

Swallowed Fluticasone Propionate Is an Effective Long-Term Maintenance Therapy for Children With Eosinophilic Esophagitis

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- OBJECTIVES:** Although effective in the treatment of eosinophilic esophagitis (EoE) in children, limited data exist on long-term safety and efficacy of swallowed topical corticosteroids. We investigated whether long-term use of swallowed fluticasone in children with EoE leads to sustained reduction in esophageal eosinophils, and endoscopic and clinical improvement.
- METHODS:** In an open-label, prospective, single-center study, we offered pediatric patients with active EoE fluticasone 2 puffs to swallow twice a day (strengths in $\mu\text{g}/\text{puff}$: 2–4 years: 44, 5–11 years: 110, ≥ 12 years: 220). Clinical, endoscopic, and histological assessments were performed at baseline and shortly after therapy. If histological remission was seen, fluticasone was continued with clinical follow-ups every 4 months and endoscopic and histological follow-ups yearly. Clinical scores were derived from eight symptoms (abdominal pain, nausea, vomiting, regurgitation, chest pain, dysphagia, food impaction, and early satiety). Endoscopic scores were derived from six features (rings, exudates, furrows, edema, stricture, and shearing). Scores were expressed as ratio (features present/total). In addition to peak eosinophils/high power field (HPF) (primary outcome), histological features (eosinophilic microabscesses, degranulation, superficial layering, basal zone hyperplasia, dilated intercellular spaces, and lamina propria fibrosis) were assessed. Median clinical and endoscopic scores and individual histologic features were compared over 4 time intervals: <4 months, 4–12 months, 13–24 months, and >24 months. Growth and adverse effects were monitored.
- RESULTS:** We enrolled 54 patients, 80% male, median age 6.5 years (range 2–17 years), 85% atopic (57% asthma, 68% allergic rhinitis, and 31% atopic dermatitis), and 74% with food allergy. Mean follow-up was 20.4 months, the longest being 68 months (5.7 years). Esophageal eosinophil counts significantly decreased (median peak eosinophils/HPF at baseline 72, <4 months: 0.5, 4–12 months: 1.75, 13–24 months: 10, and >24 months: 12, all $P<0.01$). All histological features significantly decreased from baseline to all follow-up time points (all $P<0.01$). Lamina propria fibrosis significantly decreased (% patients with fibrosis at baseline 92, <4 months: 41, 4–12 months: 50, 13–24 months: 45, and >24 months: 39, all $P<0.01$). Endoscopic features improved (score at baseline 0.37, <4 months: 0.17, 4–12 months: 0.17, 13–24 months: 0, and >24 months: 0.1, all $P<0.01$, except at >24 months: $P<0.05$). Symptoms improved (score at baseline 0.22, <4 months: 0, 4–12 months: 0.11, 13–24 months: 0.11, and >24 months: 0.11, all $P<0.05$ except at >24 months: $P=0.05$). In a mixed linear regression model that accounts for correlation of repeated observations in the patient in a per-patient analysis, we found that treatment with swallowed fluticasone led to a statistically significant and sustained decrease in peak esophageal eosinophil counts. Asymptomatic esophageal candidiasis was seen in three children but resolved with anti-fungal therapy. Height and weight z-scores followed expected growth curves.

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Received 2 November 2015; accepted 4 May 2016

CONCLUSIONS: We demonstrate that swallowed fluticasone is effective as a long-term maintenance therapy for children with EoE, without growth impediment or serious side effects.

SUPPLEMENTARY MATERIAL is linked to the online version of the paper at <http://www.nature.com/ajg>

Am J Gastroenterol advance online publication, 21 June 2016; doi:10.1038/ajg.2016.238

INTRODUCTION

Eosinophilic esophagitis (EoE) is a chronic immune-mediated inflammatory disease of the esophagus (1,2). EoE is characterized clinically by symptoms of esophageal dysfunction including abdominal pain, regurgitation, vomiting, dysphagia, and esophageal food impactions (3,4). Many children with EoE fail to thrive unless treated successfully (4). Esophageal features that can be identified endoscopically and are indicative of clinical symptoms include fixed esophageal rings (trachealization), strictures, longitudinal furrows, exudates (white plaques), and edema (5). The histological hallmark of the disease is infiltration of the esophagus by eosinophils (≥ 15 eosinophils/high power field (HPF) in the maximally involved area (2)). Per definition, both clinical and histopathological features are refractory to therapy with proton pump inhibitors (PPIs) (2). EoE is associated with histological evidence of esophageal remodeling, even in young children (6), and if left untreated can lead to esophageal dysmotility and strictures (7).

The prevalence of EoE in children has been increasing, currently approaching that of pediatric Crohn's disease and ulcerative colitis (8–11). Goals of therapy currently include improvement of clinical symptoms, remission of histopathological features, and avoidance of long-term complications. However, no FDA-approved medical therapies for EoE exist to date (12). Available therapeutic approaches include various dietary restrictions and off-label use of topical corticosteroids to the esophagus (2). Given the chronic nature of EoE, its tendency to develop progressive fibrosis over time that is reversible with therapy in children (13), and its tendency to relapse within a few weeks to months following discontinuation of therapy (14), long-term therapy for EoE is needed. Although dietary restriction therapies can be used chronically in children with EoE (15–20), their long-term acceptance by patients has been a challenge (21). Topical corticosteroids in the form of fluticasone propionate (FP) to swallow (14,22–28) or oral viscous budesonide (29–32) have been shown to be effective in children, as demonstrated by several case series and short-term randomized controlled trials, but their efficacy or safety for chronic use has not yet been established. The longest treatment duration reported is 6 months in children (28), with some efficacy of the drug still demonstrated and with minimal side effects. Whether this effect is sustained beyond 6 months is unknown.

Our objective was to investigate the long-term efficacy and safety of swallowed FP in children with EoE. We hypothesized that, in pediatric patients with EoE, maintenance therapy with swallowed FP leads to a sustained improvement of eosinophilic infiltration of the esophagus, as well as endoscopic and clinical features. We also investigated the safety of long-term swallowed FP administration in children.

METHODS

Design and participants

We conducted an open-label, prospective, single-center study of long-term swallowed FP as a maintenance therapy for children with EoE. All pediatric patients (age 2–18 years) with confirmed active EoE seen at the Mount Sinai Center for Eosinophilic Disorders between June 2006 and August 2012 were offered FP. The choice of this therapy was made by participants and their families. Options offered to everyone included an empiric elimination diet consisting of dietary removal of common EoE food triggers, an elemental diet consisting of exclusive feeding of an amino acid-based formula with one food, or FP to swallow. Each option was offered for long-term use if proven effective clinically and histologically after short-term therapy (**Figure 1**). All participants provided consent, and assent when applicable, to have all their data analyzed, following approval from the Institutional Review Board at our institution. Active EoE was defined as the presence of esophageal symptoms and histological evidence of ≥ 15 eosinophils/HPF in the maximally involved areas of the esophagus on esophagogastroduodenoscopy with biopsies despite prior therapy with a PPI per current consensus recommendations (1,2). Patients with evidence of concomitant eosinophilic gastritis and/or enteritis, active *Helicobacter pylori* infection, or parasitic infection were excluded.

For all subjects, baseline clinical, endoscopic, and histological assessments were obtained before therapy with fluticasone.

Pharmacological treatment

Therapy with FP metered dose inhaler was given as two puffs to swallow twice a day, specifically after breakfast and at bedtime. All patients were instructed to puff the medication into their mouth without synchronous deep inhalation or the use of a spacer. They were also instructed not to eat, drink, rinse their mouth, or brush their teeth for an hour in the morning and then all night at bedtime after taking fluticasone. The dose of swallowed FP was modeled according to age-specific treatment doses for asthma. Three different strengths of FP were used according to age: 2–4-year old: 44 μ g/puff; 5–11-year old: 110 μ g/puff; and ≥ 12 -year old: 220 μ g/puff. The dose was unchanged over time, unless disease relapse without an identifiable cause was observed. Correct administration was demonstrated, and proper adherence was discussed by instructing the subjects and their families to check the reading on the counter that is fitted to the back of the inhaler. At study entry, diets were liberalized for any patients who were on dietary restriction therapy for EoE.

After short-term therapy with fluticasone, patients were instructed to follow-up at our Center for repeat clinical, endoscopic,

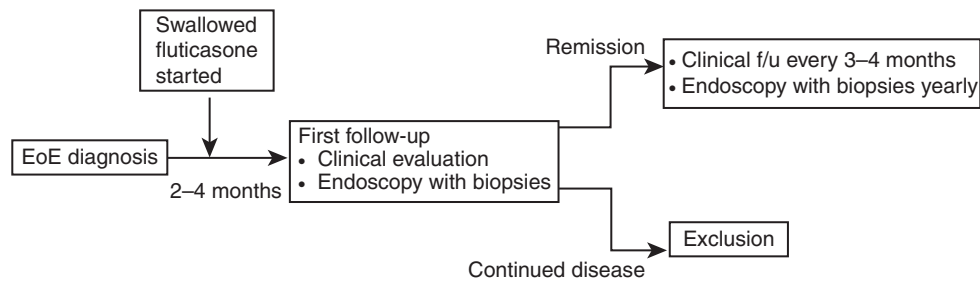


Figure 1. Flow chart of study design and decision tree.

and histological evaluations. If patients achieved disease remission with the above therapy, defined as complete resolution of their main symptom and histological clearance of esophageal eosinophils to $<15/\text{HPF}$ in the maximally involved area on biopsies, swallowed FP was continued at the same dose with clinical follow-up assessments every 4 months and endoscopic and histological follow-up assessments yearly. Additional unscheduled clinical, endoscopic, and histological evaluations were carried out in the event of re-emergence of esophageal symptoms at any time during their therapy. Clinical data that were collected at the time of endoscopic assessments were included in the analysis. Proper use of FP was demonstrated at every follow-up visit. Adherence to FP was also monitored at every follow-up visit by reminding the subjects and their families to check the reading on the counter that is fitted to the back of the inhaler and, when needed, by calling the dispensing pharmacy to check on the number of refills obtained. Potential side effects were investigated at every follow-up visit.

Although a systematic follow-up schedule was designed for the study as described above, some patients followed individualized schedules based on personal schedules and dictated by symptoms and disease activity. Patients had clinical, endoscopic, and histologic data available at all reported time points.

Study end points

The primary end point of the study was reduction over time in the peak esophageal eosinophil count in the maximally involved area of the esophagus, achieved with swallowed FP. We also reported reduction in peak eosinophil count in the distal and proximal esophagus, respectively. Secondary end points were as follows: (1) determination of histological remission over time, as measured by reduction in peak eosinophil counts to <15 , <10 , and <5 eosinophils/HPF (all three end points were considered as histological remission is currently not yet defined) (1,12); (2) improvement of esophageal histologic features other than the intraepithelial eosinophil count; (3) resolution of esophageal remodeling as determined by the presence of sub-epithelial lamina propria fibrosis; (4) improvement of endoscopic features; and (5) improvement of clinical symptoms.

