The Journal of Laryngology & Otology (2011), ***125***, *1028–1032.*

doi:10.1017/S0022215111001721

MAIN ARTICLE

Comparison of nasal cytology and symptom scores in patients with seasonal allergic rhinitis, before and after treatment

A ÖZGÜR[1], S ARSLANOĞLU[1], D ETİT[2], U DEMİRAY[1], H K ÖNAL[1]

Departments of [1]Otorhinolaryngology, and [2]Pathology, İzmir Atatürk Training and Research Hospital, Turkey

Abstract

Objective: To evaluate symptom scores and nasal smear cytology findings in seasonal allergic rhinitis patients, before and after treatment.

Methods: Twenty-nine consecutive adult patients with seasonal allergic rhinitis were evaluated prospectively. They received mometasone furoate nasal spray and cetirizine for 21 days. Nasal and ocular symptom scores were recorded before and after treatment. Nasal cytology was also assessed as a means of determining treatment.

Results: The combined use of an intranasal corticosteroid and an oral antihistamine caused a significant improvement in nasal and ocular symptom scores. Cytological evaluation revealed significant reduction in nasal eosinophil, neutrophil and goblet cell counts after three weeks' treatment.

Conclusion: Symptom scoring systems are widely used for the evaluation of drug efficacy in allergic rhinitis treatment. When investigating the disease and evaluating treatment efficacy, objective as well as subjective methods are needed. Nasal cytological assessment is a simple, objective method which provides valuable information about the nasal mucosa.

Key words: Rhinitis; Pathology; Cell Biology; Symptoms And Signs

Introduction

Allergic rhinitis is a common, immunoglobulin E (IgE) mediated disease of the nasal mucosa which significantly impairs patients' quality of life.[1–3] It is characterised by ocular and nasal symptoms, the latter including obstruction, itching, rhinorrhoea and sneezing.

Treatment efficacy is evaluated by various subjective and objective methods. The grading of nasal symptom severity is crucial in order to guide treatment choice and to facilitate assessment of treatment outcomes. Various subjective scoring systems have been described with which to grade symptom severity. Nasal cytological assessment, an objective evaluation method, may also be used as an adjunct.[4–7]

This study was designed to assess symptom scores and nasal cytology scores in patients with seasonal allergic rhinitis, before and after treatment with fluticasone proprionate and cetirizine.

Materials and methods

A prospective study was conducted on 29 adult patients with seasonal allergic rhinitis treated in the otolaryngology-head and neck surgery department of a tertiary referral hospital, between June 2007 and June 2008.

All consecutive patients aged 19–67 years with at least a two-year history of moderate or severe seasonal allergic rhinitis (based on the clinical definition and classification criteria of the Allergic Rhinitis and its Impact on Asthma (ARIA) Workshop report) were considered eligible for study inclusion.[8]

The protocol was approved by the ethics committee of the research hospital. Written, informed consent was obtained from all patients.

After detailed medical history-taking and otorhinolaryngological examination, nasal specimens were taken using disposable nasal brushes. Patients were also asked to rate the current severity of their nasal and ocular symptoms.

Patients were then prescribed oral levocetirizine dihydrochloride tablets, 5 mg once daily, and mometasone furoate monohydrate nasal spray, two sprays in each nostril (50 μg in each spray) once daily, for 21 days.

At the end of the treatment period, patients were recalled for follow up. They were again asked to rate

Accepted for publication 23 January 2011 First published online 27 July 2011

their current nasal and ocular symptom severity, and further nasal specimens were taken using the same collection technique.

Clinical symptom scores

Before and after treatment, patients rated their nasal symptoms (i.e. nasal stuffiness, rhinorrhoea, sneezing and itching) and ocular symptoms (i.e. eyelid swelling, ocular itching, redness and tearing) using a four-point scale, with 0 = no symptoms, 1 = mild symptoms, 2 = moderate symptoms and 3 = severe symptoms.

Individual and total symptom scores were then calculated. Each patient's total nasal symptom score and total ocular symptom score were calculated by summing that patient's individual nasal and ocular symptom scores, respectively.

