigger	34/50	68%
Milk	26/50	52%
Gluten	8/50	16%
Two food triggers	14/50	28%
Milk and gluten	14/50	28%
No food trigger	2/50	4%
FFGED RESPONDERS (10/10)		
One food trigger	1/10	10%
Egg	1/10	10%
Two food triggers	6/10	60%
Milk and legumes	3/10	30%
Milk and egg	3/10	30%
Three food triggers	3/10	30%
Milk, gluten, and egg	2/10	20%
Milk, gluten and legumes	1/10	10%
SFGED RESPONDERS (4/7)		
Three food triggers	1/4	25%
Milk, egg, and fish/seafood	1/4	25%
Four food triggers	1/4	25%
Gluten, egg, legumes, and fish/seafood	1/4	25%
Five food triggers	2/4	50%
Milk, gluten, egg, nuts, and fish/seafood	1/4	25%
Milk, gluten, egg, legumes, and fish/seafood	1/4	25%

Table 3. Calculations on the number of endoscopic procedures and correlative diagnostic process time required for empiric dietary interventions in ten EoE given patients.

Histologic remission rates of 40% (TFGED), 60% (FFGED) and 70% (SFGED) and a 6-week period before each endoscopy for either elimination diet or food reintroduction were assumed.

	Initial endoscopy after a SFGED	Food reintro in SFGED responders (n=7)					Total number of endoscopies	Total weeks on dietary restrictions
6	10	$7 \times 6 = 42$					52	312
	Initial endoscopy after a FFGED	Food reintro in FFGED responders (n=6)	Step-up SFGED	Food reintro in SFGED responders (n=1)				
4-6	10	$6 \times 4 = 24$	4	$1 \times 6 = 6$			44	264
	Initial endoscopy after a TFGED	Food reintro in TFGED responders (n=4)	Step-up FFGED	Food reintro in FFGED responders (n=2)	Step-up SFGED	Food reintro in SFGED responders (n=1)		
2-4-6	10	$4 \times 2 = 8$	6	$2 \times 4 = 8$	4	$1 \times 6 = 6$	42	252

Figure 1. Flow chart of patients during the study.

Figure 2. Dysphagia Symptom Scores (median $\pm$ IQR) in the included EoE patients, at baseline and after any dietary intervention, irrespective of remitted or persistent esophageal eosinophilia.

Figure 3. “Per protocol” remission rates on a TFGED (56 patients) and after step-up intervention with a FFGED (10 patients) and a SFGED (7 patients).

Figure 4. Proportion of responders to each dietary intervention and corresponding number of food triggers identified after individual food reintroduction