Histological evaluation

Hematoxylin and eosin-stained slides were blindly evaluated by two pathologists (M.G.H. and M.S.M.). All esophageal biopsies

were scanned at $\times 100$ power. Four distal esophageal, 4 proximal esophageal, 2 gastric antral, 2 gastric fundic, and 4–6 duodenal biopsies were obtained at each procedure for evaluation, in addition to any sites with macroscopically abnormal areas. From the area of maximum intraepithelial eosinophilic density, the number of intraepithelial eosinophils was then reported for the $\times 400$ HPF (0.237 mm^2) with the highest concentration of eosinophils (peak count). Eosinophils were counted both when the entire cell including the nucleus was evident and/or when a discrete cluster of eosinophilic granules was identified. The highest eosinophil count found in the distal and proximal esophageal biopsies was recorded for analysis. The following additional histological features were recorded (6): presence of eosinophilic microabscesses (defined as a cluster of ≥ 4 eosinophils), presence and extent of eosinophilic degranulation (graded under HPF as absent/mild = 0 to few extracellular granules; or extensive = moderate-to-marked degranulation), superficial layering of intraepithelial eosinophils, epithelial basal zone thickness in well-oriented sections (graded from 0 to 3 based on its proportion of the total epithelial thickness; $0 \leq 20\%$ total epithelial layer thickness, $1 = 20\%$ to $1/3$ thickness, $2 = 1/3$ – $2/3$ thickness, $3 = \geq 2/3$ to full thickness), presence of dilated intercellular spaces, and the presence of lamina propria fibrosis. Edges of the biopsy specimen and areas with crush artifact were excluded from evaluation. Histological features were assessed over time.

Endoscopic evaluation

All pre- and post-treatment endoscopies were performed by the same gastroenterologist (M.C.) who recorded six macroscopic features (rings, white plaques, furrows, decreased vascular pattern (edema), stricture, and esophageal shearing). These were then graded using the modified classification and grading system published by Hirano *et al.* for adults with EoE (5). A final score was calculated for each patient at every endoscopy by summing up the grades given for every feature and dividing it by the number of features assessed (six features). Double weight was given to rings and strictures in the final summation score, as their rare presence in the pediatric population indicates severe and advanced disease (6). Median endoscopy scores were compared over time.

Clinical evaluation

Clinical evaluations included a history and physical examination with measurements of height and weight. Up to eight clinical

symptoms (abdominal pain, nausea, vomiting, regurgitation, chest pain, dysphagia, food impaction, and early satiety) were assessed for their presence by parent and patient. A symptom score was calculated at each visit by dividing the number of clinical symptoms present per patient by the total number of symptoms assessed at that visit (up to 8 symptoms). Median clinical scores were compared over time.

Peripheral eosinophil counts

Eosinophils in peripheral blood were measured on the same day of every endoscopy and reported as cells per μL .

Safety

As candida infections and growth delay have been subjects of concern with topical corticosteroid use in children with EoE, side effects were monitored by clinical history, physical examination, and following of growth by calculating the z score for height and weight for all patients over time using the SAS program for the CDC 2000 growth charts (2 to ≤ 20 years) (<http://www.cdc.gov/nccdphp/dnpao/growthcharts/resources/sas.htm>). Occurrence of oropharyngeal or esophageal candida infection was recorded and treated without interruption of FP maintenance therapy. As alteration of the hypothalamic pituitary adrenal (HPA) axis is a concern with long-term steroid use, morning serum cortisol levels were collected when prompted by parental anxiety regarding chronic use of steroids.

Statistical analysis

Intention-to-treat analysis was performed. Statistical analysis was performed by the use of the SAS statistical software package, version 9.3. Follow-up time points were grouped by timing of endoscopies into <4 months, 4–12 months, 13–24 months, and >24 months intervals. Depending on individual initial treatment timing and follow-up schedules, participants had none, one, or multiple endoscopies per time period at the time of data analysis. Comparison for all outcomes was made from baseline (before treatment) to the four different follow-up intervals on therapy. Statistical significance between groups of data were determined by the use of the paired t -test for continuous variables. Secondary histological outcome evaluating different cutoffs for remission (<15 , <10 , and <5 eosinophils/HPF) was calculated using McNemar's test for categorical variables. The correlation coefficient between the endoscopic score and individual histological features was calculated adjusting for intraclass correlation. Mean z -scores for height and weight at baseline of all patients were compared with all follow-up times using paired t -tests. For comparisons of continuous variables between patients who started FP as first therapy and those switching from diet, the Mann–Whitney U test for non-parametric continuous data was used. Statistical significance was determined at $P < 0.05$.

Mixed linear regression model

We fitted a mixed linear regression (negative binomial) model to test the hypothesis that treatment initiation would lead to a sustained decrease in the histological outcome, the peak eosinophil count, our response variable, while accounting for correlation of repeated observations in the same patient in a per patient analysis.

Very briefly, in our models, we modeled the population averaged peak eosinophil count response with fixed effects using the predictors treat (indicating treatment initiation) and further follow-up (indicating subsequent follow-up observations), controlling for the potential confounders Diet (prior diet modification) and PPI (prior proton inhibitor treatment) and allowing for individual patient (random) intercepts and further follow-up (to account for the correlation between repeated measurement in the same patient and the variability in patient response). Over-dispersion led us to prefer a negative binomial over a poisson model. The most parsimonious model was selected based on the expected log predicted density, but inclusion of potential confounders and/or omission of the random effect to allow for individual patient long-term effects (further follow-up) did not affect the inferences.

We fitted our mixed models in the statistical software environment R (33). We used the R package rstanarm (34) as an interface to the Stan statistical programming language (35) to implement our model with Stan's Hamiltonian Monte Carlo algorithms. We used the R package shinystan (36) to explore the Monte Carlo Markov Chain output, render some graphs, and confirm model convergence. We relied on Rhat as a convergence diagnostic (37). Additional details on model formulation and building is included in the Supplementary Material (Supplementary statistical modeling information).

RESULTS

Participants

Fifty-four patients with a diagnosis of active EoE chose FP to swallow as treatment option and were enrolled in the study. Patients were predominantly male (80%) with a median age of 6.5 years (range 2–17 years). The majority of patients (85%) had other atopic conditions such as asthma (57%), allergic rhinitis (68%), and/or atopic dermatitis (31%). Seventy-four percent had a history of immunoglobulin E (IgE)-mediated food allergy (Table 1). As patients with EoE often have an atopic background, including IgE-mediated food allergy, atopic dermatitis, allergic rhinitis, and asthma, the question remains whether EoE disease severity and progression is influenced by an atopic predisposition (15,16,25,38). The primary outcome (peak eosinophil count) was compared for these atopic conditions. An underlying diagnosis of an atopic disease in a patient (asthma, allergic rhinitis, atopic dermatitis, and food allergy) did not correlate with severity of EoE measured as peak esophageal eosinophil count (P values >0.4 for all atopic features examined). Only three patients continued PPI therapy throughout their treatment with swallowed FP.

FP was the first treatment modality chosen by 15 patients; an additional 39 children had been on dietary restriction therapy before opting for swallowed FP, and their diets were fully liberalized at study entry. All but two patients who had been on a diet before changing to FP had failed dietary restriction therapy. Baseline characteristics were comparable for those two subgroups (Table 2).

Of the 54 participants, 2 were lost to follow-up and 8 withdrew from therapy with swallowed FP, 7 due to lack of histological

Table 1. Characteristics of all patients at the time of inclusion into the study

	All patients (n=54)
Gender, male/female	43/11
Age (years): median (range)	6.5 (2–17)
Atopy: no. patients (%)	46 (85%)
Asthma	31 (57%)
Allergic rhinitis	37 (68%)
Atopic dermatitis	17 (31%)
IgE-mediated food allergy: no. patients (%)	40 (74%)
Esophageal peak eosinophil count/HPF: median (range)	72 (1–220)
Blood eosinophil count (cells/ μ l): median (range)	400 (100–1,500)
HPF, high power field.	

response at various time points during therapy, and 1 for parental concern over chronic steroid use. Forty-four patients remained on treatment at the time of data analysis. All 54 patients were included in the data analysis.

Mean duration of follow-up of patients after initiation of swallowed FP was 20.4 months (1.7 years), with the longest being 68 months (5.7 years).

Efficacy

Peak esophageal eosinophil count (primary end point). Treatment with swallowed FP significantly decreased the maximum number of eosinophils/HPF in the most severely affected area of the esophagus at all follow-up time points compared with baseline (median 72 eosinophils/HPF at baseline compared with median 0.5 eosinophils/HPF at follow-up within 4 months; and 1.75 eosinophils/HPF, 10 eosinophils/HPF, and 12 eosinophils/HPF at 4–12 months, 13–24 months, and >24 months follow-ups, respectively, all $P < 0.0001$; **Figure 2a**). Separate analysis of peak eosinophils in the distal and proximal esophagus showed a comparable reduction in peak eosinophils at all time points, respectively. In the distal esophagus, median 60 eosinophils/HPF at baseline was present, compared with median 0.5 eosinophils/HPF at follow-up within 4 months, $P < 0.0001$, and 1.0 eosinophils/HPF, 8 eosinophils/HPF, and 12 eosinophils/HPF at 4–12 months, 13–24 months, and >24 months follow-ups, respectively (all P values between <0.0001 and 0.0002). As for the proximal esophagus, median 55.5 eosinophils/HPF at baseline was present, compared with median 0 eosinophils/HPF at follow-up within 4 months, $P < 0.0001$, and 0 eosinophils/HPF, 0.5 eosinophils/HPF, and 0.5 eosinophils/HPF at 4–12 months, 13–24 months, and >24 months follow-ups, respectively (all P values between <0.0001 and 0.0004; **Figure 2b, c**).

Other histologic findings. Although the peak number of eosinophils/HPF to diagnose EoE is clearly defined at ≥ 15 , no consensus on criteria for histologic remission exists to date (12). To better

Table 2. Characteristics at inclusion of patients who switched from dietary restriction therapy to FP compared to patients who started swallowed FP as their first treatment modality

	Dietary restriction therapy before fluticasone (n=39)	Fluticasone as first therapy modality (n=15)	P value
Gender, male/female	30/9	13/2	
Age (years): median (range)	6 (2–17)	7 (2–17)	0.4211
Atopy: no. patients (%)	33 (85%)	2 (87%)	
Asthma	21 (54%)	10 (67%)	
Allergic rhinitis	28 (72%)	9 (60%)	
Atopic dermatitis	15 (38%)	2 (13%)	
IgE-mediated food allergy: no. patients (%)	29 (74%)	11 (73%)	
Esophageal peak eosinophil count/HPF: median (range)	75 (38–145)	72 (1–220)	0.7869
Blood eosinophil count (cells/ μ l): median (range)	400 (200–700)	500 (100–1,500)	0.9127
FP, fluticasone propionate; HPF, high power field; IgE, immunoglobulin E.			

understand treatment response and histologic remission, resolution of eosinophilic infiltration was evaluated applying three different criteria (<15 eosinophils/HPF, <10 eosinophils/HPF, and <5 eosinophils/HPF).

The percentage of patients who achieved remission when analyzed at the various intervals (<4 months, 4–12 months, 13–24 months and >24 months) were as follows <15 eosinophils/HPF in the most severely affected area: 83%, 84%, 59%, 63%; <10 eosinophils/HPF in the most severely affected area: 75%, 78%, 51%, 58%; and <5 eosinophils/HPF in the most severely affected area: 63%, 66%, 48%, 58% (**Supplementary Table 1**).

