

Effects of Fluticasone Furoate on Clinical and Immunological Outcomes (IL-17) for Patients With Nasal Polyposis Naive to Steroid Treatment

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Abstract

Objectives: We investigated the effect of topical steroids on clinical outcomes and related immune response of chronic rhinosinusitis with nasal polyp (CRSwNP) patients and in eradicating some polyps. We want to explore a new potential mechanism linked to Th-17 cells.

Methods: Prospective, double-blind, placebo-controlled studies with 24 allergic and nonallergic patients were randomized to either placebo or fluticasone furoate for 12 weeks. Assessment of clinical response, endoscopic score with biopsies of the inferior turbinate, and polyps before and after treatment were performed. Biopsies were stained for T-cells, eosinophils, neutrophils, and IL-17A/F.

Results: Steroid treatment improved the mean symptoms scores from 7.12 to 4.02 ($P < .01$) and the polyp score from 5.13 to 3.31 ($P < .05$), but the comparison with placebo was not statistically significant in nonallergics due to insufficient study power. Steroid treatment decreased eosinophil counts on allergics but not neutrophils or T-cells. The IL-17A/F expression was higher in nonallergics with high neutrophil counts and was inclined by steroids. Compared to baselines, IL-17 cells were significantly less in allergic individuals and were not observed in allergics and with high neutrophil counts.

Conclusion: Topical steroids were more effective on certain nasal polyp phenotypes. Identification of polyp phenotype might be essential to ensure a better therapeutic response to intranasal corticosteroids.

Keywords

nasal polyps, chronic rhinosinusitis, eosinophilic rhinosinusitis, clinical outcomes research, innate immunity, nasal and sinus surgery, obstructive sleep apnea

Introduction

Chronic rhinosinusitis (CRS), a chronic inflammatory disease of the nasal mucosa and paranasal sinuses, is characterized by the symptoms of nasal obstruction, smell loss, facial pressure pain, and rhinorrhea. It has been suggested that CRS is not a single disease, and evidence exists that categorizes CRS into polypoid and nonpolypoid, on the basis of differences in cytokine profile.¹ Nevertheless, considerable controversy indicates that many patients without nasal polyps show polypoid changes of the sinus mucosa. While tissue eosinophilia and T cells demonstrating a skewing toward Th2 cytokine expression are common features of chronic rhinosinusitis with nasal polyp (CRSwNP), chronic rhinosinusitis without nasal polyp (CRSsNP) reveals Th1 polarization and a lesser degree of eosinophilic infiltration.^{2–4} The Th2-type response is associated with IgE-mediated degranulation and release of inflammatory

mediators by mast cells and basophils that are shown to contribute to excessive production of IL-4, IL-5, and IL-13 cytokines and tissue infiltration by lymphocytes and eosinophils. Nasal polyps in CRS are also portrayed by epithelial thickening and denudation, thickening of the basement membrane, glandular hyperplasia, and edematous stroma.⁵ However, apart from an allergic nature involving IgE and aeroallergens, immune response in CRS is often not complemented by obvious atopic sensitization. On the basis of an analysis of a prospective cohort study, Nakayama et al⁶ reported that patients with mucosal eosinophilia had higher

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polyp recurrence rate than patients without mucosal eosinophilia. Consequently, they concluded that presence of mucosal eosinophilia is a more important factor than nasal polyps for classifying CRS and might affect prognosis and treatment outcome. There have been reports that the majority of CRSwNP cases in Western countries have a strong tendency to recur after surgery and display eosinophil-dominant inflammation. In contrast to Western countries, more than half of the CRSwNP cases in East Asia and Japan do not present eosinophil-dominant inflammation but show features indicative of neutrophilic inflammation.⁷