Nasal cytology evaluation

Nasal cytology samples were obtained before and after treatment by scraping the surface of the middle third of the inferior turbinate, using disposable nasal brushes, in both nasal cavities. Specimens were spread on microscopy slides and fixed in 95 per cent ethyl alcohol. Preparations stained with haematoxylin and eosin and Giemsa were examined using oil immersion light microscopy (×1000).

All specimens were evaluated by the same pathologist, who was blinded to the patient's identity and clinical features. On each slide, five randomly chosen magnification fields were examined and cell counts (i.e. eosinophils, neutrophils, basophilic cells and goblet cells) recorded and graded on a four-point scale (Table I). The mean cell count per 10 high-power fields was also recorded, for each nasal cavity. Grades higher than 1+ were accepted as positive.

Data analysis

The Statistical Package for the Social Sciences version 16.0 for Windows software was used for statistical analysis. Data were evaluated using McNemar, chi-square and correlation tests. Statistical significance was accepted for p values less than 0.05.

TABLE I
NASAL CYTOGRAM GRADING

Cell count	Cell appearance	Grade
*Eosinophils & neutrophils**		
0	None	0
0.1–1.0	Occasional cells	0.5+
1.1–5.0	Few scattered cells or small clumps	1+
5.1–15.0	Moderate no of cells & larger clumps	2+
15.1–20.0	Larger clumps of cells, not covering entire field	3+
>20	Large clumps of cells covering entire field	4+
*Basophilic cells**		
0	None	0
0.1–0.3	Occasional cells	0.5+
0.4–1.0	Few scattered cells	1+
1.1–3.0	Moderate no of cells	2+
3.1–6.0	Many cells, easily seen	3+
>6.0	Large no of cells, as many as 25/HPF	4+
Goblet cells†		
0%	None	0
1–24%	Occasional to few cells	1+
25–49%	Moderate no	2+
50–74%	Many cells, easily seen	3+
75–100%	Large no, may cover entire field	4+

*Mean cells per 10 high-power fields (HPF) ×1000.
†Goblet cell count as percentage of epithelial cell count. Adapted with permission.[4] No = number

Results and analysis

We enrolled in the study 29 adult patients with documented seasonal allergic rhinitis: 11 men (38 per cent) and 18 women (62 per cent). Patients' mean age ± standard deviation (SD) was 35.55 ± 12.59 years (range, 19–67 years).

Prior to treatment, all patients had a total nasal symptom score of 10 or more (mean ± SD 15.69 ± 2.31) and a total ocular symptom score of 7 or more (mean ± SD 13.21 ± 3.83).

Following treatment, the mean ± SD total nasal symptom score was 2.69 ± 3.92. A mean ± SD nasal symptom score decrease of 13 ± 4.13 was observed; this decrease was statistically significant ($p < 0.001$). Based on improvement in nasal symptoms, treatment success was 75.9 per cent (i.e. 22 patients). Table II shows patients' nasal symptom scores before and after treatment.

Following treatment, the mean ± SD total ocular symptom score was 2.52 ± 3.58. Thus, the mean ± SD decrease in total ocular symptom score over the course of the study was 10.69 ± 5.74; this decrease was also statistically significant ($p < 0.001$) (Table II). Based on improvement in ocular symptoms, treatment success was 72.4 per cent (21 patients).

There was a statistically significant correlation between the decrease in nasal and ocular symptom scores ($p < 0.04$).

Nasal cytology examination indicated that eosinophils were present in 17 patients (58.6 per cent) before treatment and four patients (13.8 per cent) after treatment. Neutrophils were present in 26 patients (89.7 per cent) before treatment and 16 patients (55.2 per cent) after treatment. Basophilic cells were present in seven patients (24.1 per cent) before treatment and three patients (10.3 per cent) after treatment. Goblet cells were present in 17 patients (58.6 per cent) before treatment and four patients (13.8 per cent) after treatment (Table III).

