

Relationships between allergic inflammation and nasal airflow in children with seasonal allergic rhinitis

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Background: Allergic rhinitis is characterized by a T_H2 -dependent inflammation. Nasal obstruction is a typical symptom of allergic rhinitis.

Objective: To evaluate the possible relationships among nasal symptoms, allergic inflammation, including inflammatory cells and cytokine pattern, and nasal airflow in children with seasonal allergic rhinitis.

Methods: Children with seasonal allergic rhinitis and moderate-severe nasal obstruction were evaluated during the pollen season. Total symptom score, rhinomanometry, nasal lavage, and nasal scraping were evaluated in all patients. Inflammatory cells were counted by conventional staining; interleukin 5 (IL-5) and IL-8 levels were measured by immunoassay on fluids recovered from nasal lavage.

Results: Twenty children (11 boys and 9 girls; mean $\pm$ SD age, 12.9 ± 1.7 years) participated in this study. Eosinophil levels were significantly associated with total symptom score ($r = 90.6\%$, $P < .001$), IL-5 ($r = 94.9\%$, $P < .001$), and nasal flow ($r = -93.6\%$, $P < .001$). No association was elicited with IL-8 ($r = 9.4\%$, $P = .69$). In a multivariate analysis that included eosinophils, neutrophils, and IL-5, eosinophil levels were shown to be the only independent predictor of nasal flow.

Conclusions: This study demonstrates the close connection between T_H2 cytokines and eosinophil infiltration. In addition, there is clear evidence concerning the relationship among nasal symptoms, eosinophil infiltration, and nasal airflow. These findings constitute evidence of the relationship between nasal airflow impairment and eosinophilic inflammation in children with seasonal allergic rhinitis.

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INTRODUCTION

Mucosal infiltration by inflammatory cells, including T cells, mast cells, and eosinophils, characterizes allergic rhinitis.¹ The cytokine pattern is typically characterized by a T_H2 polarization, since increased levels of T_H2 -derived cytokines, including interleukin 3 (IL-3), IL-4, IL-5, IL-13, and granulocyte-macrophage colony-stimulating factor, have been broadly described in allergic rhinitis.² T_H2 -derived cytokines, mainly IL-5, account for recruiting, activating, and prolonging survival of eosinophils in airways. Actually, eosinophil infiltration may be considered a reliable marker of allergic inflammation. On the other hand, IL-8 is associated with typical neutrophil accumulation.³ Previously, neutrophilic infiltration has been described in children with seasonal allergic rhinitis.⁴

Nasal obstruction constitutes a leading symptom in children with allergic rhinitis. Allergic inflammation, mucosal congestion, and mucous hypersecretion cause nasal obstruction.

Allergic inflammation induces a swollen mucosa. Vasodilation causes engorgement of sinusoidal capacitance vessels. Rhinorrhea and increased mucous production contribute to impair nasal airflow. Furthermore, nasal obstruction may be objectively measured by rhinomanometry.

We previously showed relationships among nasal symptoms, allergic inflammation, and airflow impairment in adults with seasonal allergic rhinitis.⁶ The aim of this study was to investigate the possible relationships among nasal symptoms, inflammatory cells, cytokines, and nasal airflow in children with seasonal allergic rhinitis.

MATERIALS AND METHODS

Patients

Children with seasonal allergic rhinitis due to pollen sensitization were prospectively and consecutively evaluated. A detailed clinical history and a complete physical examination were performed for each child. The diagnosis of seasonal allergic rhinitis was made, according to international guidelines,⁷ on the basis of patient history and positive skin prick test results for pollen allergens only. In our geographic area, the most important pollen allergens include grasses and *Parietaria judaica*.⁴ Ten healthy children constituted the control population in this study.

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Study Design

The study was conducted during the pollen season, when patients were symptomatic. In our geographic area, pollen peak occurs during the spring.⁴ Only patients who met all the following criteria were eligible for inclusion in the study: boys and girls younger than 17 years, history of seasonal allergic rhinitis due to pollen allergen exposure for the previous 2 years, sensitization to grasses and/or *Parietaria* only, rhinitis symptoms for the 2 previous weeks, a total symptom score (TSS) of more than 6 at baseline (particularly with moderate-severe nasal obstruction), and informed consent obtained by parents before study entry. Exclusion criteria were the following: sensitization to perennial allergens, diagnosis of asthma, acute and chronic upper respiratory tract infections within 30 days before the study, anatomic nasal disorders (ie, septum deviation), nasal polyps, use of antibiotics, nasal or oral corticosteroids within the previous 4 weeks, and use of antihistamines and oral and nasal decongestants within the previous week. Symptom evaluation, rhinomanometry, nasal scraping, and nasal lavage were performed in all patients. The study was approved by the institutional review board.

