

Short communication

Relationships between allergic inflammation and nasal airflow in children with persistent allergic rhinitis due to mite sensitization

Background: Allergic rhinitis is associated with Th2-dependent inflammation. Nasal obstruction is the most typical symptom in children with mite allergy.

Objectives: The aim of this study was to evaluate the possible relationships among nasal symptoms, allergic inflammation, including inflammatory cells and cytokine pattern, and nasal airflow in children with persistent allergic rhinitis because of mite sensitization.

Methods: Twenty children (13 males and seven females, mean age 13.4 ± 1.6 years) with persistent rhinitis because of mite allergy were evaluated. All of them had moderate–severe grade of nasal obstruction. Total symptom score (TSS), rhinomanometry, nasal lavage, and nasal scraping were obtained in all subjects. Inflammatory cells were counted by conventional staining; interleukin (IL)-5, and IL-8 were measured by immunoassay on fluids recovered from nasal lavage.

Results: Eosinophils were significantly associated with TSS ($R = 74.4\%$, $P = 0.0002$), with IL-5 ($R = 90.6\%$, $P < 0.0001$) and with nasal flow ($R = -69\%$, $P = 0.0007$), but not with IL-8 ($R = 0.1\%$, $P = 0.995$). Eosinophil levels were shown to independently predict nasal flow ($P < 0.001$), with flow decreasing linearly for increasing eosinophils, together with a significant effect of neutrophils ($P = 0.016$, linear increase in flow) and a borderline effect of IL-8 ($P = 0.063$, linear increase in flow).

Conclusions: This study demonstrates the close association between IL-5 concentration and eosinophil infiltration. In addition, there is clear evidence concerning the relationship between eosinophil infiltration and nasal airflow. Thus, nasal eosinophils can be regarded as the most important predictor of upper airway function. These findings constitute first evidence of the relationship between nasal airflow impairment and Th2-related eosinophilic inflammation in children with persistent allergic rhinitis because of mite sensitization.

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Allergic rhinitis is characterized by an inflammatory response as experimental evidence supports a direct relationship between the immunoglobulin E-mediated reaction and the resulting inflammation (1).

The immunopathological feature of allergic rhinitis is based on mucosal infiltration by inflammatory cells, including T cells, mast cells, and eosinophils (2). The cytokine pattern is typically characterized by a Th2 polarization, as increased levels of Th2-derived cytokines, including interleukin (IL)-3, IL-4, IL-5, IL-13, and GM-CSF, have been broadly described in allergic rhinitis (2). Th2-derived cytokines, mainly concerning IL-5, account for recruiting and activating eosinophils in airways. Actually, eosinophil infiltration may be considered as a reliable marker of allergic inflammation. On the contrary, IL-8 is associated with typical neutrophil accumulation (3).

Allergic rhinitis and its impact on asthma (ARIA) document considers a new rhinitis classification: persist-

ent rhinitis is particularly suitable in childhood, as mite sensitization is dominant in this age (4). Nasal obstruction is the leading symptom in children with persistent allergic rhinitis (5). Allergic inflammation, mucosal congestion, and mucus hypersecretion contribute to cause nasal obstruction (6). Allergic inflammation induces a swollen mucosa. Vasodilation causes engorgement of sinusoidal capacitance vessels. Rhinorrhea and increased mucus production contribute to impair nasal airflow.

Nasal obstruction may be objectively measured by rhinomanometry.

We previously showed relationships among nasal symptoms, allergic inflammation, and airflow impairment in adult subjects suffering from allergic rhinitis because of pollen sensitization (7).

The aim of this study was to investigate the possible relationships among nasal symptoms, inflammatory cells, cytokines, and nasal airflow in children with persistent allergic rhinitis because of mite sensitization.

Material and methods

Patients

Twenty children with persistent allergic rhinitis because of mite sensitization were prospectively and consecutively evaluated: 13 males and seven females (mean age 13.4 ± 1.6 years). A detailed clinical history and a complete physical examination were carried out for each child.

The diagnosis of persistent allergic rhinitis was made, according to ARIA guidelines (4), on the basis of history and skin prick test positivity for mite allergens (i.e. *Dermatophagoides pteronyssinus* and *D. farinae*) only.

