

# Nasal Resistance and Allergic Inflammation Depend on Allergen Type

Matteo Gelardi<sup>a</sup> Alessandro Maselli Del Giudice<sup>a</sup> Teresa Candreva<sup>a</sup>  
Maria Luisa Fiorella<sup>a</sup> Michaela Allen<sup>b</sup> Catherine Klersy<sup>b</sup> Gian Luigi Marseglia<sup>b</sup>  
Giorgio Ciprandi<sup>c</sup>

<sup>a</sup>Dipartimento di Oculistica e Otorinolaringoiatria, Università di Bari, Bari, <sup>b</sup>Clinica Pediatrica e Servizio di Biometria ed Epidemiologia Clinica, Università di Pavia, IRCCS Policlinico San Matteo, Pavia, and

<sup>c</sup>Dipartimento Patologie Testa Collo, Azienda Ospedale-Università San Martino, Genoa, Italy

## Key Words

Allergen sensitization • Allergic rhinitis classifications • Nasal airflow resistance • Nasal cytology

## Abstract

**Background:** Allergic rhinitis is characterized by inflammatory cell infiltrate and increased nasal airflow resistance. **Objective:** The aim of this study was to evaluate the relationship between sensitization type, inflammatory cell pattern, and nasal airflow resistance in a group of rhinitics with monosensitization. **Methods:** Seventy-seven subjects (40 males and 37 females, with a mean age of  $33 \pm 4.4$  years) suffering from allergic rhinitis were studied. Skin prick test, nasal cytology and electronic rhinomanometry were assessed in all subjects. **Results:** The number of subjects monosensitized to house dust mites was 23, to grasses 20, to cypress 17, to *Parietaria* 11, and to olive tree 6. Significant differences were observed between each type of allergen sensitization concerning both the nasal airflow resistance ( $p = 0.002$ ) and the nasal cytology pattern: eosinophils ( $p = 0.004$ ), degranulated eosinophils ( $p = 0.002$ ), mast cells ( $p = 0.006$ ) and degranulated mast cells ( $p = 0.008$ ). Furthermore, goblet cells were higher in house dust mite-sensitized subjects compared with the pollen-sensitized group ( $p = 0.018$ ), in which a prevalence of eosinophils, degranulated eosinophils, mast cells and degranulated mast cells was observed

( $p = 0.049$ ,  $p < 0.001$ ,  $p = 0.022$  and  $p = 0.007$ , respectively). Nasal resistances were higher in the pollen group ( $p = 0.001$ ).

**Conclusions:** This study provides evidence that inflammatory cell pattern and nasal resistance depend on the type of allergen sensitization.

Copyright © 2006 S. Karger AG, Basel

## Introduction

Allergic rhinitis is the most frequent immunoglobulin-E-mediated disease. Its prevalence is high in the general population and still on the increase [1–3].

Allergic rhinitis is classically subdivided into seasonal (SAR) and perennial allergic rhinitis (PAR), depending on the type of allergen and the occurrence of symptoms during the year [4]. SAR is caused by outdoor allergens, mainly pollens, whereas PAR is due to indoor allergens, including house dust mites (HDM), pets and cockroaches. This classification has recently been revised by the ARIA (Allergic Rhinitis and its Impact on Asthma) workshop [5]. The new classification into ‘intermittent’ and ‘persistent’ forms does not consider the type of allergen, but rather the duration of symptoms (days/week and consecutive weeks) and the impact on life quality. On the basis of these considerations, we recently performed a study on a large population of subjects who perceived isolated nasal symptoms

in order to evaluate the clinical and functional aspects in relation to the type of allergic rhinitis and the causal allergen(s) [6]. The large majority of subjects (80%) had a mixed allergic rhinitis, being sensitized both to seasonal and perennial allergens [6]. Moreover, a seasonal variability of the symptom score was detectable both for SAR and PAR patients (SAR patients were more symptomatic during the spring-summer season, while PAR patients worsen during autumn-winter), whereas mixed allergic rhinitis patients did not show seasonal variations [6].

It is well known that allergen exposure activates mast cells with a consequent release of preformed mediators, mainly histamine and leukotrienes, and cytokines capable of inducing the recruitment and activation of inflammatory cells, including eosinophils, neutrophils and Th2 lymphocytes [7]. These inflammatory events lead to the onset of typical nasal symptoms, including itching, sneezing, rhinorrhea and obstruction. Recently, the relationship between nasal obstruction, Th2-dependent inflammation and nasal airflow limitation was evidenced both in adults and children suffering from allergic rhinitis [8–10].