When distal and proximal esophageal peak eosinophil counts were analyzed separately, similar results were seen with respect to the remission rate: Distal esophagus (<4 months, 4–12 months, 13–24 months and >24 months): <15 eosinophils/HPF—83%, 88%, 63%, 63%; <10 eosinophils/HPF—75%, 84%, 56%, 58%; <5 eosinophils/HPF—63%, 69%, 48%, 58%. Proximal esophagus (<4 months, 4–12 months, 13–24 months and >24 months): <15 eosinophils/HPF—88%, 84%, 81%, 79%; <10 eosinophils/HPF—88%, 81%, 81%, 79%; <5 eosinophils/HPF—88%, 72%, 81%, 74%.

Of the 43 patients who achieved remission at the first follow-up endoscopy, a total of 13 patients had relapse during their follow-up course, 6 of whom had multiple relapses, for a total of 25 relapse occurrences for all patients (**Supplementary Figure 1**). Eighteen of 25 relapse occurrences were noted to mainly affect the distal esophagus: median eosinophils/HPF 37 in the distal esophagus (range 15–182) and 2 in the proximal esophagus (range 0–99). Reasons for relapse in these patients were non-adherence to swallowed

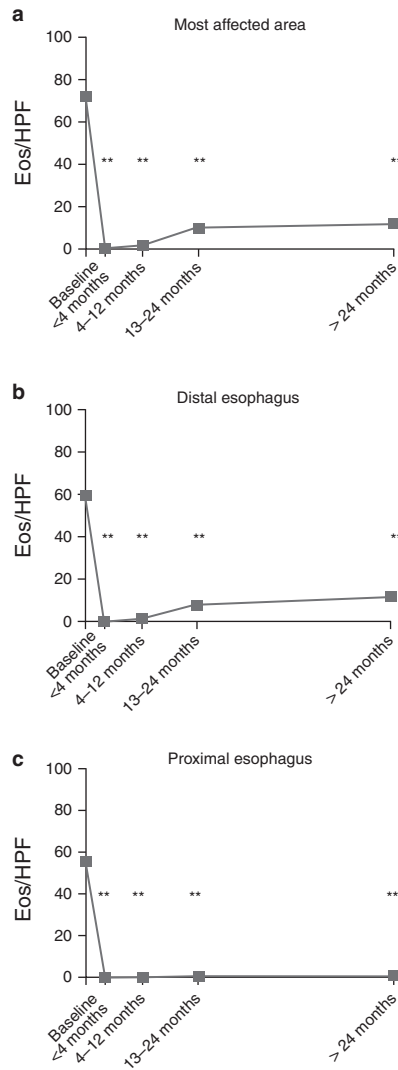


Figure 2. Primary outcome: median peak eosinophils/HPF at baseline compared with 4 follow-up intervals, (a) most affected area of the esophagus, (b) distal esophagus, (c) proximal esophagus, ** $P < 0.01$. HPF, high power field.

FP therapy (four patients), incorrect technique when the children took over the responsibility for medication administration as they got older (3 patients), and unidentified cause (6 patients, one of whom responded to an increase in the dose as he grew into the next dosing category, and another responded to addition of a PPI).

When present, each individual histologic feature showed significant improvement compared with baseline (Table 3a). Peak eosinophil count significantly correlated with the presence of secondary histologic features (eosinophilic microabscesses, eosinophil superficial layering, dilated intercellular spaces, basal zone hyperplasia, eosinophilic degranulation, and lamina propria fibrosis). Lamina propria adequate for evaluation was present in 75% of biopsies. At baseline, fibrosis of the lamina propria was noted in 91.5% of patients. The percentage of patients with lamina propria fibrosis decreased to 41% and remained similarly low at all follow-up periods (Table 3a).

Endoscopic findings. The majority of children had white plaques (58.3%), furrows (75.5%), and edema (67.4%) on endoscopy. White plaques and furrows rapidly and significantly responded to treatment. Resolution of edema was less pronounced short term but showed a significant reduction between 4 and 24 months of treatment. Shearing was present in 4.2% of patients at baseline and quickly resolved with therapy. Fixed rings were found in 10.2% of patients, resolved after 4–12 months of FP treatment and did not recur, except in one patient in his second year of treatment with FP in the absence of esophageal eosinophilia. Rings cleared again on subsequent follow-ups. A stricture was found in 1/54 (1.85%) of patients at baseline. After 7 months of treatment with FP, the stricture resolved and did not recur.

The cumulative score of endoscopic features significantly improved with swallowed FP at all time points (Table 3b). Furthermore, the endoscopic score significantly correlated with esophageal eosinophil counts and other histological features (correlation coefficient for peak eosinophils $<15/\text{HPF}$: -0.60476 , $P < 0.0001$; eosinophilic microabscesses: 0.64566 , $P < 0.0001$; eosinophilic degranulation: 0.62673 , $P < 0.0001$; eosinophil superficial layering: 0.61638 , $P < 0.0001$; dilation of intercellular spaces: 0.63599 , $P < 0.0001$; basal zone hyperplasia: 0.68110 , $P < 0.0001$; lamina propria fibrosis: 0.51159 , $P < 0.0001$).

Clinical findings. At baseline, most patients (88.9%) had at least one of eight assessed symptoms (abdominal pain, nausea, vomiting, regurgitation, chest pain, dysphagia, food impaction, and early satiety). With swallowed FP treatment, patients experienced a significant decrease in the symptom score at all follow-up periods up to 2 years (Table 3c).

Per patient analysis and modeling. In our linear mixed effects model, initiation of treatment with swallowed fluticasone led to a statistically significant and sustained decrease in peak eosinophil counts. We present the detailed regression results of the most parsimonious model in Supplementary Table 2 and explain the findings with a representative patient with a starting peak eosinophil count of 80/HPF, treatment initiation may suppress the peak eosinophil count to 10 at the first endoscopy appointment and stays in remission at further follow-up appointments.

Numerically, the regression coefficient for treatment (2.1) equals the difference of the log of a starting peak eosinophil count (80 for a representative patient), minus the log of the peak eosinophil count at the first appointment (10 for typical patient): $\log(80) - \log(10) = 2.1$ (regression coefficient for treatment). More formally stated, in our mixed effects negative binomial regression model, the difference in the logs of expected peak eosinophil counts is expected to change by the respective regression coefficient, given the other predictor variables in the model are held constant.

Our model specifically investigated the hypothesis of sustained effect by contrasting the first follow-up vs. subsequent follow-up. The estimate of the posterior median the regression coefficient for further follow-up supports the hypothesis of sustained reduction in peak eosinophil count in our cohort. For our exemplary patient, the peak eosinophil count would remain below 15.

Table 3. Secondary outcomes: percent of patients with the presence of (a) histologic, (b) endoscopic, and (c) clinical features at baseline and at follow-up intervals

a	Baseline (n=54)	<4 months (n=24)	4–12 months (n=33)	13–24 months (n=27)	>24 months (n=20)
% Patients with histologic feature					
Eosinophilic microabscesses	53.85	8.33**	6.25**	7.41**	21.05**
Eosinophil superficial layering	44.23	0.00	9.38**	14.81**	21.05**
Dilated intercellular spaces	84.62	17.39**	15.63**	25.93**	21.05**
Basal zone hyperplasia	92.31	33.33**	31.25**	44.44**	47.37**
Eosinophilic degranulation	53.85	4.17**	6.25**	11.11**	21.05**
Lamina propria fibrosis	91.49	41.18**	50.00**	45.45**	38.89**
b	Baseline	<4 months	4–12 months	13–24 months	>24 months
% Patients with endoscopic feature					
Fixed rings	10.20	0.00	0.00	3.70	0.00
White plaques	58.33	13.04*	24.24	29.63	30.00
Furrows	75.51	26.09**	12.12**	18.52**	30.00*
Edema	67.44	56.52	46.67*	14.81*	26.32
Stricture	1.85	0.00	0.00	0.00	0.00
Shearing	4.17	0.00	0.00	0.00	0.00
Endoscopic feature score: median (range)	0.37 (0–1.33)	0.17 (0–0.67)**	0.17 (0–0.67)**	0.00 (0–0.67)**	0.10 (0–0.67)*
c	Baseline	<4 months	4–12 months	13–24 months	>24 months
% Patients with clinical symptom					
Abdominal pain	38.89	16.67	34.38	26.92	30.00
Nausea	14.81	8.33	9.38	15.38	15.00
Vomiting	18.52	0.00	6.25	11.54	25.00
Regurgitation	38.89	0.00	12.50	23.08	10.00
Chest pain	18.52	12.50	12.50	7.69	5.00
Dysphagia	35.19	12.50	12.50	15.38	5.00
Food impaction	22.22	12.50	0.00	15.38	5.00
Early satiety	18.52	0.00	0.00	3.85	0.00
Clinical symptom score: median (range)	0.22 (0–0.67)	0.00 (0–0.44)**	0.11 (0–0.44)*	0.11 (0–0.33)*	0.11 (0–0.33)

The features are also summarized in a score (median/range) for endoscopic and clinical data at baseline and follow-up intervals. * $P < 0.05$, ** $P < 0.01$.

However, the model estimated wide credible intervals for sustained effect, together with significant patient variability, this means that the effect of swallowed fluticasone is not consistent for all patients. Inter-patient variability may best explain the inter-individual differences (as seen in **Figure 3**) in patient responses, as a mixed effects model allowing patient-specific trajectories fit our data better than the simpler model with only fixed effects. Our models showed that diet modification and prior treatment with PPIs do not affect our inferences.

Peripheral eosinophil counts. Median peripheral eosinophil count was 400/ μ l (range 100–1,500/ μ l) at baseline. No significant change in peripheral eosinophil counts was noted at any follow-up

time point (median 300–350/ μ l at all follow-up time points, all P values between 0.05 and 0.52).

Safety. Asymptomatic candidal esophageal infection was seen in three patients at 4–12, 13–24, and >24 months, respectively. Esophageal candidiasis resolved with fluconazole therapy as confirmed by repeat endoscopy with biopsies, while FP therapy was continued.

Comparison of mean baseline height z -scores with mean height z -scores recorded at the four follow-up time periods revealed no significant deviation from the projected growth curves (P values 0.1479, 0.9602, 0.2263, and 0.1703 at 4–12, 13–24, and >24 months, respectively). A similar result was seen for weight z -scores

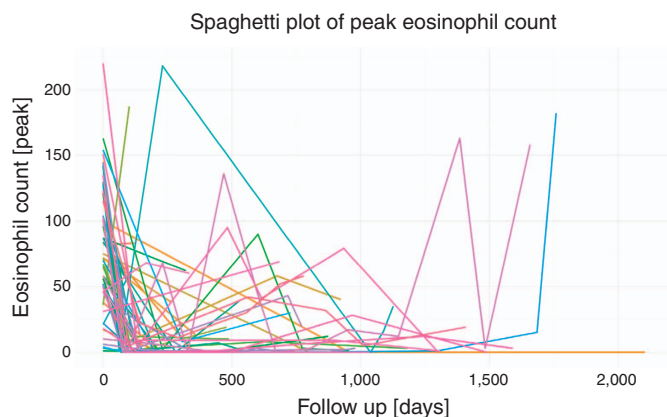


Figure 3. The peak eosinophil count (peak esophageal eosinophils/HPF) is plotted against patient follow-up (in days) with individual patient trajectories indicated by colored lines. After treatment initiation with swallowed fluticasone, the peak eosinophil count decreases and remains low, with a minimal increase into the second year (possibly due to either decreasing adherence or treatment efficacy) and increasing uncertainty as fewer patients were followed beyond 2 years. HPF, high power field.