Skin Prick Testing

Sensitization was evaluated by performing skin prick testing as stated by the Italian Society of Allergy and Clinical Immunology.⁸ The allergen panel consisted of the following: house dust mites (*Dermatophagoides farinae* and *Dermatophagoides pteronyssinus*), cat, dog, grasses mix, Compositae mix, *Parietaria officinalis*, birch, hazel tree, olive tree, *Alternaria tenuis*, *Cladosporium*, and *Aspergillus* mix; the concentration of allergen extracts was 100 IR/mL (Stallergenes, Milan, Italy).

Nasal Symptoms

The following symptoms were evaluated by the patient, answering the questions posed by the investigator: nasal obstruction, sneezing, rhinorrhea, and itchy nose. Each symptom was evaluated on the following scale: 0, absent; 1, mild (symptom was present but was not annoying or troublesome); 2, moderate (symptom was frequently troublesome but did not interfere with either normal daily activity or sleep); and 3, severe (symptom was sufficiently troublesome to have interfered with normal daily activity or sleep). The TSS, which is the sum of each individual symptom, was also considered.

Table 1. Patient Characteristics*

Characteristic	Seasonal allergic rhinitis patients (n = 20)	Controls (n = 10)
Eosinophils	10.3 (3.2)	0.2 (0.1)
Neutrophils	21.6 (5.4)	1.2 (0.3)
IL-8, pg/mL	662.6 (127.7)	247.8 (44.8)
IL-5, pg/mL	6.4 (1.6)	1.3 (0.6)
Total symptom score	9.4 (1.8)	0.2 (0.1)
Nasal flow, mL/s	358.0 (62.7)	542.8 (79.4)

* Mean (SD) are reported.

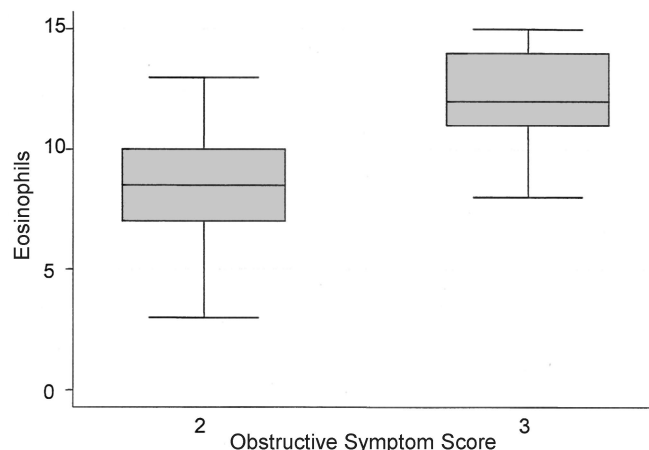


Figure 1. Eosinophil levels according to obstructive symptoms score (t test $P = .001$). Boxes indicate median; error bars, 25th and 75th percentiles.

Rhinomanometry

Nasal airflow was measured by active anterior rhinomanometry. Children wore a tight-fitting facemask and, with the mouth closed, breathed through one nostril. A sensor, placed in the contralateral nostril, recorded data on prenasal and postnasal pressures via airflow and pressure transducers. The instrument (ZAN 100 Rhino; ZAN, Messgeraete GmbH, Germany) was connected to a personal computer. The signals of transnasal airflow and pressure were amplified, digitized, and saved for statistical analysis. Nasal airflow was reported as the sum of recorded airflow through the right and left nostrils in milliliters per second at a pressure difference of 150 Pa across the nasal passage. Four or more airflow measurements were performed for each patient, and the mean was recorded when reproducible values were achieved.

Nasal Cytologic Analysis

Nasal cytologic specimens were obtained by scraping the head of the inferior turbinate with a cotton swab, as described in previous reports.^{4,6} Briefly, after the nasal scraping, the cotton tip of the swab was immersed in a plastic tray with phosphate-buffered saline and transferred to a 10-mL polypropylene tube. The recovered fluids were centrifuged at 220g per minute for 10 minutes, and each pellet was resuspended in phosphate-buffered saline (2 mL). Cell suspensions were filtered to reduce the quantity of mucus, and cytospin slides were prepared by using standard techniques. Smears were stained with Diff-Quik stain and were analyzed by an optic microscope (Olympus U-SPT; Olympus Italy, Rome, Italy). The number of inflammatory cells was expressed as a mean of 10 optical fields at $\times 100$ magnification. Samples were examined in a blinded fashion.