Thus, the primary purpose of this study was to verify whether patients with allergic rhinitis monosensitized either to HDM or pollen had a different nasal cytology pattern and nasal airflow resistance. Other endpoints were to assess the association between nasal cytology and nasal resistances and to describe the relationship between both the nasal cytology and the nasal resistances with the single allergens involved.

## Methods

### Study Design

The study was conducted during the pollen season, corresponding to spring in our region, when patients were symptomatic [6, 8–10]. We included subjects with allergic rhinitis among patients seen at the ENT Clinic of Bari (Southern Italy). In this observational study, we enrolled only those subjects reporting symptoms of rhinitis with monosensitization to one pollen or HDM. Subjects with acute upper respiratory infections, anatomic nasal defects (i.e. septum deviation), nasal polyps, asthma and/or bronchial hyperreactivity, and using nasal or oral corticosteroids, nasal or oral decongestants, and antihistamines during the previous 4 weeks were excluded.

The diagnosis of allergic rhinitis was made on the basis of a history of nasal symptoms and positive skin prick test according to validated criteria [4, 5].

On the basis of the sensitizing allergen, we subdivided the patients further into two groups: pollen-sensitized and HDM-sensitized subjects.

Skin prick tests, nasal endoscopy and rhinomanometry were performed in all subjects. The study was approved by the Institu-

tional Review Board of the University of Bari, and an informed consent was obtained from all patients.

### Skin Prick Test

Allergy was assessed by the presence of sensitization to the most common classes of aeroallergens by performing a skin prick test. It was performed as stated by the European Society of Allergy and Clinical Immunology: sensitization was considered when the wheal diameter was equal or greater than 3 mm [11].

The allergen panel consisted of the following: house dust mites (*Dermatophagoides farinae* and *Dermatophagoides pteronyssinus*), cat, dog, grass mix, *Compositae* mix, *Parietaria judaica*, birch, hazel tree, olive tree, *Alternaria tenuis*, *Cladosporium*, and *Aspergilli* mix; the concentration of allergen extracts was 100 IR/ml (Stallergenes, Milan, Italy).

### Nasal Cytology

Cytological samples were obtained by scraping with a Rhino-Probe™. The samples were collected from the medial portion of the inferior middle turbinate. After fixing with absolute alcohol for 3 min and drying, the samples were stained using the May-Grünwald-Giemsa (Carlo Erba, Milan, Italy) method, then mounted on covered slides and examined under a microscope (Nikon E600, Nikon, Italy). Cell count was performed on 10 microscopic samples at high-power magnification ( $\times 1,000$ ) in immersion [12]. Samples were examined blindly by 2 different readers.

### Rhinomanometry

Nasal airflow resistance was measured by active anterior electronic rhinomanometry. Patients wore a tight-fitting facemask and breathed through one nostril with their mouth closed. A sensor, placed in the contralateral nostril, recorded data on pre- and postnasal pressures via airflow and pressure transducers. The instrument (Rhinomanometer Menfis, Amplifon, Italy) was connected to a personal computer. The signals of transnasal airflow and pressure were amplified, digitalized and saved for statistical analysis.

Nasal resistance was measured in milliliters per second as the sum of the recorded airflow through the right and left nostrils at a pressure difference of 150 Pa across the nasal passage.

Four or more airflow measurements were performed for each patient, and the mean value was recorded when reproducible values were achieved. Normal values are  $<0.50$  Pa/ml/s [13].

### Statistical Analysis

Data were described as medians and 25th–75th percentiles if continuous, and as counts and percentages if categorical. For the purpose of the analysis, cell levels were dichotomized according to the median of their distribution. Continuous variables and categorical variables were compared between HDM and pollen groups by means of the Mann-Whitney U test and the Fisher exact test, respectively. The association of the cytologic pattern (dichotomized) and baseline nasal resistances (log transformed) was assessed by means of a general linear regression model, while controlling for the presence of seasonal or perennial allergy. Finally, cell concentrations were compared between the single allergens by means of the Kruskal-Wallis test (nonparametric analysis of variance) and the Mann-Whitney U test for post hoc comparisons. Due to the explorative nature of this analysis, no correction