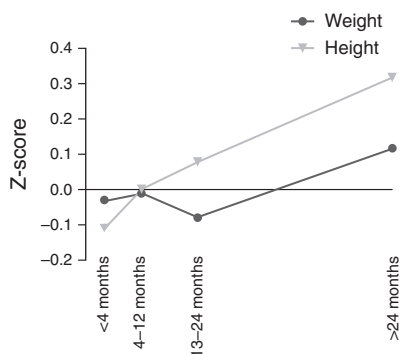


Figure 4. Mean z-scores for height and weight at baseline for all patients were compared with mean z-scores at all follow-up periods. A positive z-score indicates net growth/weight gain above the predicted growth curves, and negative z-scores indicate less growth/weight gain as predicted by predicted growth curves. All time points were not significant compared with baseline.

of patients while taking swallowed FP ($P=0.7685, 0.9270, 0.4708, 0.5670$ at 4–12, 13–24, and >24 months, respectively; **Figure 4**).

Data on morning serum cortisol measurements were collected on nine patients. Morning cortisol levels ranged from 5.1 to 23.6 μL (median 10.1 μL). Two patients were found to be at the lower and higher end of the normal range for their age. These patients had no clinical findings, had normal growth, and a complete endocrine work-up was negative.

DISCUSSION

This is an important clinical trial that demonstrates that swallowed FP leads to a sustained long-term remission of EoE in

children, defined as a reduction in peak eosinophil counts in the most affected area of the esophagus. This long-term oral steroid treatment was well tolerated and safe. This is particularly encouraging considering that to date no satisfying alternative long-term treatment options other than significant dietary modifications are available (1,2).

In our prospective study of pediatric patients with EoE, swallowed FP led to sustained improvement of histopathological, endoscopic, and clinical markers of disease severity. Treatment response of all disease markers in all patients was most pronounced at the first short-term follow-up (at <4 months) but was maintained at subsequent follow-up time points for several months, the longest being 68 months. In addition, using a linear mixed effects model, we found that initiation of treatment with swallowed fluticasone led to a statistically significant and sustained decrease in peak eosinophil counts. Using the model, our confidence in the sustained effect of swallowed fluticasone obviously diminishes for later follow-ups where we have less data. We have less data beyond the third follow-up, not because of attrition (we lost only 6 out of 54 patients) but because patients were enrolled consecutively and not all have had years of follow-up yet. An update with additional long-term results as these become available in our ongoing trial is planned.

Although other pediatric studies have shown short-term response to FP using comparable dose ranges (22–27), this study adds to the literature by showing that this short-term effect can be maintained safely in pediatric patients for at least 2 years. Our findings concur with and surpass previous studies analyzing the efficacy of topical corticosteroids in inducing remission and histopathological, endoscopic, and symptom improvement with short-term treatment (22,23,25,26,39,40).

Various factors likely played into this favorable study outcome. Throughout the study, correct administration was demonstrated at length, strategies to ensure and enhance adherence were reviewed and discussed at every follow-up visit by the treating physician (M.C.), containers were checked when necessary and pharmacies were called to verify refills, and treatment was supervised by the caregivers in most patients. In addition, the design of our study, allowing patient and parent choice of therapy (diet vs. swallowed FP) likely improved the outcomes, as families were sharing the responsibility in the treatment process.

Clinical symptoms were recorded and tracked throughout the study. However, symptom severity measures were not used, and symptom report was based on patient and parent re-collection. This limitation in our study highlights the need for a standardized and validated clinical symptom questionnaire designed for pediatric patients with EoE.

Although open-label design is always a limitation, the fact that the families chose the therapy modality was not reflected in their characteristics. Atopy and food allergy were comparable between patients who started swallowed FP as first treatment modality vs. those who were on dietary restriction therapy first (**Table 2**). Any change or restriction in diet was regarded as dietary modification in our study. The degree of modification was highly variable in our patients and included elemental diet, empiric 6-food elimination diet, or test-directed elimination diets. These dietary

therapies were conducted at various points, including in the distant past before fluticasone was offered, and the diet had been already liberalized by then. Dietary therapy is known to be extremely difficult to maintain and adhere to, especially as patients get older and become more independent. Inconsistent adherence to diet was found to be a problem in some of our patients. All diets were fully liberalized at the start of swallowed fluticasone.

Enrollment into our study started in 2006, before understanding the role PPIs have in EoE and before recommendations on PPI use in the consensus recommendations were available. Three of our patients continued PPI after start of swallowed FP, whereas all other patients discontinued PPI at enrollment into the study. Exclusion of those three patients did not change the primary and secondary outcomes. The patients remained in the group for the intention-to-treat analysis. A recent publication by Rajan *et al.* (41) also did not find influence of PPI on eosinophilia or tissue remodeling.

As histological definition of remission is not yet established, we assessed different peak eosinophil counts (<15, <10, and <5 eosinophils/HPF) for histologic remission as one of our secondary end points to validate established practice (18,25,42–45). Applying stringent criteria for definition of remission, the majority of patients reached and maintained peak eosinophil counts of <5 eosinophils/HPF after initiation of treatment to the longest follow-up period. At all follow-up time points, 75% of patients in remission had a peak of <5 eosinophils/HPF, suggesting that true resolution of disease with swallowed FP might be associated with close to absent eosinophils in tissue biopsies and indicating that the doses we used, although generally not very high, were very effective.

We have demonstrated the effect of swallowed FP on fibrosis of the lamina propria in a previous study (13). In this study, we were again able to demonstrate long-term complete resolution of lamina propria fibrosis in >50% of treated patients, starting as early as <4 months (Table 3a). As discussed above, lamina propria adequate for evaluation was present in 75% of biopsies. These results are not typically seen in adult patients with EoE (45). It is possible that esophageal fibrosis in adult patients, as it has often developed over a longer period of time and undergone multiple disease remission and relapse cycles, is less responsive to treatment than in pediatric patients.

A recently published study investigating the long-term control of esophageal remodeling in patients with EoE supports the observation that, although esophageal remodeling is present in children and is associated with inflammation, a substantial and sustainable response to swallowed topical steroids can be found. The authors described the main effect of swallowed topical steroids on fibrosis being exerted primarily via control of inflammation, as lower fibrosis scores correlated with decreased number of esophageal eosinophils, rather than with treatment duration of swallowed topical steroids (41). This finding supports our observation that a decrease in peak esophageal eosinophil levels is significantly correlated with LP fibrosis reversal, with the underlying mechanism likely being inflammation control.

Interestingly, relapse was more pronounced and frequent in the distal portion of the esophagus. All patients with relapse in the distal esophagus had decreased amounts of swallowed FP intake, either

because of non-adherence, poor technique, or outgrown doses. It is possible that this observation is due to less contact time distally in the esophagus with smaller doses, as topical corticosteroid therapies were demonstrated to exert their function locally in the esophagus (46). Our results demonstrate the necessity for optimal treatment adherence and dosing. We also demonstrate that transition of medication administration from caregiver to child may affect efficacy of the drug and should be addressed during visits.

Our study design did not plan for dose increases as children grew. To evaluate whether growth might lead to outgrowing of the dose and resulting relapses, we analyzed all relapse occurrences for all patients. We found that only one of the six patients with unidentified reasons for relapse responded to an increase in the dose of FP. We can therefore conclude that the relatively lower FP dose resulting from growth and weight gain is enough to maintain remission in most patients if good adherence and technique are achieved.

As for the four patients with disease relapse over time without an identified etiology, it is possible that they have developed resistance to steroids over time. Rajan *et al.* hypothesize that there may be distinct EoE phenotypes including “transient responders”, which may help explain our observation (41).

Although short-term efficacy of swallowed FP has been previously demonstrated (14,22–28), significant uncertainty regarding long-term safety and efficacy remained concerning this off-label use of an otherwise very well established and safe medication. Two main concerns of long-term treatment with swallowed FP often raised are recurrent candida infections and alteration of the HPA axis with suppressed growth (47). Our safety data showed a very low rate of local esophageal candida infections (three patients) that resolved with treatment without recurrence and without the need to discontinue swallowed FP. No impact on growth was noted in our patients. To better address questions about effects on the HPA axis, measurement of serum morning cortisol was started after completion of the study period. Cortisol levels were within the normal range for their age. None of the children showed clinical signs of adrenal insufficiency or delayed growth, and endocrine work-up was negative. In a recent publication by Butz *et al.* (28), saliva cortisol levels did not show significant alteration in patients treated with high-dose swallowed FP. Swallowed FP in the doses used in this study can be considered of low risk for long-term treatment of EoE in children, although further studies evaluating the effect of swallowed FP on the HPA axis, such as with an ACTH stimulation test, are warranted.

Other limitations to our study are its open-label, non-randomized design and the lack of a control group. However, a recently published randomized, double-blind placebo-controlled trial evaluating treatment with high-dose swallowed FP did not detect a significant placebo effect, validating our approach (28). In addition, a concern with the lack of randomization is selection bias that may limit the strength of our conclusions. However, given clinical evidence from randomized trials in adults (48–52) and children (22,23,25–28), equipoise among experts in the field regarding the effectiveness of swallowed FP for EoE in children may have been eroded to the point that a randomized trial could be ethically problematic, especially in minors (53). Furthermore, randomization of

our patients into diet vs. swallowed FP, rather than giving them the choice, would have led to low adherence in several patients, making comparisons of therapy difficult. As detailed above, even though we allowed the families the choice of therapy, our analysis showed similar population characteristics among those that chose swallowed FP vs. diet as their first treatment modality. Therefore, we believe that our trial offers the first and best available evidence in support of long-term chronic use of swallowed FP to treat EoE in children and will allow for the first time comfort in prescribing swallowed FP as a long-term therapeutic option for children with EoE, given its long-term efficacy and safety profile. Further investigation using larger multi-center trials is warranted to confirm optimal long-term dosing regimens while providing maintenance of disease remission and minimizing potential side effects in children with EoE.

CONFLICT OF INTEREST

Guarantor of the article: Mirna Chehade, MD, MPH.

Specific author contributions: Acquisition of data; analysis and interpretation of data; drafting of the manuscript; and critical revision of the manuscript for important intellectual content: Doerthe A. Andreae; acquisition of data; analysis and interpretation of data; and critical revision of the manuscript for important intellectual content: Matthew G. Hanna; acquisition of data; analysis and interpretation of data; and critical revision of the manuscript for important intellectual content: Margret S. Magid; statistical analysis and critical revision of the manuscript for important intellectual content: Stefano Malerba; statistical analysis; and interpretation of data; and critical revision of the manuscript for important intellectual content: Michael H. Andreae; statistical analysis and critical revision of the manuscript for important intellectual content: Emilia Bagiella; study concept and design; acquisition of data; analysis and interpretation of data; drafting of the manuscript; and critical revision of the manuscript for important intellectual content; statistical analysis; obtained funding; administrative, technical, or material support; and study supervision: Mirna Chehade.