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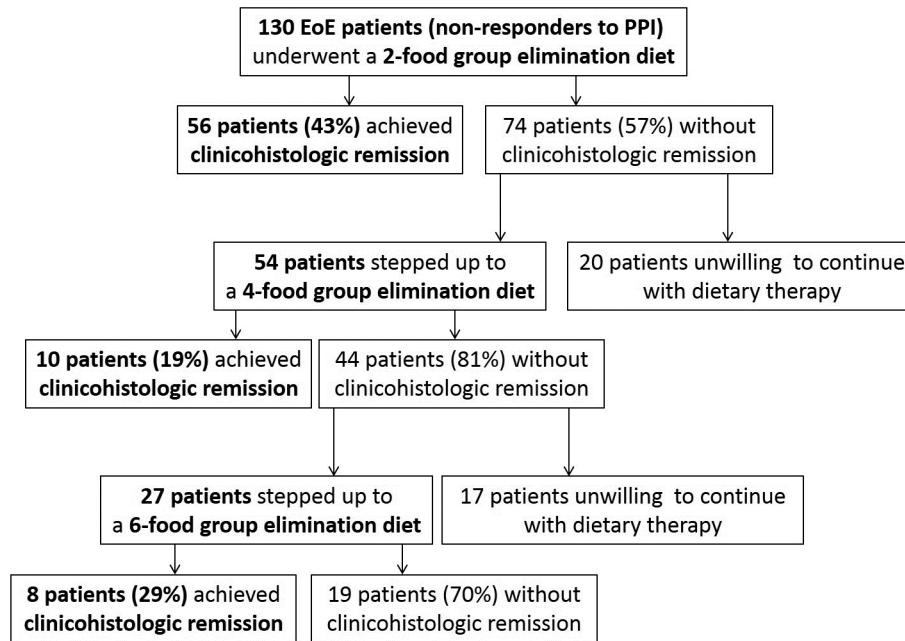
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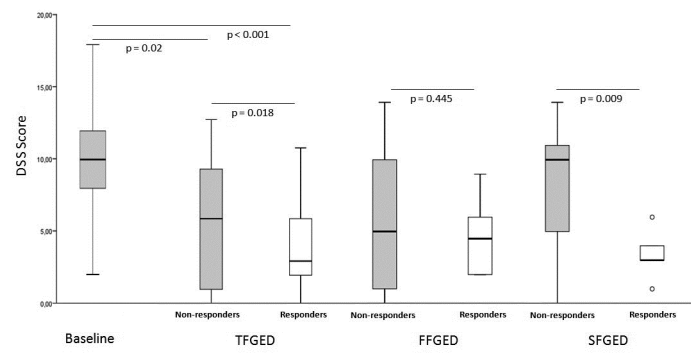
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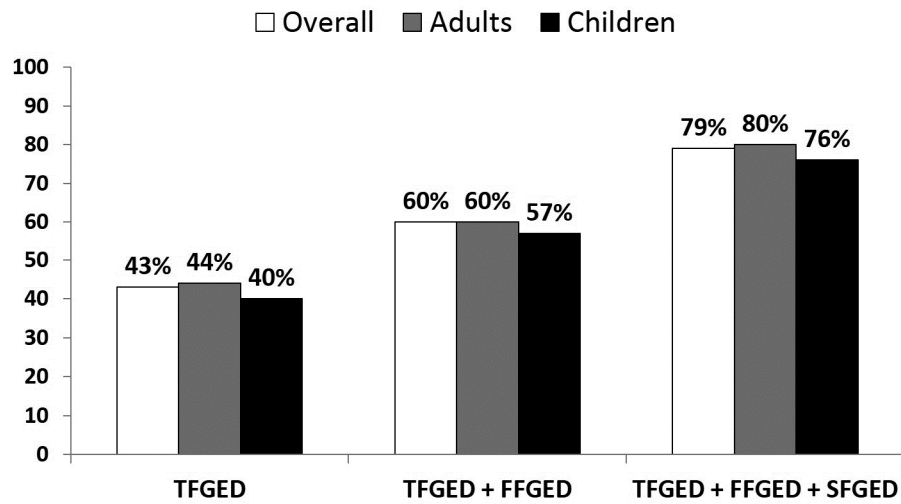
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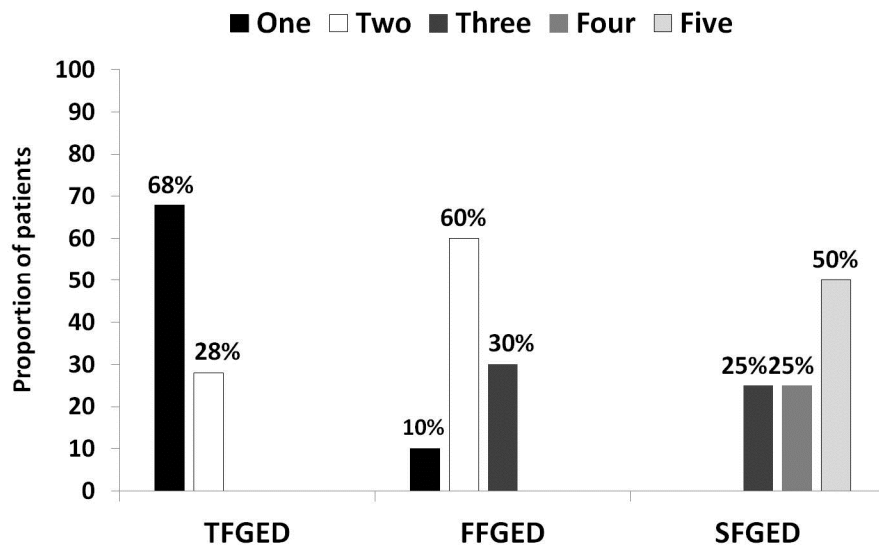
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Supplementary Table 1. Clinico-histological remission rates estimated for the whole series of 130 recruited patients, in the hypothetical case every participant completed the whole study protocol with no dropout patients.

	Clinico-histological remission rates	
	"Per protocol"	Data imputation
TFGED	56 / 130 (43.07%)	56 / 130 (43.07%)
TFGED + FFGED	66 / 100 (60%)	70 / 130 (53.84%)*
TFGED + FFGED + SFGED	74 / 93 (79.56%)	88 / 130 (67.69%)**

* Data imputation was based on the theoretical assumption than 14 patients out of those 74 who failed to TFGED would have started a FFGED and obtained an overall response rate of 19% (the same observed for the 54 participants with complete data).

** To estimate overall potential response to a SFGED in the entire group of 130 recruited patients, we have consider that 60 patients should started on a SFGED (excluding from the initial 130 patient series those 56 with response to a TFGED and 14 who responded to a FFGED). Imputing the same response rated documented for the patients who truly underwent to a SFGED, clinico-pathological after a SFGED should have be achieved in 18 patients, being 88 the overall number of patients that achieved histological remission of EoE at any stage of the 2-4-6 step up protocol (56 + 14 + 18).