**Table 1.** Comparison of the cytologic pattern in patients with pollen and HDM sensitization

| Variables                | Pollen     | HDM        | p value <sup>1</sup> |
|--------------------------|------------|------------|----------------------|
| Goblet cells             | 30 [20–60] | 60 [35–70] | 0.018                |
| >40                      | 18 (33)    | 17 (71)    | 0.003                |
| Neutrophils              | 75 [50–75] | 75 [75–90] | 0.244                |
| >75                      | 12 (22)    | 9 (37)     | 0.177                |
| Eosinophils              | 75 [50–75] | 50 [50–75] | 0.049                |
| >75                      | 7 (13)     | 0 (0)      | 0.093                |
| Degranulated eosinophils | 50 [30–80] | 10 [5–40]  | 0.000                |
| >50                      | 25 (46)    | 5 (21)     | 0.044                |
| Mast cells               | 25 [0–50]  | 0 [0–50]   | 0.022                |
| >25                      | 26 (48)    | 9 (37)     | 0.463                |
| Degranulated mast cells  | 10 [0–60]  | 0 [0–10]   | 0.007                |
| >10                      | 24 (44)    | 6 (25)     | 0.133                |
| Lymphocytes              | 50 [25–75] | 50 [30–75] | 0.964                |
| >50                      | 19 (35)    | 7 (29)     | 0.795                |
| Activated lymphocytes    | 10 [0–25]  | 0 [0–30]   | 0.407                |
| >0                       | 28 (52)    | 9 (37)     | 0.327                |
| Plasma cells             | 0 [0–0]    | 0 [0–0]    | 0.112                |
| >0                       | 12 (22)    | 2 (8)      | 0.205                |

Cell populations are described and analyzed both on a continuous scale and after dichotomization according to their median values. Values indicate medians with 25th–75th percentiles in brackets for continuous variables, and counts with percentages in parentheses for categorical variables.

<sup>1</sup> The Mann-Whitney U test was used to compare median values and Fisher's exact test to compare counts.

for multiple tests was applied. Stata 8 (StataCorp, College Station, Tex., USA) was used for computation. A 2-sided p value <0.05 was considered statistically significant.

## Results

### Patients

Seventy-seven monosensitized symptomatic subjects with allergic rhinitis were prospectively and consecutively evaluated during the pollen season: 40 males and 37 females, with a mean age of  $33 \pm 4.4$  years. All patients were monosensitized. They were grouped according to the presence of pollen or HDM sensitization for the analysis of the primary endpoint of the study.

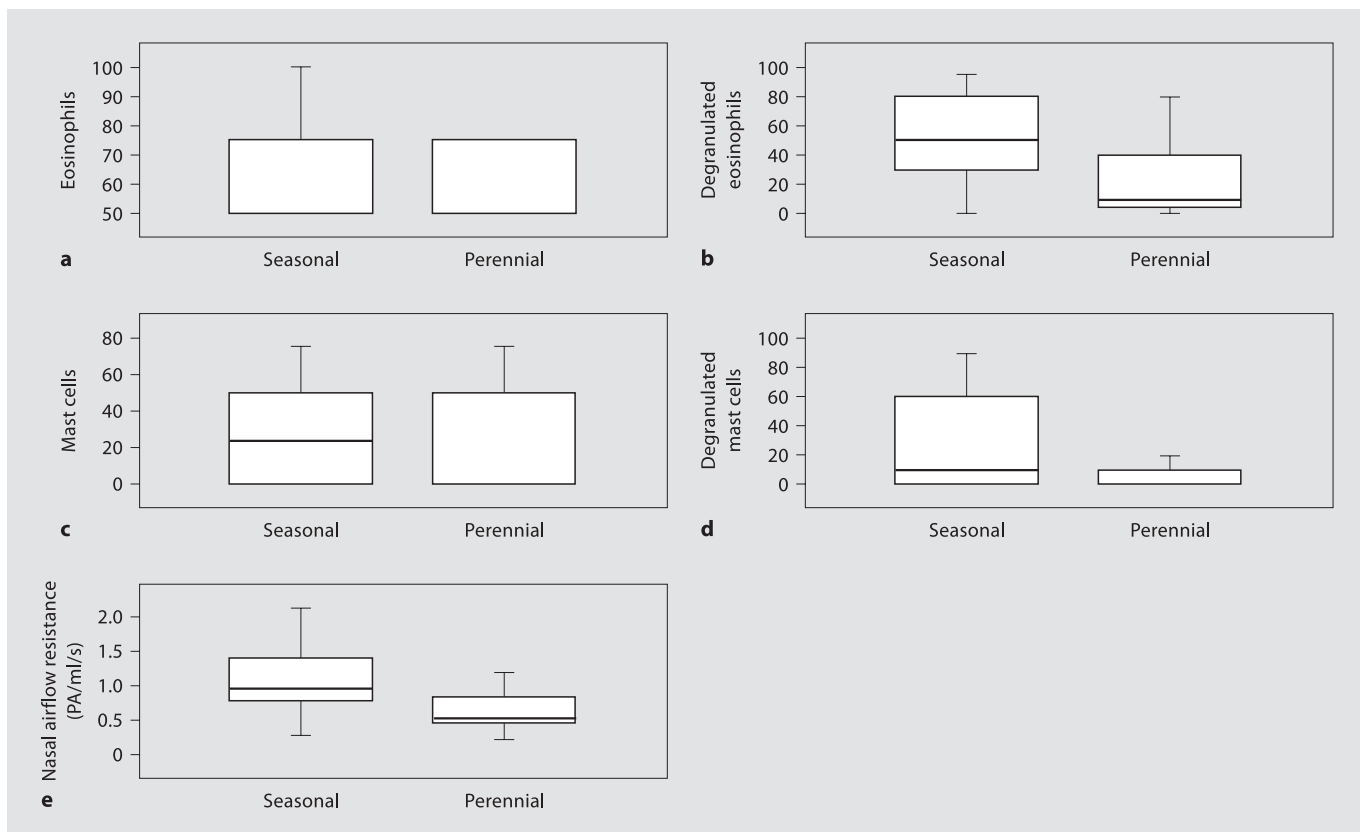
### Association of Pollen or HDM Sensitization and Nasal Cytology or Nasal Airflow Resistance