Financial support: Supported in part by grants UL1TR000067 and 5KL2TR001071-03 from the National Center for Advancing Translational Sciences, National Institutes of Health.

Potential competing interests: None.

Study Highlights

WHAT IS CURRENT KNOWLEDGE

- ✓ Swallowed steroids have been shown to be safe and effective as a short-term therapy in children with eosinophilic esophagitis (EoE).
- ✓ However, their efficacy for chronic use has not been established.

WHAT IS NEW HERE

- ✓ Swallowed steroids are safe and effective when used as a long-term maintenance therapy in children with EoE.
- ✓ Treatment positively affects histological, endoscopic, and clinical features of EoE.

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Swallowed Fluticasone Propionate is an Effective Long-Term Maintenance Therapy for Children with Eosinophilic Esophagitis: Supplemental statistical analysis

Introduction

Below follows an abbreviated summary of our model building. We build a mixed linear models to test the hypothesis of a *sustained* decrease in peak eosinophil count after treatment initiation. To account for patient variability and the correlation of repeated observations we include a random effect for endoscopy follow up visit

Load EoE data

We load the data from the Rdata file *EoEdata9Feb.Rdata*

Dataset as table

Patient	Endoscopy	Date	Eos	Diet	PPI	Endoscopy.factor	treat	furtherfollowup	Followup
1	1	2012-04-30	52	1	0	1	0	0	0
1	2	2012-07-23	2	1	0	2	1	0	84
2	1	2009-08-10	82	1	0	1	0	0	0
2	2	2009-12-14	83	1	0	2	1	0	126
3	1	2010-05-10	38	0	0	1	0	0	0
3	2	2010-12-17	0	0	0	2	1	0	221

This is an observational open label study of 54 subjects, each with several (between 1 to 6) follow up endoscopies at variable intervals. The outcome is continuous (Eos), a repeated measure of eosinophil *count*, showing on average a response to treatment over time.

Diet indicated if patient was pretreated with diet PPI indicated concurrent treatment with proton inhibitor

Endoscopy codes for the follow up visit endoscopy, either as integer or as factor.

treat indicates start of treatment (after first visit) furtherfollowup codes for endoscopy visits after the second. Followup is days of follow up at which Eos was measured (in days).

Explore response

Table of mean and medians

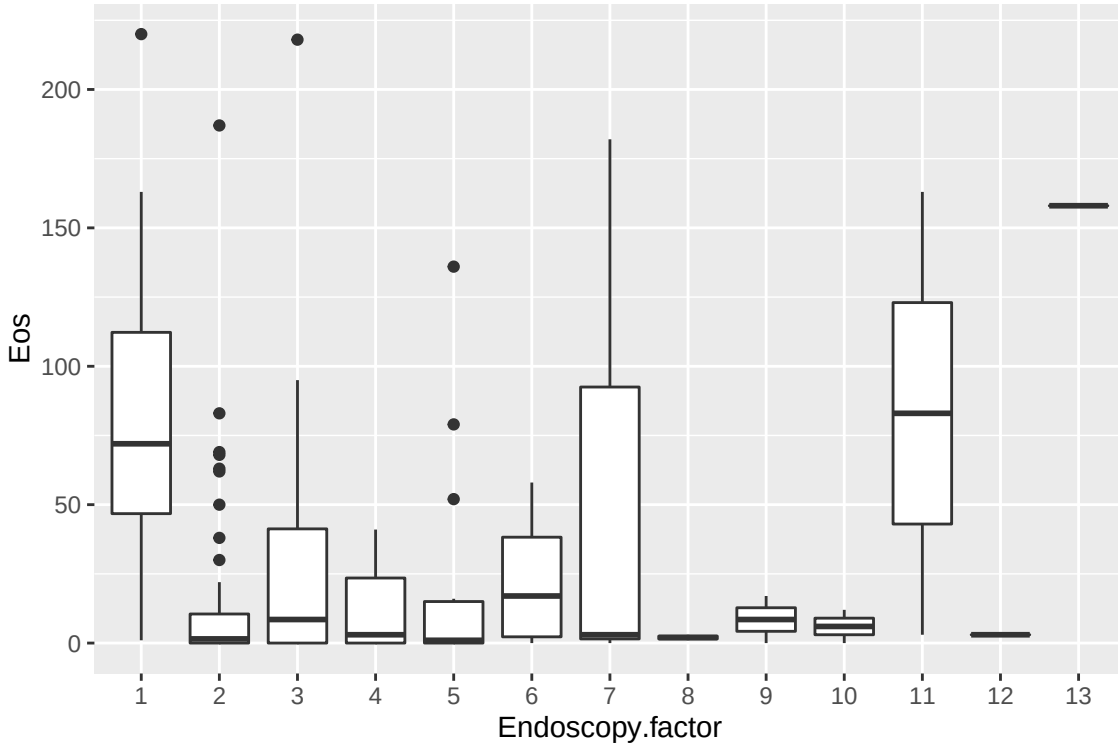
Endoscopy	Number	MedianEos	meanEos
1	54	72.0	78.46
2	54	1.5	14.65

Endoscopy	Number	MedianEos	meanEos
3	30	8.5	27.50
4	19	3.0	11.32
5	14	1.0	21.64
6	8	17.0	22.25
7	3	3.0	61.67
8	2	2.0	2.00
9	2	8.5	8.50
10	2	6.0	6.00
11	2	83.0	83.00
12	1	3.0	3.00
13	1	158.0	158.00

This table list the median Eos count by endoscopy.

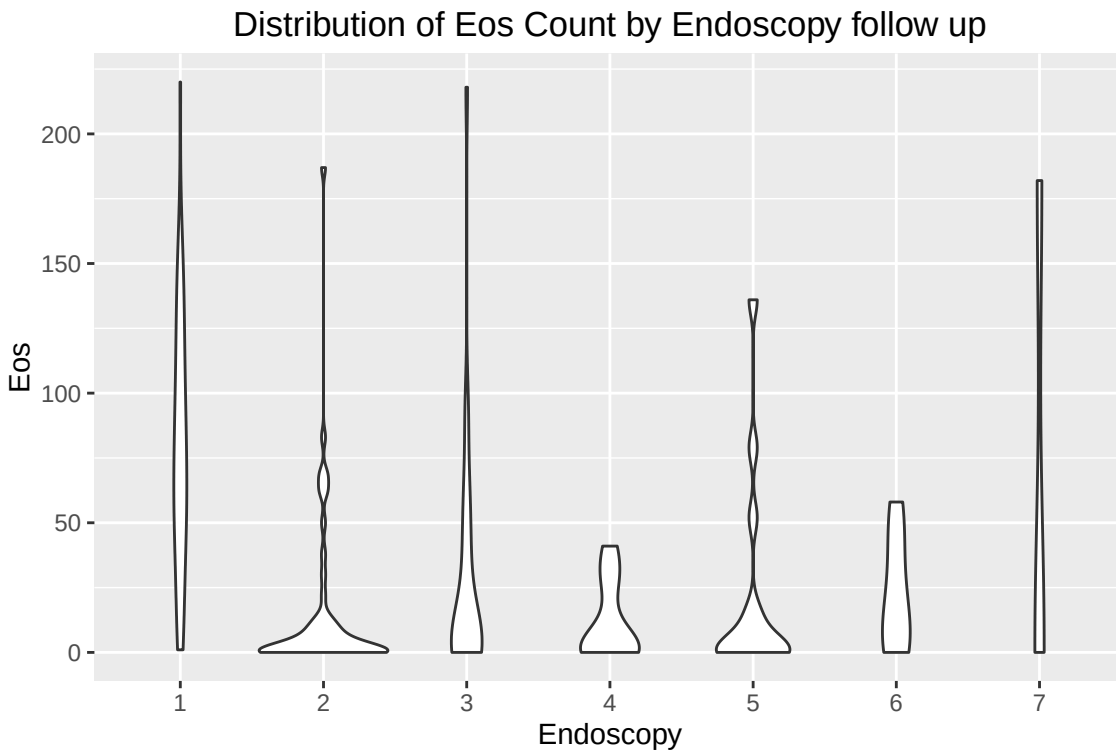
Box and violin plots

Boxplot



This boxplot shows the distribution of Eos peak counts by endoscopy.

Violin plot



We look at the distribution of peak eosinophil counts by endoscopy appointment, (limiting ourselves to the first 7 follow up endoscopies for better visualization).

Figure 1 for manuscript

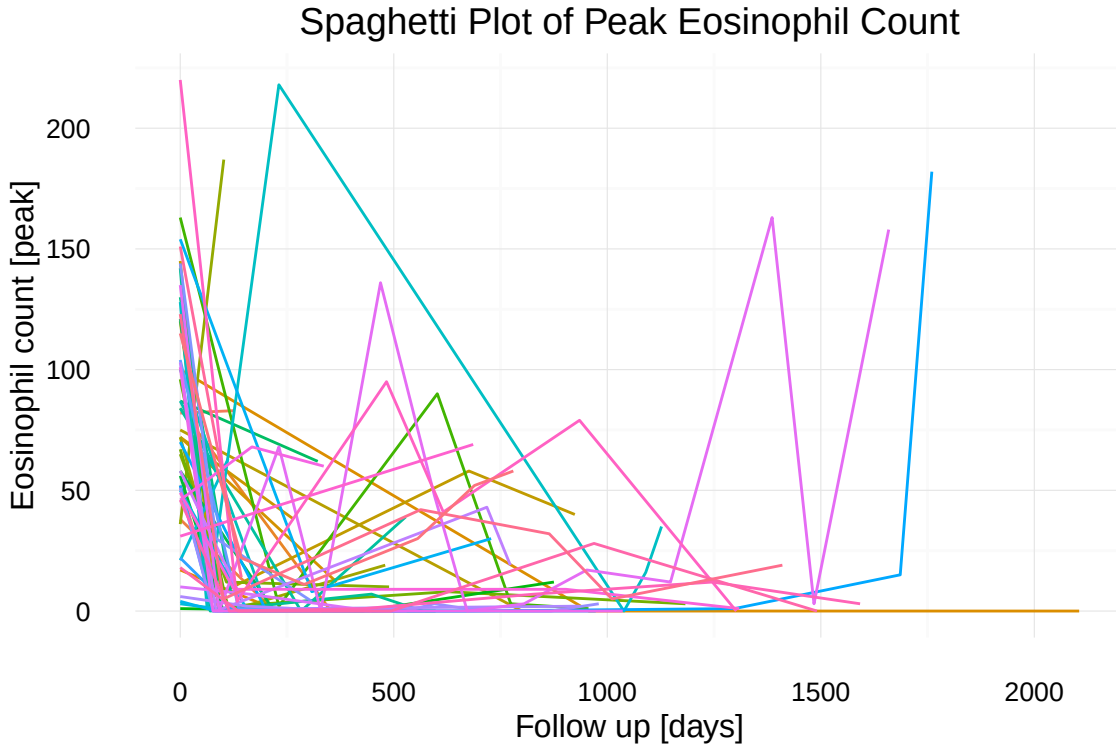


Figure 1: Above, the peak eosinophil count is plotted against patient follow up [in days] with individual patient trajectories indicated by colored lines. After treatment initiation with swallowed fluticasone, the peak eosinophil count drops and remains low, possibly with a slight increase into the second year (possibly due to either decreasing compliance or treatment efficacy) and increasing uncertainty as fewer patients were followed beyond two years.