Th17 cells play a key role in the pathogenesis of allergic diseases because of their ability to release IL-17A cytokine and orchestrate innate and adaptive immune responses. IL-17A upregulates the expression of several proinflammatory cytokines, including tumor necrosis factor α (TNF- α), IL-1 β , IL-6, and IL-8, and is involved in the recruitment, activation, and migration of neutrophils.^{8,9} It is interesting to note that myeloperoxidase-positive cells (neutrophils) and myeloperoxidase and IL-8 concentrations are increased in CRSsNP and CRSwNP, suggesting that neutrophils are also implicated in nasal polyp pathogenesis.¹⁰ Further, patients with nasal polyp also have increased levels of IL-17 expression and orchestrate a neutrophilic inflammation.¹¹ Thus, characterization of allergic versus nonallergic phenotypes of CRS may be very helpful in understanding the underlying pathophysiology and may be used to target treatment.

Corticosteroids have a well-established role in the medical management of nasal polyposis. Treatment with topical steroid sprays or drops has been shown to be effective in reducing sinus inflammation and nasal polyp size and improve symptoms associated with CRS.¹²⁻¹⁴ Studies have demonstrated that short courses of oral steroids are of clinical benefit in CRS with nasal polyps and in cases of severe CRS when rapid symptomatic improvement is required.^{13,15} Although steroids have been noted to be of value in the treatment of NP associated with CRS, a considerable number of patients appear to be not responding to steroid treatment and eventually need surgery. Even after surgery, some of the cases exhibit recurrence of disease. As part of their search for predictors expecting the response to surgery in combination with perioperative oral glucocorticoids in CRS patients, Ebbens et al¹⁶ reported that CRS patients with higher levels of eosinophils are less likely to suffer from postoperative recurrent sinonasal disease when treated postoperatively with inhaled corticosteroids. Furthermore, the benefit of steroids for nonpolypoid CRS has been difficult to demonstrate,¹⁷ probably reflecting the heterogeneous nature of nonallergic rhinitis. The aim of this study was to investigate steroid (fluticasone furoate [Avamys GSK, Mississauga Canada]) responsiveness relative to inflammatory mediators present in the polyps, clinical outcomes, and related immune response of CRSwNP

patients. We also wanted to explore a new potential mechanism linked to Th-17 cells.

Methods

Study Design

This study was conducted as a prospective, double-blind, placebo-controlled investigation that enrolled 24 patients (8 female and 16 male; 29-64 years of age; mean age, 40.5 years) who never used topical or oral corticosteroids with mild to moderate nasal polyp volume. Fifteen allergic and 7 nonallergic patients treated either with placebo ($n = 4$) or fluticasone furoate (Avamys GSK) ID, BID ($n = 18$) for 12 weeks. Two patients dropped out before the second biopsy, and an additional patient lost his symptoms score sheet. All participants gave written informed consent, and the study was approved by the central institutional review board, Mississauga, Ontario, Canada.

The diagnosis of chronic rhinosinusitis with nasal polyps was based on history, clinical examination, and nasal endoscopy and conforms to the clinical definition,¹ which is inflammation of the nose and the paranasal sinuses with a minimum duration of 12 weeks and characterized by 2 or more symptoms such as blockage, nasal congestion, discharge facial pressure, reduction or loss of smell with endoscopic signs (presence or not of polyps) and CT changes.

The atopic status was determined by using the skin prick testing for 14 common aeroallergens. All patients were tested prospectively by Dr Dube, a certified allergist. Subjects with polyposis class I and II who never received intranasal topical steroids were eligible to participate in the study. Consecutive patients seen in clinic met the inclusion criteria. Patients were not eligible to take part in the study if they received oral or topical steroids in the past. No patients had sinus surgery in the past. Immunosuppressed and cystic fibrosis patients were excluded from this study. Active and nonactive medications were provided by the company, and we were blind to the process. Randomization was performed by the study coordinator.

Endoscopic Score

Nasal polyp score was measured¹⁸ with level I being under the middle turbinate, II extension between the middle and inferior turbinate, and level III at the floor of the nasal cavity. Assessment of clinical response was obtained with the endoscopic score summation of both side of the nasal cavity.