Ten normal children were considered as control population in this study.

Study design

The study was conducted during the autumn–winter season, when patients were symptomatic.

Only patients who met all the following criteria were eligible for inclusion into the study: men and females aged < 17 years, history of persistent allergic rhinitis because of mite allergen exposure for the previous 2 years, monosensitization to house dust mites, rhinitis symptoms for the two previous weeks, total symptom score (TSS) minimum value > 6 at baseline, particularly with nasal obstruction moderate–severe (see below), and an informed consent obtained by parents prior to study entry. Exclusion criteria were: sensitization to other allergens, diagnosis of asthma, acute and chronic upper respiratory infections within 30 days before the study, anatomic nasal disorders (i.e. septum deviation), nasal polyps, use of antibiotics, nasal or oral corticosteroids within the previous 4 weeks, and use of antihistamines within the previous week.

Symptom assessment, rhinomanometry, nasal scraping, and nasal lavage were performed in all subjects.

The study was approved by the Institutional Review Board.

Skin prick test

Sensitization was assessed by performing skin prick test. It was performed 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 *D. pteronyssinus*), cat, dog, grasses mix, *Compositae* mix, *Parietaria officinalis*, birch, hazel tree, olive tree, *Alternaria tenuis*, *Cladosporium*, and *Aspergilli* mix; the concentration of allergen extracts was 100 I.R./mL (Stallergenes, Milan, Italy).

Nasal symptoms

The following symptoms were assessed by the patient, answering the questions made 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). Total symptom score being the sum of each individual symptom was considered.

Rhinomanometry

Nasal airflow was measured by active anterior rhinomanometry. Patients wore a tight-fitting facemask, and with the mouth closed,

breathed through one nostril. A sensor, placed in the contralateral nostril, recorded data on pre- 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 right and left nostrils in mm/s 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 cytology

Nasal cytologic specimens were obtained by scraping the head of the inferior turbinate with a cotton swab, as described in previous reports (7, 9). Briefly, after the nasal scraping, the cotton tip of the swab was immersed in a plastic tray with phosphate-buffered saline (PBS) and transferred to a 10 ml polypropylene tube. The recovered fluids were centrifuged at 220 g/min for 10 min, and each pellet was re-suspended in PBS (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 optic microscope (Olympus U-SPT, Olympus, Rome, Italy). The number of inflammatory cells was expressed as a mean of 10 optical fields at 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 (7, 9). This was performed with the patient's head bent backwards 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° (7, 9).

Cytokine assessment

The cytokines were measured with enzyme-linked immunosorbent assay (ELISA) method (R & D Systems Inc., Minneapolis, MN, USA). This assay employed the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for each cytokine was precoated onto a microplate. Study samples and standard dilutions of each specific cytokine were assayed separately in duplicate according to the ELISA kit manufacturer's instructions. Standards and samples were pipetted into the wells and each cytokine was bound by the immobilized antibody. After removing unbound substances, an enzyme-linked polyclonal antibody specific for each cytokine was added to the wells. After removing unbound antibody-enzyme reagent, a substrate solution was added to the wells and color developed in proportion to the amount of cytokine bound in the initial step. Then, color development was stopped and the intensity of the color measured. The color intensity was read at a wavelength of 490 nm using a spectrophotometer (Molecular Device Corp., Sunnyvale, CA, USA). The average net optical density of the standards was plotted, and the amount of each cytokine in each sample was evaluated by interpolation from the standard curve. Results were the means of duplicate samples. The inter- and intra-assay coefficients of reproducibility for each cytokine were as follows: IL-5, 2–4, and 2–7%, IL-8, 4–8, and 1–4%. The sensitivity of the commercially prepared ELISA was < 1 pg/ml for IL-5 and < 10 pg/ml for IL-8.

Statistical analysis

Descriptive statistics were computed as mean and standard deviation (SD) for continuous variables and as absolute and relative (%) frequencies for categorical variables. Comparison of eosinophil levels between symptoms scores was performed by means of Student *t*-test, while eosinophil levels were related to continuous variables by means of linear regression. In this case model adequacy was verified by residual analysis. A multivariate linear model was fitted to assess the independent association of eosinophil levels and nasal flow, while controlling for other patients characteristics, after checking for collinearity. The choice between collinear variable was performed based on pathophysiologic considerations. Stata 8 (StataCorp, College Station, TX) was used for computation. A two-sided *P*-value <0.05 was considered statistically significant.