Supplemental Table: Regression coefficients final model B

Coefficient	Rhat	lower 95%	posterior median	upper 95%
(Intercept)	1	4.02	4.45	4.92
treat	1	-2.89	-2.12	-1.39
furtherfollowup	1	-0.61	0.26	1.10
b[(Intercept) Patient:1]	1	-1.36	-0.14	1.02
b[furtherfollowup Patient:1]	1	-2.23	-0.11	2.06

Supplemental Table: Posterior median estimates (50%) for fixed effects and exemplary random effects regression coefficients are shown above with their corresponding 95% upper and lower bounds, followed by an exemplary random effect for patient 1. For our mixed effects negative binomial regression model, the difference in the logs of expected peak eosinophilic counts is expected to change by the respective regression coefficient, given the other predictor variables in the model are held constant. **In a representative patient with a starting peak eosinophilic count of 80, this might mean a reduction to a peak eosinophilic count of 10, which is mostly sustained in further follow ups.** Numerically, -2.1, the reduction in log Eos count estimated by the regression coefficient for treatment initiation, equals the difference between $\log(80)$ and $\log(10)$. The consistently low Rhat values below 1.1 suggest adequate model convergence.

Model

Our mixed model was formulated as

Formula

$$\text{logit}(\mu_{ij}) = \beta_0 + \beta_1 * \text{treat}_{ij} + \beta_2 * \text{furtherfollowup}_{ij} + \beta_3 * \text{Diet}_i + \beta_4 * \text{PPI}_i + b_{0ij} + b_{1ij} * \text{furtherfollowup}_{ij}$$

With μ_{ij} the eosinophil (peak count) for i-th patient at the j-th appointment and treat_{ij} a patient specific indicator for the second follow up visit, and $\text{furtherfollowup}_{ij}$ an indicator for visits beyond the second, respectively, (both included as a fixed effect and as a random effect (grouped by patient_i) and accounting for the correlation among repeated observations in the same subject), Diet_i is an indicator of pre-treatment with diet for the ith patient prior to initiation of oral flovent therapy and PPI_i indicating concurrent therapy with proton pump inhibitor.

Fitting advanced hierarchical models with classical software packages can be challenging, so we used a Bayesian hierarchical modelling approach with the default priors of the software package rstanarm (Gabry, J. & Goodrich, B. (2016), ‘rstanarm: Bayesian Applied Regression Modeling via Stan’, R package version 2.9.0-1.). The models were run with 2000 iterations and six chains in parallel and converged quickly as evidenced by the Rhat conversion diagnostic being smaller than 1.05 for all parameters, evidence for good mixing in graphical plots of the MCMC chains. Our models were robust to changes in our prior specification.

We build our model sequentially, testing the inclusion of additional fixed effects (concurrent proton pump inhibitor treatment and concurrent diet modification) by comparing the expected log predicted density for a new dataset and found that including these predictors improved the model fit. We found that the inclusion of random effects as described above improved the model fit, indicating that the additional variability may be best explained by between patient variability.

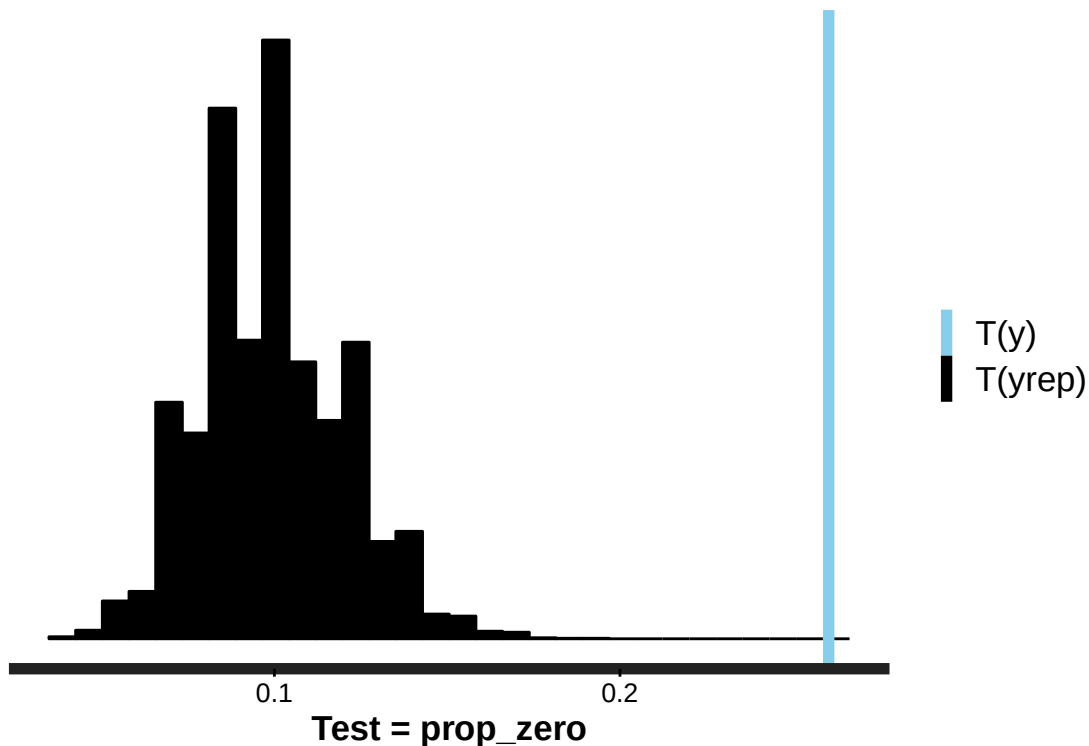
We explored our model assumptions, recognizing that our inferences of the mean effect of treatment will likely be robust to minor violations of normality and homoscedascity in our mixed effects model; Comparing the fitted and predicted values to the observed values, we observed shrinkage (a pulling of the fitted and predicted values towards the mean values of the parameter), as is typical in hierarchical models, leading the improved convergence and model stability by regularization (Gelman, Andrew, et al. Bayesian data analysis. Vol. 2. Boca Raton, FL, USA: Chapman & Hall/CRC, 2014.).

Poisson model

```
## stan_glmmer(formula = Eos ~ treat + furtherfollowup + Diet + PPI +
##           (1 + furtherfollowup | Patient), data = EoEdata9Feb2016,
##           family = "poisson", iter = myiter, chains = mycores - 2,
##           cores = mycores - 2)
##
## Estimates:
##           Median MAD_SD
## (Intercept)      4.3    0.3
## treat           -1.7    0.0
## furtherfollowup -0.6    0.4
## Diet            -0.3    0.3
## PPI              0.1    0.6
##
## Error terms:
## Groups Name          Std.Dev. Corr
```

```
## Patient (Intercept)      0.97
##      furtherfollowup 1.87      -0.01
## Num. levels: Patient 54
##
## Sample avg. posterior predictive
## distribution of y (X = xbar):
##      Median MAD_SD
## mean_PPD 36.9      0.6
```

Posterior predictive checks



The proportion of zeros in the peak Eos counts in the observed data is around 30% (vertical blue line). In the replicated datasets for the poisson model, zeros occur much less frequently. This may point to overdispersion and we explored alternative model (negative binomial) to account better for the large number of zeros in the observed data. The negative binomial distribution allows the mean and variance to differ, (unlike in the poisson model where they are constraint to be the same).

Negative binomial models

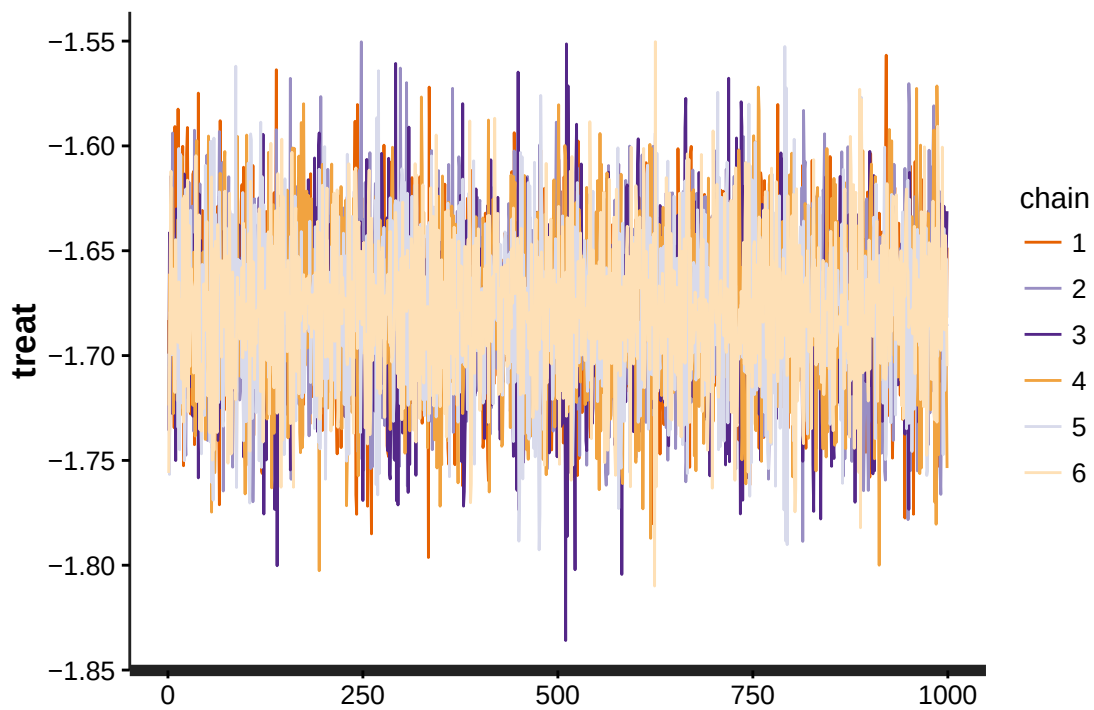
Model A

```
## stan_glmmer(formula = Eos ~ treat + furtherfollowup + Diet + PPI +
##      (1 + furtherfollowup | Patient), data = EoEdata9Feb2016,
##      family = neg_binomial_2, iter = 2000, chains = 4, cores = 4)
##
## Estimates:
##      Median MAD_SD
```

```
## (Intercept)      4.7    0.4
## treat           -2.1    0.4
## furtherfollowup  0.3    0.4
## Diet            -0.3    0.4
## PPI              0.3    0.8
## overdispersion  0.5    0.1
##
## Error terms:
## Groups Name          Std.Dev. Corr
## Patient (Intercept)  0.63
##      furtherfollowup 0.83    0.61
## Num. levels: Patient 54
##
## Sample avg. posterior predictive
## distribution of y (X = xbar):
##      Median MAD_SD
## mean_PPD 46.7   11.6
```