Nasal Lavage

A lavage of the nasal cavity was performed using 5 mL of physiologic saline, according to standard methods described elsewhere.^{4,6} This was performed with the patient's head bent

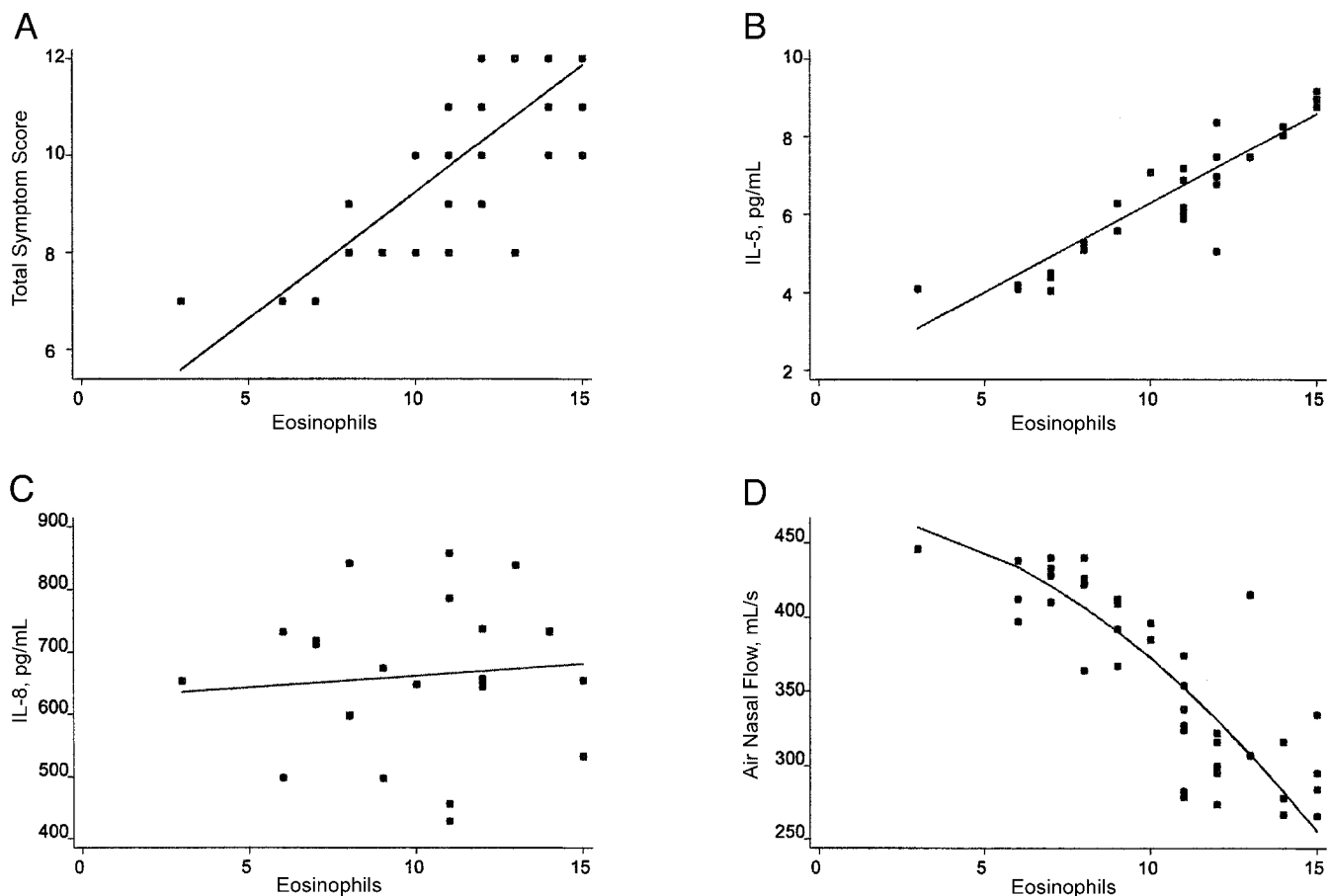


Figure 2. Association of eosinophil levels with total symptom score (A), interleukin 5 (IL-5) (B), IL-8 (C), and air nasal flow (D). Continuous lines represent linear fit. Correlation coefficients and *P* values are reported in the text.

backward during closure of the soft palate. The recovered fluid was comparable in all groups; it was collected to evaluate cytokine levels and stored at -20° .^{4,6}

Cytokine Evaluation

The cytokine levels were measured with the enzyme-linked immunosorbent assay (ELISA) method (R&D Systems Inc, Minneapolis, MN). Study samples and standard dilutions of each specific cytokine were assayed separately in duplicate according to the ELISA kit manufacturer's instructions. Results were the means of duplicate samples. The interassay and intra-assay coefficients of reproducibility for each cytokine were 2% to 4% and 2% to 7% for IL-5 and 4% to 8% and 1% to 4% for IL-8, respectively. The sensitivity of the commercially prepared ELISA was less than 1 pg/mL for IL-5 and less than 10 pg/mL for IL-8.