Patients with pollen sensitization (n = 54) were monosensitized to cypress, grasses, olive tree or *P. judaica*. Patients with HDM sensitization (n = 23) were monosensitized to *Dermatophagoides* only.

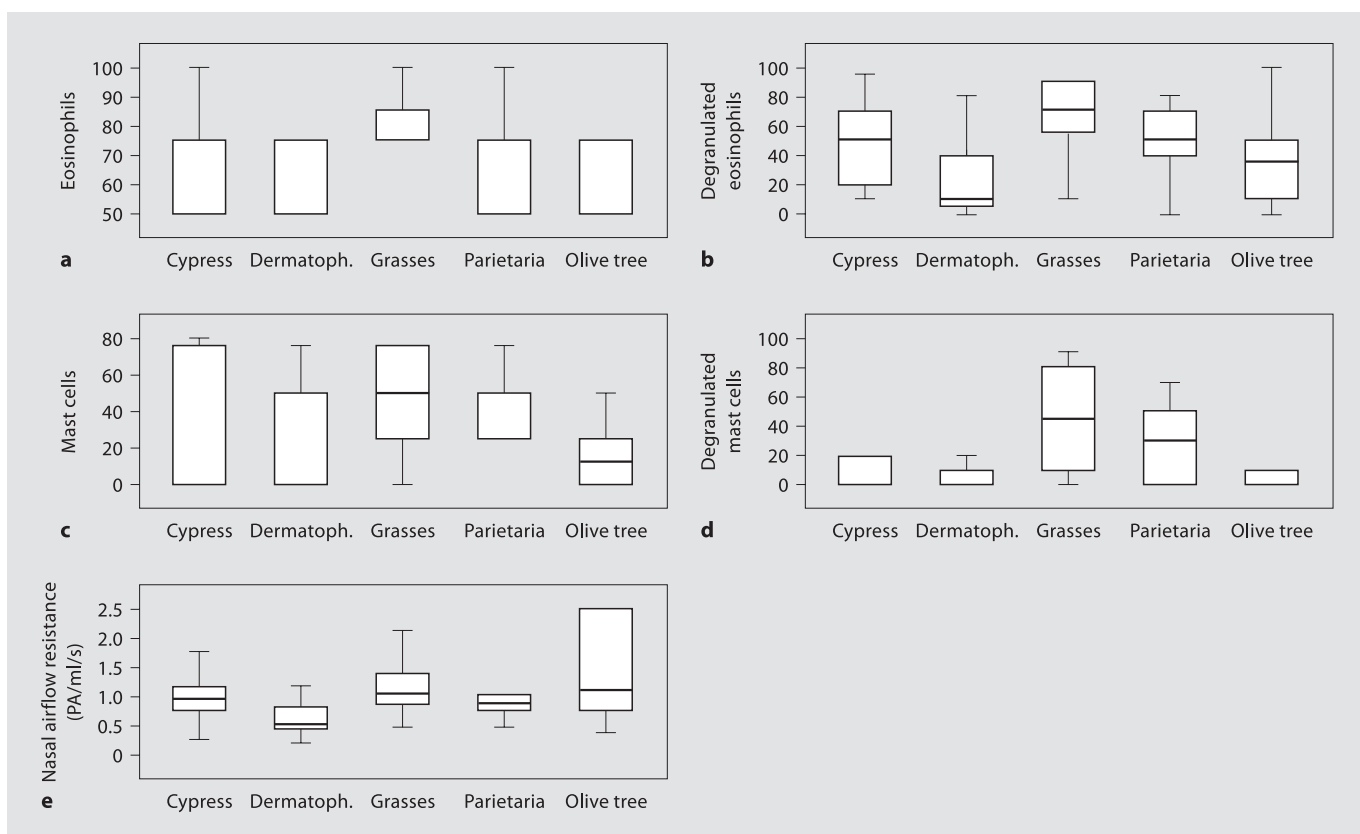
Cell concentrations in the pollen group and the HDM group are summarized in table 1, both on a continuous scale and as proportions of patients with increased concentrations (above the median value). Goblet cell concentration was significantly higher in the HDM group (p = 0.018), with 71% of patients with increased values in the HDM group, as compared with 33% in the pollen group (p = 0.003), whereas eosinophil, degranulated eosinophil, mast cell and degranulated mast cell levels were significantly higher in the pollen group (p = 0.049, p < 0.001, p = 0.022 and 0.007, respectively; fig. 1).