Nasal Symptoms Score

All patients completed a weekly questionnaire with a score sheet from 0 to 3, with the worst symptoms being at 3 and

covering smell, sneezing, nasal obstruction, pruritus, and rhinorrhea. Addition of these 5 scores consisted of the global clinical score. The presence of epistaxis and dryness was also followed. The nasal symptom score used in this study was the same score used by Schering-Merck for Nasonex. The global score was not the addition of the other scores but an answer to how the treatment benefited them.

Nasal Biopsies

Following the endoscopic scoring, topical 4% xylocaine (AstraZeneca Canada Inc, Mississauga, Canada) and between 5 and 1 ml of injected xylocaine 1% with 1:100 000 dilution of ephedrine was used at the sites of biopsy. Study subjects ($n = 22$) were biopsied from the inferior turbinate and from the polyps. The patients then received placebo or topical corti16

costeroid treatment (fluticasone furoate ID, BID) for a period of 12 weeks. The dosage of the fluticasone was 27.5 microgram/spray, 2 sprays in each nostril, once a day, and alternate sides were used to prevent the confounding factor of scarring and inflammation. At the end of corticosteroid treatment period, patients were then biopsied again. Biopsies were taken alternatively on the right and left side before and after treatment.

Fresh biopsy specimens obtained before and after treatment with topical corticosteroids were fixed in paraformaldehyde and then processed for paraffin embedding. Tissue blocks were sectioned at 5 μ m thickness onto poly-L-lysine-coated slides. Inflammatory cell infiltrate in tissues was determined by routine hematoxylin and eosin staining (H&E). Inflammatory cell phenotypes were identified by immunocytochemistry (ICC) using monoclonal antibodies: anti-EG2 (eosinophilic cationic protein detecting eosinophils), anti-elastase (neutrophils), anti-CD4 (helper T cells), and anti-CD8 (cytotoxic T cells). Tissue sections were also processed for immunostaining of IL-17A and IL-17F.

Immunohistochemistry

Tissue sections were deparaffinized in xylene and dehydrated in ethanol series. Slides were washed in PBS for 5 minutes and processed for antigen retrieval using citrate buffer. Endogenous peroxidase activity was blocked with hydrogen peroxide in PBS for 30 minutes. Sections were then washed in PBS, blocked using DAKO universal blocking reagent for 60 minutes at room temperature. Sections were incubated overnight at 4°C with primary anti-EG2, or anti-elastase, or anti-CD4, or anti-CD8, or anti-IL-17A, or anti-IL-17F antibodies. The primary antibody was replaced by nonimmune serum for negative controls. After incubation with biotinylated secondary antibodies, sections were washed 3 times with PBS, incubated

with streptavidin-horse radish peroxidase complex, and then developed using diaminobenzidine substrate (DAB) as per the manufacturer's instructions. Nucleus was stained blue with Gill II hematoxylin.

Quantification

Stained sections were inspected using an Olympus light microscope, and digital images were captured by a BX51 Olympus microscope attached to a CoolSNAP-Procolor digital camera (Cارسen Groups, Markham, Canada) using the Image Pro-Plus 6.0 system (Media Cybernetics, Rockville, Maryland, USA) and stored electronically. Immunostained biopsy sections were counted in a blinded fashion in coded random order through use of an Olympus microscope, and positive cell counts were obtained using computer-assisted image analysis and were normalized to the total area of the biopsy. Results are expressed as positive cells/mm².

Statistical Analysis

An ANOVA on change from baseline adjusted for baseline value to compare mean values for pre- and posttreatment of allergic or nonallergic subjects. Paired *t* test was used to compare clinical symptoms score for nasal obstruction, rhinorrhea, sneezing, and daytime fatigue. Correlation coefficient was calculated for IL-17 expression and neutrophil cell count in nasal polyps. One-way ANOVA with post hoc Bonferroni test was used to compare statistical significance on subjects treated with steroids for different time points.