Decreases in eosinophils, neutrophils, goblet cells and total cytology scores were statistically significant; the decrease in basophilic cells was not statistically significant. There was a statistically significant correlation between the decrease in total cytology scores and the decrease in eosinophil and neutrophil scores ($p < 0.01$). However, there was no significant correlation

https://doi.org/10.1017/S0022215111001721 Published online by Cambridge University Press

TABLE II
TOTAL SYMPTOM SCORES PRE- AND POST-TREATMENT

Score	Pre-Rx	Post-Rx	Reduction	*p*
TNS	15.69 ± 2.31	2.69 ± 3.92	13.00 ± 4.18	<0.001
TOS	13.21 ± 3.83	2.52 ± 3.58	10.69 ± 5.74	<0.001

Data represent means ± standard deviations unless otherwise specified. Rx = treatment; TNS = total nasal symptoms; TOS = total ocular symptoms

between the decrease in total cytology score and the decrease in basophilic cell and goblet cell scores ($p > 0.05$).

Discussion

Allergic rhinitis is a 'symptomatic disorder of the nose, induced after allergen exposure, by an IgE-mediated inflammation of the nasal membranes'.[8]

The diagnosis of allergic rhinitis is based on a combination of typical allergic symptoms, physical examination signs and diagnostic test results. Performing nasal smear cytology provides additional support for the diagnosis. Some authors consider an increase in eosinophil count, on nasal smear cytology, to be a diagnostic criterion, but there is no consensus.[9–11] The Allergic Rhinitis and its Impact on Asthma initiative has proposed that intermittent allergic rhinitis be diagnosed when symptoms last less than four days a week, or less than four consecutive weeks. Moderate or severe disease is characterised by the presence of one or more of the following: abnormal sleep; impairment of daily activities, leisure and/or sport; impairment of school or work; and troublesome symptoms.[12]

The patients enrolled in our study complained of symptoms in the same months of the year for at least two years, lasting no more than four weeks. They also fulfilled the criteria for the diagnosis of moderate or severe disease.

All our patients were given an intranasal corticosteroid (mometasone furoate) and an oral H_1-antihistamine (levocetirizine). At the end of a three-week course of treatment with these drugs, patients' mean total nasal symptom score had improved by 86.8 per cent, and their mean total ocular symptom score by 80.9 per cent ($p < 0.001$). These results are consistent with those of Meltzer *et al.*, who found a decrease in mean nasal symptom scores, compared with baseline measurements, in patients with seasonal allergic rhinitis treated with mometasone furoate.[7] In another study, patients receiving levocetirizine showed an overall total symptom score improvement of 86 per cent in the first week of treatment and 47 per cent over the entire treatment period, compared with placebo.[13] Lee *et al.* found a 43 per cent decrease in symptom scores in children (aged six to 12 years) with perennial allergic rhinitis treated with oral antihistamine only (levocetirizine).[14] Several studies have shown that mometasone furoate improves the mean total ocular symptom score of patients suffering seasonal allergic rhinitis.[15–17]

TABLE III
NASAL CYTOLOGY SCORES PRE- AND POST-TREATMENT

Score	Pre-Rx	Post-Rx	*p*
Eosinophils	1.17 ± 1.18	0.41 ± 0.61	0.002
Neutrophils	2.05 ± 1.14	1.36 ± 1.20	0.006
Basophilic cells	0.43 ± 0.65	0.25 ± 0.67	0.289
Goblet cells	0.83 ± 0.92	0.17 ± 0.46	0.001
Total	4.48 ± 1.68	2.19 ± 1.79	0.001

Data represent means ± standard deviations unless otherwise specified. Rx = treatment

Normal nasal mucosa consists of epithelial cells, goblet cells and basal cells. There are usually no eosinophils or basophilic cells in the superficial layer. A moderate number of neutrophils and a few bacteria may be seen.

In nasal mucosa affected by allergic rhinitis, eosinophils are encountered at all ages.[4] In Meltzer and colleagues' multicentre study, nasal eosinophilia was present in 64 to 86 per cent of patients enrolled in seasonal allergic rhinitis studies.[18] In another study assessing adult patients with seasonal allergic rhinitis and a nasal cytology grading of at least 1+, eosinophilia was found in 81 per cent of patients, basophilic cells in 42 per cent, neutrophils in 64 per cent and bacteria in 28 per cent.[19]

In our study, a nasal cytology grading of 1+ or more was considered as eosinophilia, and was found in 58.6 per cent of patients. Nasal eosinophilia was reduced to 13.8 per cent following treatment with oral levocetirizine dihydrochloride (5 mg daily) and mometasone furoate monohydrate (200 µg daily). Compared with pre-treatment values, this decrease in nasal eosinophilia was statistically significant ($p < 0.001$).