Model B

Model convergence



The traceplot indicates good mixing of the chains

Explore in shinystan

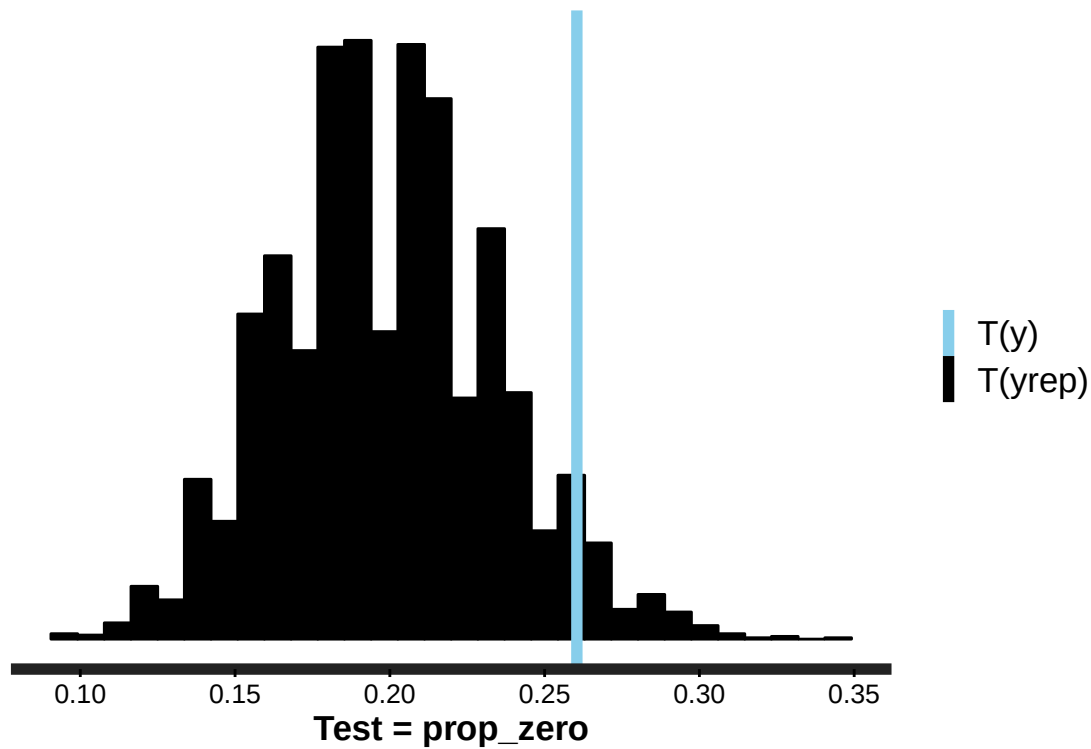
```
require(shinystan)
fit <- fit3
launch_shinystan(fit)
```

We explored the model fits in the interactive R package **shinystan** and found no evidence to suggest non-convergence, with consistently low Rhat values below 1.1.

Model B results

```
## stan_glmmer(formula = Eos ~ treat + furtherfollowup + (1 + furtherfollowup |
##   Patient), data = EoEdata9Feb2016, family = neg_binomial_2,
##   iter = 2000, chains = 4, cores = 4)
##
## Estimates:
##               Median MAD_SD
## (Intercept)      4.4    0.2
## treat          -2.1    0.4
## furtherfollowup  0.3    0.4
## overdispersion  0.5    0.1
##
## Error terms:
## Groups Name          Std.Dev. Corr
## Patient (Intercept)    0.63
##       furtherfollowup 0.89    0.60
## Num. levels: Patient 54
##
## Sample avg. posterior predictive
## distribution of y (X = xbar):
##               Median MAD_SD
## mean_PPD 45.2    11.0
```

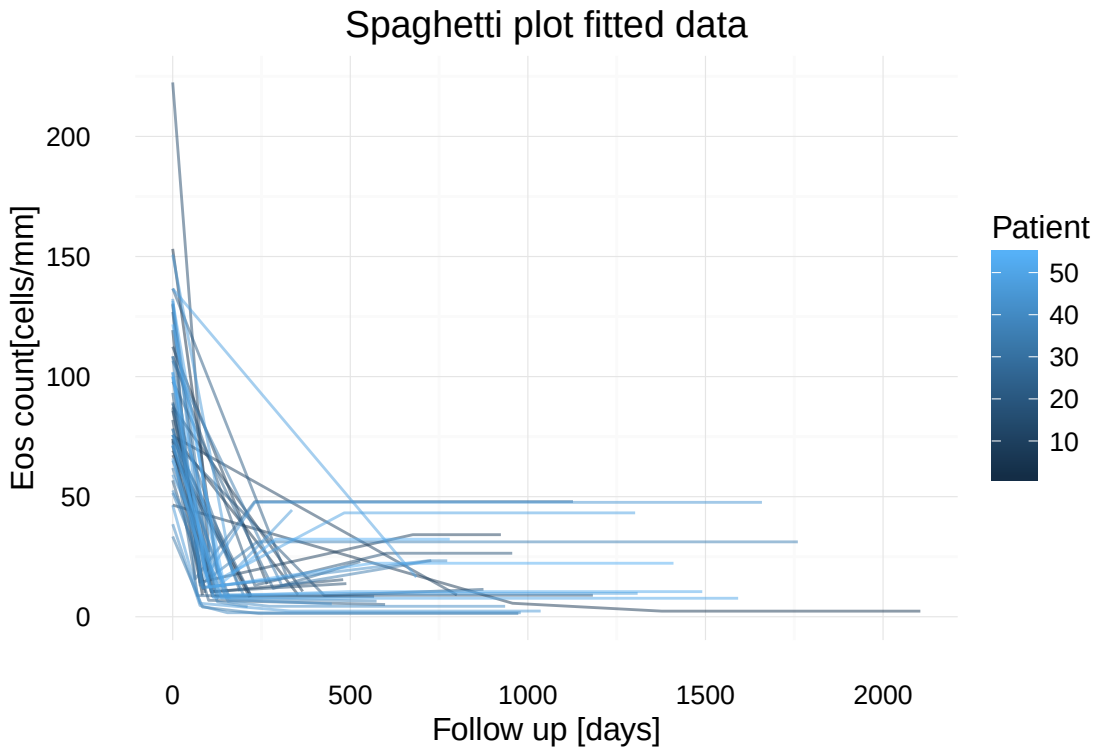
Posterior predictive check for negative binomial model B



We again compare the proportion of zero counts occurring in the observed data (blue vertical line) and in the replicated datasets for the new negative binomial model. Ideally the blue line falls in the center of the black histogram, indicating that the proportion of zero counts in the replicated sets falls around the observed value. The proportion of zeros in the observed data falls obviously not in the center of the histogram, but it is closer, suggesting a better fit. Some difference is expected in this type of model.

Plot of fitted values for negative binomial model B

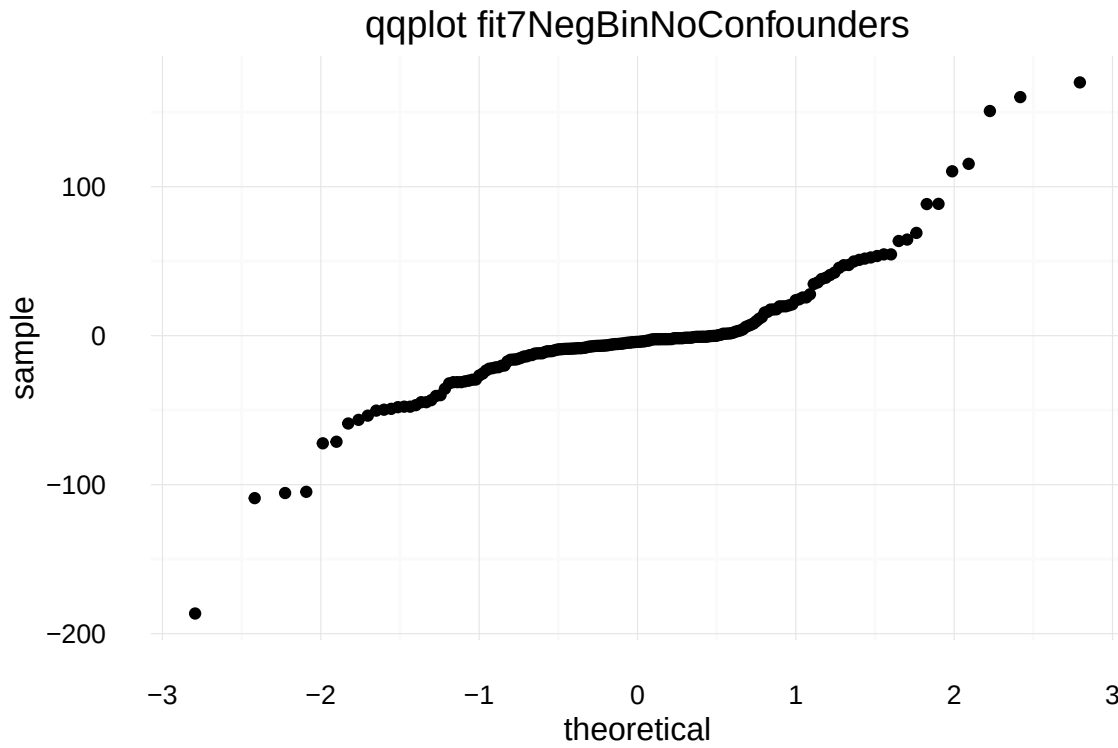
```
## [1] "SpaghettiFittedPlot.md"
```



This plot shows a resonalbe fit of the model by comparing the observed with the fitted Eos count.

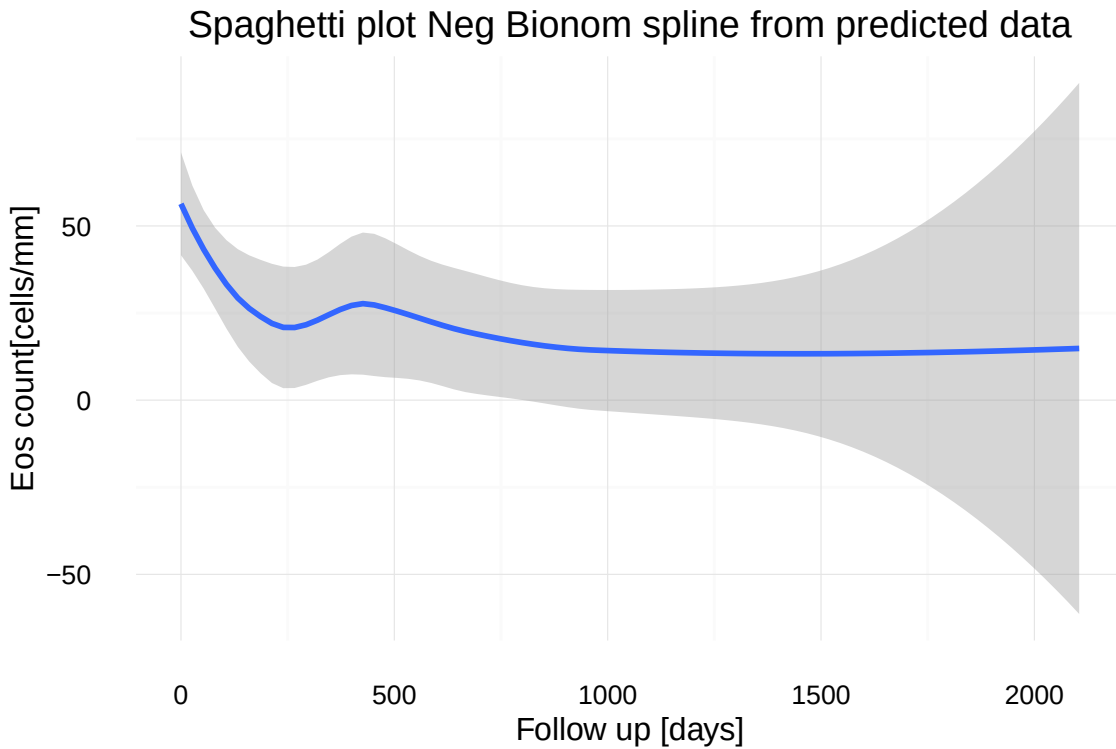
Normal distribution of residuals

```
## [1] "myqqplot.md"
```



This plot indicates violations of some assumptions (e.g. normality), but these are expected and their absence would also not be reassuring.