Results

Patients characteristics are reported in Table 1, together with data on a cohort of normal children for comparison. Obstructive symptoms were rated as 2 (moderate) in nine patients and as 3 (severe) in 11 patients.

Eosinophil levels were significantly higher in patients with obstructive symptoms score 3 (12.1 (SD 2.0) vs. 8.3 (SD 2.2), (*P* = 0.001) (Fig. 1). Moreover eosinophils were significantly associated with TSS (*R* = 74.4%,

Table 1. PAR children characteristics

Characteristic	PAR patients* (<i>n</i> = 20)	Controls* (<i>n</i> = 10)
Eosinophils	10.4 (2.8)	0.2 (0.1)
Neutrophils	21.0 (5.5)	1.2 (0.3)
IL-8 pg/ml	655.6 (124.4)	247.8 (44.8)
IL-5 pg/ml	6.3 (1.7)	1.3 (0.6)
Total symptoms score	9.0 (1.6)	0.2 (0.1)
Nasal flow (cm ³ /s)	355.1 (58.3)	542.8 (79.4)

PAR, persistent allergic rhinitis.

*Mean and (SD) are reported.

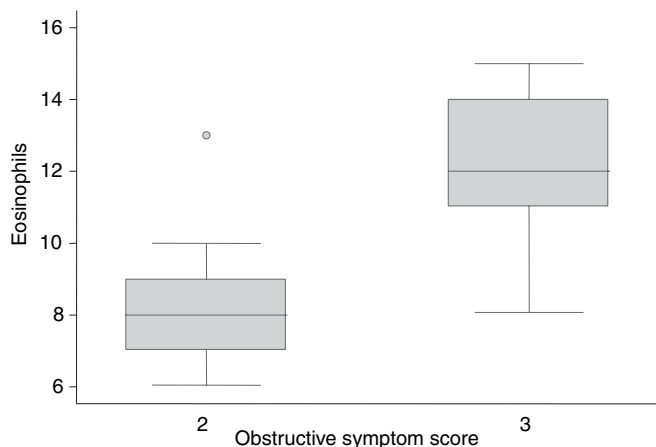


Figure 1. Box and Whisker plot (median, 25th & 75th percentiles and extremes) comparing eosinophil levels according to obstructive symptoms score (Student *t*-test *P* = 0.001).

P = 0.0002), with IL-5 (*R* = 90.6%, *P* < 0.0001) and with nasal flow (*R* = -69%, *P* = 0.0007), but not with IL-8 (*R* = 0.1%, *P* = 0.995) (Fig 2A–D). At a multivariate analysis including eosinophils, neutrophils, and IL-5, eosinophil levels were shown to independently predict nasal flow (*P* < 0.001), with flow decreasing linearly for increasing eosinophils, together with a significant effect of neutrophils (*P* = 0.016, linear increase in flow) and a borderline effect of IL-8 (*P* = 0.063, linear increase in flow). Model explained variation *R*² was high and was computed to 67%. Obstructive symptoms, TSS and IL-5 were not included in the model because of multiple collinearity.

Discussion

The present study aimed at investigating possible relationships among nasal symptoms, inflammatory cells, cytokines collected from nasal lavage, and nasal airflow in children with persistent allergic rhinitis due to mite sensitization.

Nasal obstruction is the leading symptom in children with persistent allergic rhinitis due to mite sensitization.

The pathogenesis of nasal obstruction is complex and consists of three main events: inflammatory mucosal edema, vascular congestion, and mucus hypersecretion (10). In this regard, airway obstruction constitutes the symptom more representative of allergic inflammation. Allergic inflammation is characterized by a Th2-polarization, as Th2-derived cytokines are significantly increased in patients with allergic rhinitis.

On the basis of these issues, the present study suggests some considerations concerning the link between allergic inflammation and nasal airflow impairment.

First, there is a significant positive relationship between nasal eosinophil infiltration and IL-5 levels, confirming previous findings (11). This aspect highlights the pathogenetic role exerted by Th2-cytokines, such as IL-5, in accumulating eosinophils.