In a study performed by Lee *et al.*, patients' nasal smear eosinophilia was decreased by 23.47 per cent following cetirizine treatment, by 6.5 per cent following levocetirizine treatment and by 2.85 per cent following placebo administration.[14] In Meltzer and colleagues' multicentre study, fluticasone proprionate treatment (100 µg twice daily) resulted in a 33 per cent reduction in the nasal eosinophilia ratio.[18] Ciprandi *et al.* conducted a double-blind, controlled study of 30 patients with seasonal allergic rhinitis, comparing the efficacy of levocetirizine, desloratadine and placebo treatment.[20] On completion of two weeks' treatment, the levocetirizine group showed a statistically significant

https://doi.org/10.1017/S0022215111001721 Published online by Cambridge University Press

decrease in nasal eosinophilia, while the desloratadine and placebo groups showed no significant decrease. Another study by the same group found a statistically significant decrease in nasal eosinophilia following treatment with mometasone furoate (200 μg daily).[21]

In seasonal allergic rhinitis patients, nasal cytology also reveals a significant increase in neutrophil counts during the pollen season. In the nasal cytological evaluations of both healthy and allergic individuals, a neutrophil predominance (73 per cent ± 10) was present. Nasal cytology specimens from allergic patients reveal significantly more total cells, neutrophils and eosinophils, compared with normal individuals.[22] In a study of an experimental allergic rhinitis model in guinea pigs, nasal lavage cytology showed increased numbers of eosinophils, neutrophils and mononuclear cells.[23] Meltzer *et al.* found baseline nasal neutrophilia in 60–80 per cent of patients with untreated seasonal allergic rhinitis; after two to four weeks' treatment with a topical steroid, the proportion was 50–70 per cent, a statistically significant decrease compared with placebo ($p < 0.05$).[18] Another study observed a highly significant decrease in neutrophils, in seasonal allergic rhinitis patients treated with mometasone furoate.[7] The present study identified nasal neutrophilia in 26 patients (89.7 per cent) before treatment and 16 patients (55.2 per cent) after treatment; this decrease was statistically significant ($p = 0.006$).

- **In allergic rhinitis, both objective and subjective measures are used for diagnosis and evaluation of treatment efficacy**
- **Nasal smear cytology may be a useful additional, objective assessment method for patients with allergic rhinitis, as an adjunct to symptom scoring systems**

Nasal mucosa contains approximately 200–400 basophilic cells per cubic millimetre. Most of these cells are located in the lamina propria. Three types of basophilic cells are present in the nasal mucosa: basophil leukocytes and two different types of mast cell.[4] In patients with allergic rhinitis, basophilic cell distribution on the nasal mucosal surface has been shown to increase within 5 to 24 hours of allergen exposure. This increase has been found to correlate with nasal symptom scores.[24,25] Meltzer *et al.* found a basophilic cell percentage of 29–57 per cent in seasonal allergic rhinitis patients pre-treatment, falling to 8–31 per cent after treatment with various doses of fluticasone proprionate (50–800 μg).[18] In our study, nasal basophilic cells were present in seven patients (24.1 per cent) before treatment and three patients (10.3 per cent) after treatment; this decrease was statistically insignificant ($p = 0.289$).

In an experimental allergic rhinitis model, Nakaya *et al.* have reported goblet cell hyperplasia and submucosal collagen deposition.[26] In contrast, Berger *et al.* reported that the increased mucus secretion observed on the inferior turbinates of patients with perennial allergic rhinitis was dependent upon increased goblet cell functional activity, rather than increased goblet cell numbers.[27] Meltzer *et al.* found no significant difference in nasal goblet cell counts before and after treatment with fluticasone proprionate aqueous nasal spray.[18] In the present study, goblet cells were present in 17 patients (58.6 per cent) before treatment and four patients (13.8 per cent) after treatment; this decrease was statistically significant ($p = 0.001$).