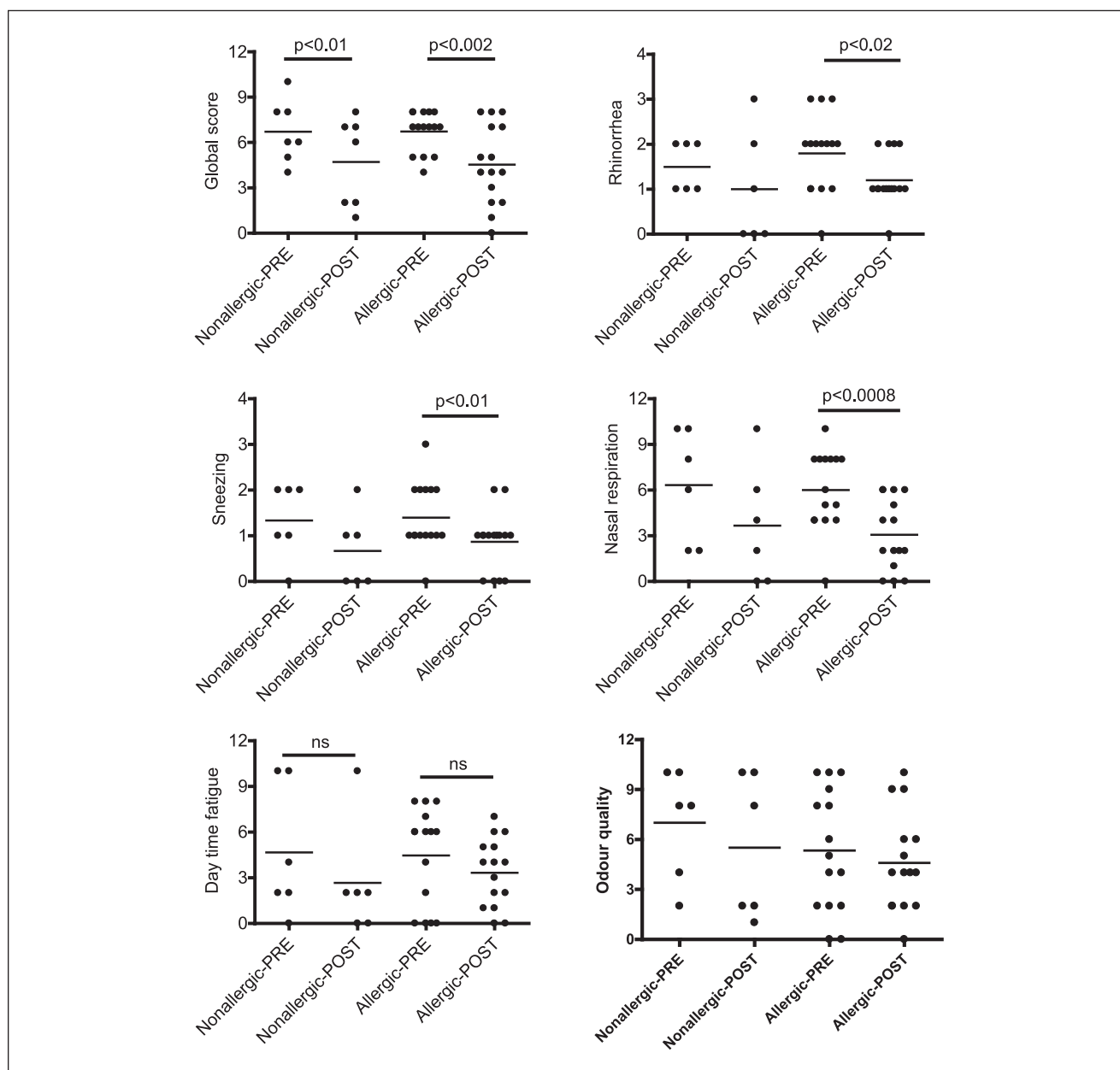
Results

A total of 24 patients were randomized, and 22 completed the study from autumn 2011 to spring 2013. Fifteen were allergic and 7 nonallergic, with a mean age of 46 years old (SD, 16). Treatment with fluticasone furoate nasal spray (FFNS) was well tolerated, with no unusual or unexpected events. Only dryness was reported in 2 patients, who finally completed the study without interruption of treatment.