Spline Model B



Model C

```
## stan_glmmer(formula = Eos ~ treat + furtherfollowup + Diet + (1 +  
##   furtherfollowup | Patient), data = EoEdata9Feb2016, family = neg_binomial_2,  
##   iter = 2000, chains = 4, cores = 4)  
##  
## Estimates:  
##           Median MAD_SD  
## (Intercept)      4.7   0.4  
## treat          -2.1   0.4  
## furtherfollowup  0.3   0.4  
## Diet           -0.3   0.4  
## overdispersion  0.5   0.1  
##  
## Error terms:  
## Groups Name      Std.Dev. Corr  
## Patient (Intercept)  0.63  
##   furtherfollowup  0.87   0.62  
## Num. levels: Patient 54  
##  
## Sample avg. posterior predictive  
## distribution of y (X = xbar):  
##           Median MAD_SD  
## mean_PPD 45.7   11.2
```

Model D

```
## stan_glmmer(formula = Eos ~ treat + furtherfollowup + PPI + (1 +
##   furtherfollowup | Patient), data = EoEdata9Feb2016, family = neg_binomial_2,
##   iter = 2000, chains = 4, cores = 4)
##
## Estimates:
##           Median MAD_SD
## (Intercept)      4.4   0.2
## treat          -2.1   0.4
## furtherfollowup  0.3   0.4
## PPI             0.3   0.7
## overdispersion  0.5   0.1
##
## Error terms:
##   Groups  Name          Std.Dev. Corr
## Patient (Intercept)    0.63
##   furtherfollowup 0.83    0.59
## Num. levels: Patient 54
##
## Sample avg. posterior predictive
## distribution of y (X = xbar):
##           Median MAD_SD
## mean_PPD 46.1   11.6
```

Model E

```
## stan_glmmer(formula = Eos ~ treat + furtherfollowup + Diet + PPI +
##   (1 | Patient), data = EoEdata9Feb2016, family = neg_binomial_2,
##   iter = 2000, chains = 4, cores = 4)
##
## Estimates:
##           Median MAD_SD
## (Intercept)      4.7   0.4
## treat          -2.2   0.4
## furtherfollowup  0.5   0.4
## Diet           -0.3   0.4
## PPI             0.7   0.6
## overdispersion  0.4   0.1
##
## Error terms:
##   Groups  Name          Std.Dev.
## Patient (Intercept) 0.72
## Num. levels: Patient 54
##
## Sample avg. posterior predictive
## distribution of y (X = xbar):
##           Median MAD_SD
## mean_PPD 48.3   13.7
```

Model F

```
## stan_glmmer(formula = Eos ~ treat + furtherfollowup + (1 | Patient),
```

```
##      data = EoEdata9Feb2016, family = neg_binomial_2, iter = 2000,
##      chains = 4, cores = 4)
##
## Estimates:
##              Median MAD_SD
## (Intercept)    4.5    0.3
## treat         -2.2    0.4
## furtherfollowup 0.6    0.4
## overdispersion 0.4    0.1
##
## Error terms:
## Groups Name      Std.Dev.
## Patient (Intercept) 0.8
## Num. levels: Patient 54
##
## Sample avg. posterior predictive
## distribution of y (X = xbar):
##              Median MAD_SD
## mean_PPD 47.8    13.7
```

Compare Models

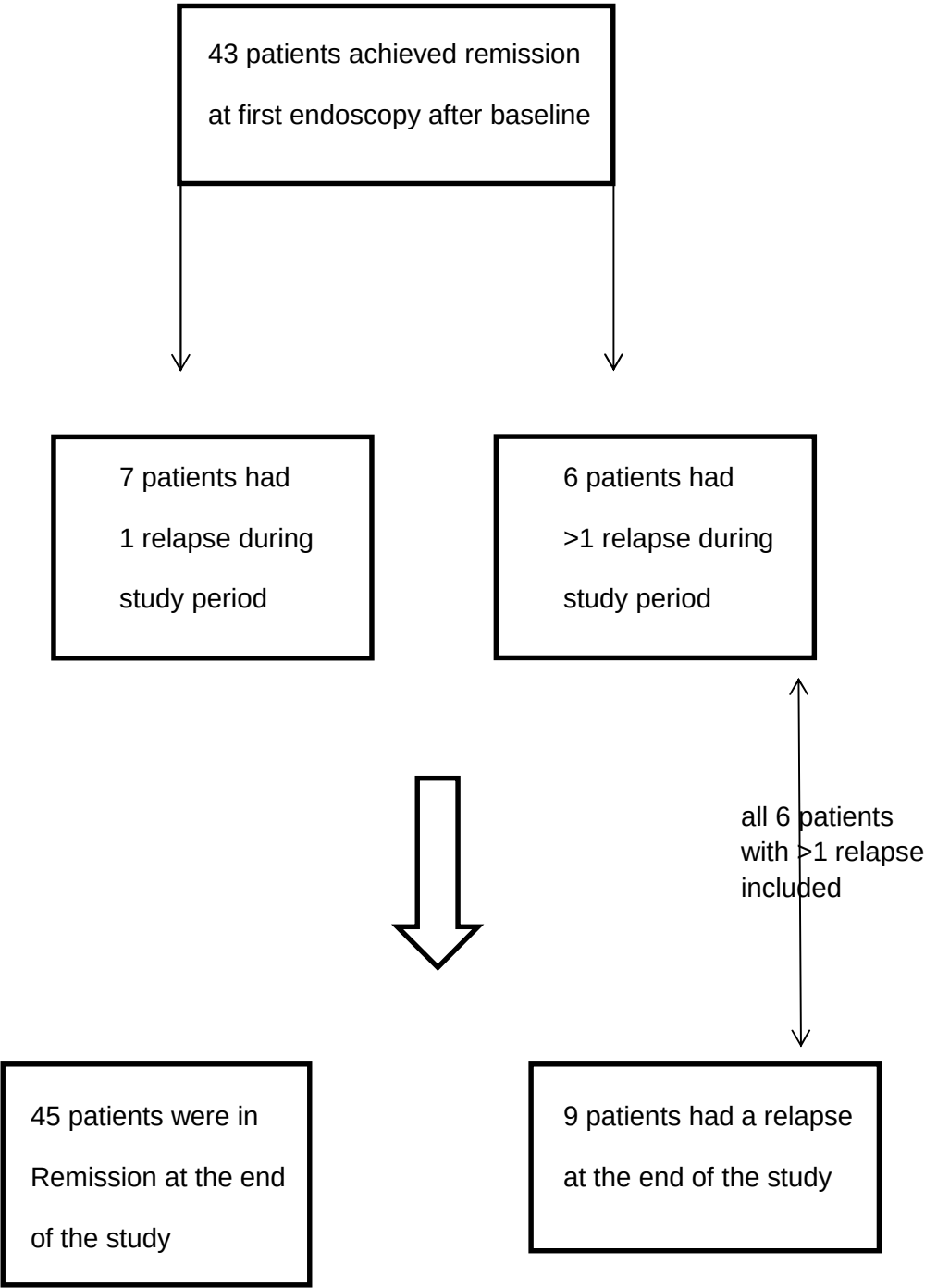
The LOO Information Criterion (LOOIC) has the same purpose as the Aikaike Information Criterion (AIC) that is used by frequentists. Both are intended to estimate the expected log predicted density (ELPD) for a new dataset.

	looic	se_looic	elpd_loo	se_elpd_loo	p_loo	se_p_loo
## modelB	1550.9	58.7	-775.4	29.4	27.3	3.9
## modelC	1552.5	58.7	-776.2	29.4	27.3	3.7
## modelA	1556.0	59.0	-778.0	29.5	27.7	4.0
## modelF	1564.1	59.1	-782.0	29.5	23.3	3.5
## modelE	1566.8	59.0	-783.4	29.5	22.8	3.4

The model fit is very similar and the inferences are the same.

1 **Online Repository**

2 **Supplemental Figure 1:** Flow diagram detailing remission and relapse rates over the study
3 period.



Supplemental Table 1: Number and percentage of patients that had eosinophil counts <15 eos/HPF at the respective follow-up time points. Also seen is the number of patients with eosinophil counts <10 eos/HPF and <5 eos/HPF. Patients with <5 eos/HPF are included in the groups <10 eos/HPF and <15 eos/HPF, and patients with <10 eos/HPF are included in the group <15 eos/HPF.

	<4 months (n=24)	4-12 months (n=32)	13-24 months (n=27)	>24 months (n=19)
Number (%) of patients with peak eosinophil count:				
<15 eos/HPF	20 (83)	27 (84)	16 (59)	12 (63)
<10 eos/HPF	18 (75)	25 (78)	14 (51)	11 (58)
<5 eos/HPF	15 (63)	21 (66)	13 (48)	11 (58)

Supplemental Table 2: Posterior median estimates (50%) for fixed effects and exemplary random effects regression coefficients are shown above with their corresponding 95% upper and lower bounds, followed by an exemplary random effect for patient 1. For our mixed effects negative binomial regression model, the difference in the logs of expected peak eosinophil counts is expected to change by the respective regression coefficient, given the other predictor variables in the model are held constant. In a representative patient with a starting peak eosinophil count of 80, this might mean a reduction to a peak eosinophil count of 10, which is mostly sustained in further follow-ups. Numerically, -2.1, the reduction in log Eos count estimated by the regression coefficient for treatment initiation, equals the difference between $\log(80)$ and $\log(10)$. The consistently low Rhat values below 1.1 suggest adequate model convergence.

Coefficient	Rhat	lower 95%	posterior median	upper 95%
(Intercept)	1	4.02	4.45	4.92
treat	1	-2.89	-2.12	-1.39
furtherfollowup	1	-0.61	0.26	1.10
b[(Intercept) Patient:1]	1	-1.36	-0.14	1.02
b[furtherfollowup Patient:1]	1	-2.23	-0.11	2.06