Secondly, this study supplies evidence concerning the linear correlation between eosinophils and airflow obstruction as the more the eosinophils infiltrating nasal mucosa the more nasal airflow impairment occurs.

Thirdly, there is correlation among symptom score, eosinophil infiltration grade, IL-5 concentrations, and nasal airflow impairment.

All these aspects underline the close association regarding the relationship between the structure and the function of the nose as highlighted by ARIA document and confirmed by Demoly et al. (12). In other words, as nasal mucosa is more inflamed as nasal airflow is more impaired.

Results by multivariate regression clearly indicate a strong relationship of upper airway function with nasal eosinophilic inflammation. Therefore, nasal eosinophils may be considered as important predictor of airway function.

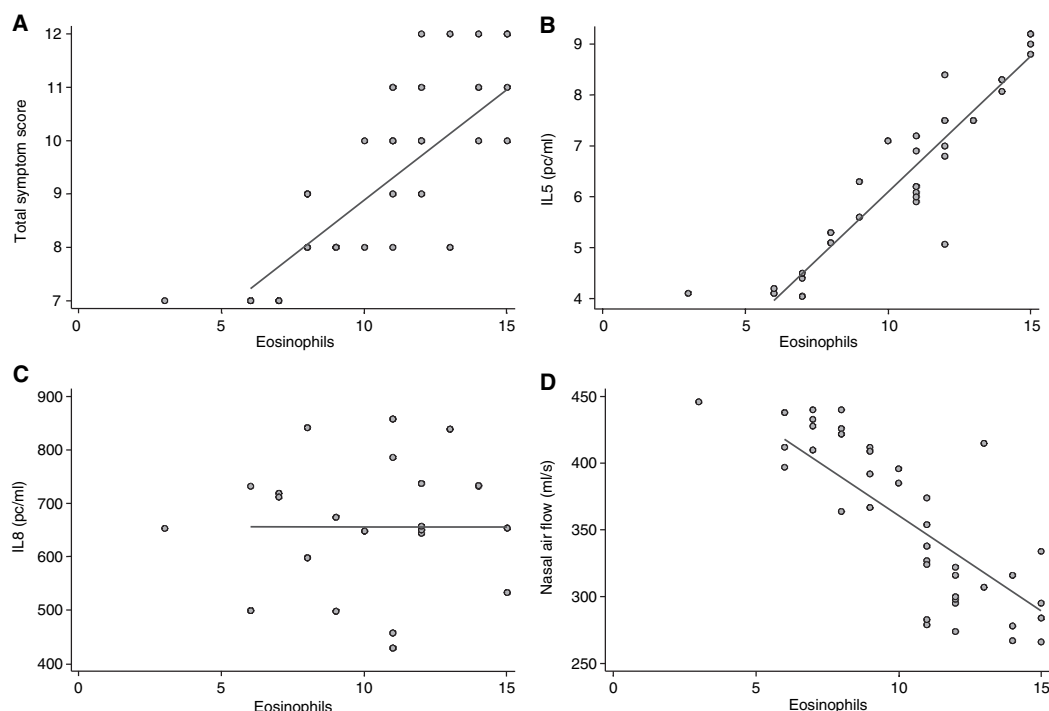


Figure 2. Scatter plots showing the association of eosinophil levels with total symptom score (A), interleukin (IL)-5 (B), IL8 (C) and nasal flow (D). Continuous lines represent linear fit. Correlation coefficients and *P*-values are reported in text.

This issue probably represents the most important outcome of the present study as it documents the close association between allergic inflammation and nasal obstruction.

Furthermore, present findings have to be added to our previous survey in allergic rhinitis. Very recently, we provided evidence, in a group of 50 patients with seasonal allergic rhinitis, of the relationships among nasal obstruction, eosinophil infiltration, Th2-derived

cytokines, and nasal airflow in patients with seasonal allergic rhinitis (7).

In conclusion, the present study provides first evidence of the relationships among nasal symptoms, eosinophil infiltration, IL-5 levels, and nasal airflow impairment in children with persistent allergic rhinitis because of mite sensitization. Therefore, we retain that these data may be considered convincing proof of the close link existing between allergic rhinitis and nasal airflow.

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