Clinical symptoms score (See Table 1) compared from the beginning and the end of the study showed a benefit for nasal obstruction, rhinorrhea, sneezing, and daytime fatigue (Figure 1) on paired *t* test. The use of topical steroids reduced the global symptom score from 3.48 to 2.33 ($P = .0003$) and improved the combined (right and left) polyp score grade from 4.83 to 2.75 ($P < .0001$). Comparison between allergic and nonallergic patients demonstrated significant benefits for rhinorrhea, sneezing, and congestion in the allergic group (Figure 1). The benefit as measured on the global symptoms score was more pronounced for the allergic subjects ($P < .002$). Relief of nasal obstruction was the most robust benefit and was sustained from 4

Table I. Clinical Symptoms Scores in Allergic Versus Nonallergic Patients.^a

Group	Global Score	Rhinorrhea	Sneezing	Nasal Respiration	Daytime Fatigue	Odor Quality
Nonallergic pretreatment	6.71 ± 0.77	1.5 ± 0.22	1.33 ± 0.33	6.3 ± 1.49	4.66 ± 1.76	7.0 ± 1.34
Nonallergic posttreatment	4.71 ± 1.11 ^b	1.0 ± 0.51	0.66 ± 0.33	3.66 ± 1.58	2.66 ± 1.52	5.5 ± 1.74
Allergic pretreatment	6.73 ± 0.34	1.8 ± 0.22	1.4 ± 0.19	6.0 ± 0.66	4.46 ± 0.82	5.33 ± 0.94
Allergic posttreatment	4.53 ± 0.68 ^c	1.2 ± 0.14 ^d	0.86 ± 0.16 ^e	3.06 ± 0.60 ^f	3.33 ± 0.58	4.6 ± 0.76

^aValues are mean ± SEM.^bSignificantly ($P < .01$) lower than nonallergic pretreatment.^cSignificantly ($P < .002$) lower than allergic pretreatment.^dSignificantly ($P < .02$) lower than allergic pretreatment.^eSignificantly ($P < .01$) lower than allergic pretreatment.^fSignificantly ($P < .0008$) lower than allergic pretreatment.**Figure I.** Clinical symptoms as reported by the patients in allergic versus nonallergic subjects.

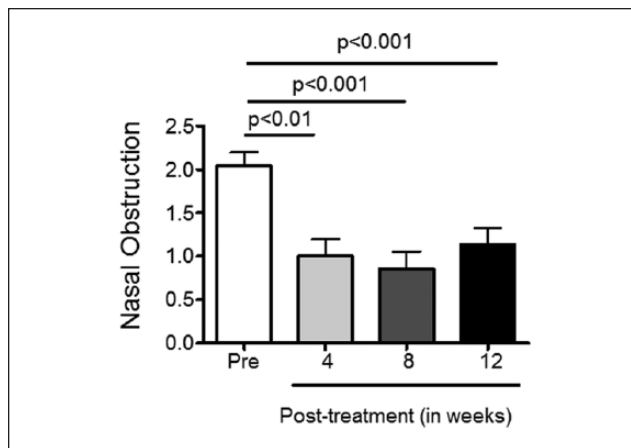


Figure 2. Impact of fluticasone furoate nasal spray (FFNS) on nasal obstruction scores for all patients over 12 weeks.