In the present study, the total cytology score was defined as the sum of the eosinophil, basophil, neutrophil and goblet cell scores. Our patients' mean ± SD total cytology score was 4.48 ± 1.68 before treatment and 2.19 ± 1.79 after treatment. There have been no previous reports evaluating these four cell groups together in seasonal allergic rhinitis patients; therefore, we could not compare our total cytology scores with other authors' findings. However, our internal comparison of total cytology scores before versus after treatment revealed a significant decrease ($p < 0.001$). Moreover, there was a significant correlation between the decrease in total cytology scores and the decrease in eosinophil and neutrophil scores ($p < 0.01$). There was no significant correlation between the decrease in total cytology scores and the decrease in basophilic cell and goblet cell scores ($p > 0.05$).

Conclusion

In the present study of patients with seasonal allergic rhinitis, we found a correlation between nasal cytological parameters and nasal and ocular symptoms before and after treatment. On completion of seasonal allergic rhinitis therapy, we observed significant improvements both in nasal and ocular symptoms and in nasal inflammatory cell counts. Symptom scoring and nasal cytological evaluation provided significant information both for diagnosis and for assessment of treatment efficacy.

The collection of nasal smears (for cytologic evaluation) is simple and well tolerated by patients. However, such smears are not commonly used in clinical trials. Nasal cytology may potentially be a useful additional investigation for the standard evaluation and diagnosis of allergic rhinitis. However, studies with larger patient series would be required in order to assess this.

References

1 Stuck BA, Czajkowski J, Hagner AE, Klimek L, Verse T, Hörmann K *et al.* Changes in daytime sleepiness, quality of life, and objective sleep patterns in seasonal allergic rhinitis: a controlled clinical trial. *J Allergy Clin Immunol* 2004;**113**:663–8
2 Leynaert B, Neukirch C, Liard R, Bousquet J, Neukirch F. Quality of life in allergic rhinitis and asthma. A population-based study of young adults. *Am J Respir Crit Care Med* 2000;**162**:1391–6
3 Bauchau V, Durham SR. Prevalence and rate of diagnosis of allergic rhinitis in Europe. *Eur Respir J* 2004;**24**:758–64

https://doi.org/10.1017/S0022215111001721 Published online by Cambridge University Press