Statistical Analysis

Descriptive statistics were computed as mean $\pm$ SD for continuous variables and as absolute and relative frequencies for categorical variables. Comparison of eosinophil levels between symptom scores was performed by the *t* test,

whereas eosinophil levels were related to continuous variables by linear regression. In this case, model adequacy was verified by residual analysis. A multivariate linear model was fitted to evaluate the independent association of eosinophil levels and nasal flow, while controlling for other patients characteristics, after checking for collinearity. The choice of collinear variable was performed based on pathophysiologic considerations. Stata 8 statistical software (StataCorp, College Station, TX) was used for computation. A 2-sided *P* < .05 was considered statistically significant.

RESULTS

Twenty children (11 boys and 9 girls; mean $\pm$ SD age, 12.9 ± 1.7 years) participated in this study. The recruited children completed all study procedures, and all samples were included in the statistical analysis. Patients characteristics are reported in Table 1, together with data on a cohort of 10 healthy children for comparison. Obstructive symptoms were rated as 2 in 10 patients and as 3 in the other 10 patients.

Eosinophil levels were significantly higher in patients with an obstructive symptoms score of 3 (12.3 ± 2.2 vs 8.3 ± 2.8 ,

$P = .002$) (Fig 1). Furthermore, eosinophil levels were significantly associated with TSS ($r = 90.6\%$, $P < .001$), IL-5 ($r = 94.9\%$, $P < .001$), and nasal flow ($r = -93.6\%$, $P < .001$), where the association was better described by the introduction of the quadratic term. No association was elicited with IL-8 ($r = 9.4\%$, $P = .69$; Fig 2A–D). In a multivariate analysis, including eosinophils, neutrophils, and IL-5, eosinophil levels were shown to be the only independent predictor of nasal flow through a quadratic term ($P = .04$), with flow decreasing quadratically for increasing eosinophil levels, without any contribution of neutrophils ($P = .77$) or IL-8 ($P = .37$). Model-explained variation R^2 was 91%. Obstructive symptoms, TSS, and IL-5 were not included in the model due to multiple collinearity.

DISCUSSION

Allergic rhinitis is characterized by a T_H2 -polarized immune response. T_H2 -derived cytokines have been previously investigated in patients with seasonal allergic rhinitis, particularly IL-5, which is critical for eosinophil accumulation in inflamed tissues.^{9,10} On the other hand, neutrophils are recruited early after allergen exposure following chemotactic signals exerted by IL-8.¹¹

Allergic inflammation accounts for the occurrence of nasal symptoms, especially nasal obstruction. Nasal obstruction constitutes a crucial symptom, because it may affect quality of life, bronchial hyperreactivity, ostiomeatal complex, and eustachian tube patency, thus allowing the occurrence of several sequelae, including rhinosinusitis, otitis media, and asthma.¹² The pathogenesis of nasal obstruction is complex and consists of 3 main events: inflammatory mucosal edema, vascular congestion, and mucus hypersecretion.¹²

Activated inflammatory cells release several mediators, including histamine and leukotrienes, and T_H2 -derived cytokines that cause the occurrence of nasal obstruction. In this regard, nasal obstruction constitutes a symptom particularly representative of allergic inflammation. In this study, we demonstrated that there is a significant positive relationship between eosinophils and IL-5 in children with seasonal allergic rhinitis, confirming previous findings in adults.⁶ This aspect highlights the pathogenetic role exerted by eosinophil accumulation and T_H2 -derived cytokine polarization in allergic rhinitis.

The most important result of the present study is the evidence concerning the positive relationship among eosinophil infiltration, cytokine concentrations, and nasal obstruction considered both as symptom and airflow. This issue underlines the close connection between allergic inflammation and nasal airflow impairment in children with nasal obstruction due to seasonal allergic rhinitis. In other words, a clear structure-function relationship is detectable at nasal level during allergic inflammation. In addition, eosinophil levels were shown to be the only independent predictor of nasal flow. This issue confirms the main pathogenetic role played by eosinophils in nasal tissues. Furthermore, eosinophils are associated with symptom severity as evidenced by the relationship between eosinophilic infiltration and TSS.

In conclusion, the present study provides the first evidence to our knowledge of the relationships among nasal obstruction symptom, eosinophil infiltration, T_H2 -derived cytokines, and nasal airflow in children with seasonal allergic rhinitis. Therefore, we maintain that, taken together, these data clearly demonstrate the association of T_H2 -dependent inflammation with nasal obstruction. Further studies should address this issue by investigating the effects of treatments.

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