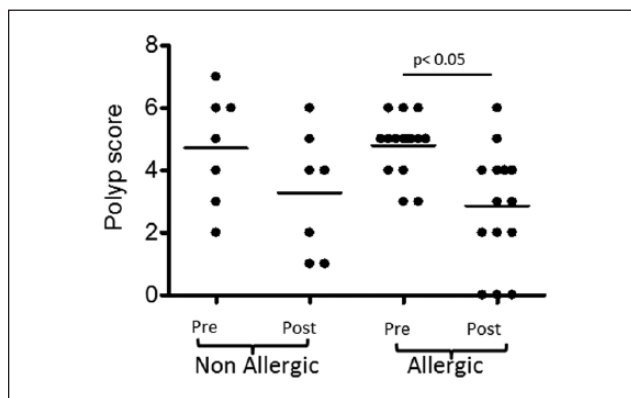


Figure 3. Evolution of pre- and posttreatment of polyp scores.

to 12 weeks in both groups of patients (Figure 2). The bilateral polyp grade score reduction was more pronounced in the allergic patients compared to nonallergic ($P < .05$) (Figure 3); however, there was a trend for the treated group but no significance compared to the placebo group due to insufficient study power.

To investigate the effect of topical steroids on immune response of CRSwNP patients, biopsies from polyps were taken before and after 12 weeks of treatment with steroids/placebo and then processed to identify eosinophils, neutrophils, and IL-17A and IL-17F.

Allergic polyps have more eosinophils compared to nonallergic polyps, but there was no difference in the number of neutrophils between the 2 groups at baseline. The use of topical steroids was also associated with a decrease in eosinophil counts ($P < .05$) in allergic individuals but not with neutrophils. However, there was no significant change in the number of inflammatory cells in nonallergic subjects. The expression of IL-17A and IL-17F

at the baseline was identified in biopsies from all patients; however, the expression of IL-17A and IL-17F in tissue taken from nonallergic was significantly higher compared to allergic polyps ($P < .005$) and was positively correlated ($R = 0.67$) with neutrophil count ($P < .05$). In allergic individuals, there was a trend toward a decrease ($P = .57$) in the number of IL-17A and IL-17F in response to steroids compared to placebo. In patients with no evidence of allergy, the number of IL-17A and IL-17F in polyp tissue did not show any change in response to topical steroids when compared to baseline and placebo ($P > .05$).

Discussion

This study was designed to assess the efficacy and evaluate the possible predictors of clinical response to treatment with fluticasone furoate. The nasal spray formulation of FF is a highly potent corticosteroid approved for therapeutic use in seasonal allergic rhinitis and perennial allergic rhinitis in Canada.¹⁹⁻²¹ Subjects in this study had endoscopically verified bilateral nasal polyps, with a mean total bilateral grade score of approximately 5.00 and achieved reduction of their polyp volume and related nasal symptoms with the use of this intranasal corticosteroid (INCS). Although we have evaluated a very small group of patients, it seems appropriate to consider that the clinical benefit was better for the allergic subjects. The control of the clinical symptoms associated to the allergic rhinitis in addition to polypoid volume reduction probably explains the better relief of rhinorrhea, sense of smell, and nasal obstruction. It is well accepted that nonallergic rhinitis has less benefit from INCS.²² The lesser reduction of polyp volume in nonallergic patient probably also accounts for persistence of the symptomatology. However, a greater number of patients and a more prolonged treatment could have alleviated the clinical difference.

Our results suggest that the use of topical steroids was more effective on certain phenotypes of nasal polyps, particularly those associated with eosinophils. When we examined the association between the clinical response to steroids and the immunological response (eosinophil count and IL-17 expression), we demonstrated a positive correlation ($P < .05$) between eosinophils response and symptoms scores but not polyps score. We have also identified a reverse correlation between the expression of IL-17A and the level of symptom scores and polyp scores, particularly in nonallergic subjects. Therefore, the cell lineage Th-17 cells that produce IL-17A, a cytokine involved in neutrophilic infiltration and local fibrosis, seems to be a very important modulator of polyp phenotype and could induce some resistance to INCS.^{23,24} We hope that further studies with a larger group of patients will clarify the phenotyping of CRSwNP and patient selection for treatment.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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