4 Howarth PH, Persson CG, Meltzer EO, Jacobson MR, Durham SR, Silkoff PE. Objective monitoring of nasal airway inflammation in rhinitis. *J Allergy Clin Immunol* 2005;**115**:414–41
5 Akerlund A, Andersson M, Leflein J, Lildholdt T, Mygind N. Clinical trial design, nasal allergen challenge models, and considerations of relevance to pediatrics, nasal polyposis, and different classes of medication. *J Allergy Clin Immunol* 2005; **115**:460–82
6 Okuda M. Grading the severity of allergic rhinitis for treatment strategy and drug study purposes. *Curr Allergy Asthma Rep* 2001;**1**:235–41
7 Meltzer EO, Jalowayski AA, Orgel HA, Harris AG. Subjective and objective assessments in patients with seasonal allergic rhinitis: effects of therapy with mometasone furoate nasal spray. *J Allergy Clin Immunol* 1998;**102**:39–49
8 Bousquet J, Van Cauwenberge P, Khaltaev N. Allergic rhinitis and its impact on asthma. *J Allergy Clin Immunol* 2001;**108**: 147–334
9 Gelardi M, Fiorella ML, Russo C, Fiorella R, Ciprandi G. Role of nasal cytology. *Int J Immunopathol Pharmacol* 2010;**23**: 45–9
10 Gelardi M, Russo C, Fiorella ML, Fiorella R, Canonica GW, Passalacqua G. When allergic rhinitis is not only allergic. *Am J Rhinol Allergy* 2009;**23**:312–15
11 Settipane RA. Rhinitis: a dose of epidemiological reality. *Allergy Asthma Proc* 2003;**24**:147–54
12 Bousquet J, Khaltaev N, Cruz AA, Denburg J, Fokkens WJ, Togias A *et al*. Allergic Rhinitis and its Impact on Asthma (ARIA) 2008. *Allergy* 2008;**63**:8–160
13 Potter PC. Levocetirizine is effective for symptom relief including nasal congestion in adolescent and adult (PAR) sensitized to house dust mites. *Allergy* 2003;**58**:893–9
14 Lee CF, Sun HL, Lu KH, Ku MS, Lue KH. The comparison of cetirizine, levocetirizine and placebo for the treatment of childhood perennial allergic rhinitis. *Pediatr Allergy Immunol* 2009; **20**:493–9
15 Bielory L. Ocular symptom reduction in patients with seasonal allergic rhinitis treated with the intranasal corticosteroid mometasone furoate. *Ann Allergy Asthma Immunol* 2008;**100**:272–9
16 Origlieri C, Bielory L. Intranasal corticosteroids and allergic rhinoconjunctivitis. *Curr Opin Allergy Clin Immunol* 2008;**8**: 450–6
17 Anolik R, Pearlman D, Teper A, Gates D. Mometasone furoate improves nasal and ocular symptoms of seasonal allergic rhinitis in adolescents. *Allergy Asthma Proc* 2009;**30**:406–12
18 Meltzer EO, Orgel HA, Rogenes PR, Field EA. Nasal cytology in patients with allergic rhinitis: effects of intranasal fluticasone propionate. *J Allergy Clin Immunol* 1994;**94**:708–15
19 Meltzer EO, Orgel HA, Bronsky EA, Furukawa CT, Grossman J, LaForce CF *et al*. A dose-ranging study of fluticasone propionate aqueous nasal spray for seasonal allergic rhinitis assessed by symptoms, rhinomanometry, and nasal cytology. *J Allergy Clin Immunol* 1990;**86**:221–30
20 Ciprandi G, Cirillo I, Vizzaccaro A, Civardi E, Barberi S, Allen M *et al*. Desloratadine and levocetirizine improve nasal symptoms, airflow, and allergic inflammation in patients with perennial allergic rhinitis: a pilot study. *Int Immunopharmacol* 2005; **5**:1800–8
21 Ciprandi G, Tosca MA, Passalacqua G, Canonica GW. Intranasal mometasone furoate reduces late-phase inflammation after allergen challenge. *Ann Allergy Asthma Immunol* 2001;**86**: 433–8
22 Lim MC, Taylor RM, Naclerio RM. The histology of allergic rhinitis and its comparison to cellular changes in nasal lavage. *Am J Respir Crit Care Med* 1995;**151**:136–44
23 Yamasaki ML, Sasaki K, Mizutani N, Nabe T, Sakura Y, Matsumoto T *et al*. Pharmacological characterization of the leukocyte kinetics after intranasal antigen challenge in a guinea pig model of allergic rhinitis. *Inflamm Res* 2001;**50**:474–82
24 Borres MP, Irander K, Björkstén B. Metachromatic cells in nasal mucosa after allergen challenge. *Allergy* 1990;**45**:98–103
25 Saito H, Howie K, Wattie J, Denburg A, Ellis R, Inman MD *et al*. Allergen-induced murine upper airway inflammation: local and systemic changes in murine experimental allergic rhinitis. *Immunology* 2001;**104**:226–34
26 Nakaya M, Dohi M, Okunishi K, Nakagome K, Tanaka R, Imamura M *et al*. Prolonged allergen challenge in murine nasal allergic rhinitis: nasal airway remodeling and adaptation of nasal airway responsiveness. *Laryngoscope* 2007;**117**:881–5
27 Berger G, Moroz A, Marom Z, Ophir D. Inferior turbinate goblet cell secretion in patients with perennial allergic and nonallergic rhinitis. *Am J Rhinol* 1999;**13**:473–7

Address for correspondence:
Dr Seçil Arslanoğlu,
İnönü Cad No 556/3,
35290 İzmir, Turkey

Fax: +90232 243 15 30
E-mail: secilarslanoglu@hotmail.com

Dr S Arslanoğlu takes responsibility for the integrity of the content of the paper
Competing interests: None declared