**Fig. 1.** Cell levels according to pollen/HDM sensitization for eosinophils (a), degranulated eosinophils (b), mast cells (c), degranulated mast cells (d) and nasal airflow resistance (e). In all cases, a significant association between cell levels or resistances and sensitization type was present (see text for details).

**Fig. 2.** Cell levels according to single allergen sensitization for eosinophils (a), degranulated eosinophils (b), mast cells (c), degranulated mast cells (d) and nasal airflow resistance (e). In all cases, a significant association between cell levels or resistances and sensitization type was present (see text for details). Dermatoph. = *Dermatophagoides*.



1



2

**Table 2.** Association of cell population and nasal airflow resistances (Pa/ml/s) while controlling for the presence of pollen or HDM association

| Variables                | Median           | p value <sup>1</sup> |
|--------------------------|------------------|----------------------|
| Goblet cells             |                  | 0.333                |
| ≤ 40                     | 0.90 (0.53–1.16) |                      |
| >40                      | 0.90 (0.58–1.40) |                      |
| Neutrophils              |                  | 0.222                |
| ≤ 75                     | 0.95 (0.74–1.20) |                      |
| >75                      | 0.90 (0.46–1.20) |                      |
| Eosinophils              |                  | 0.675                |
| ≤ 75                     | 0.90 (0.55–1.20) |                      |
| >75                      | 0.98 (0.89–1.35) |                      |
| Degranulated eosinophils |                  | 0.184                |
| ≤ 50                     | 0.87 (0.51–1.08) |                      |
| >50                      | 0.98 (0.80–1.40) |                      |
| Mast cells               |                  | 0.081                |
| ≤ 25                     | 0.80 (0.48–1.20) |                      |
| >25                      | 0.98 (0.80–1.40) |                      |
| Degranulated mast cells  |                  | 0.350                |
| ≤ 10                     | 0.88 (0.49–1.20) |                      |
| >10                      | 0.98 (0.80–1.37) |                      |
| Lymphocytes              |                  | 0.682                |
| ≤ 50                     | 0.90 (0.56–1.28) |                      |
| >50                      | 0.89 (0.58–1.20) |                      |
| Activated lymphocytes    |                  | 0.027                |
| ≤ 0                      | 0.90 (0.74–1.40) |                      |
| >0                       | 0.89 (0.53–1.12) |                      |
| Plasma cells             |                  | 0.916                |
| ≤ 0                      | 0.90 (0.58–0.19) |                      |
| >0                       | 0.98 (0.51–1.40) |                      |

Values indicate medians with 25th–75th percentiles in parentheses.

<sup>1</sup> Analysis adjusted for pollen/HDM sensitization in a general linear regression model.

No other cell population appeared to differ significantly between the groups, although activated lymphocytes and plasma cells were more prevalent in the patients with pollen sensitization.

Finally, nasal resistances were significantly higher in the pollen group ( $p = 0.001$ ), with 33 patients (61%) showing increased values as compared with 4 (17%) in the HDM group ( $p < 0.001$ ; fig. 1).

#### Nasal Cell Population and Nasal Airflow Resistances

The association of cell populations and nasal resistance is shown in table 2. Higher levels of mucipar cells, eosinophils, degranulated eosinophils, mast cells, degranulated mast cells and plasma cells tended to be re-

lated to higher baseline resistances (although a borderline nonsignificance was observed for mast cells and a moderate significance was attained for activated lymphocytes only), while the inverse was true for neutrophils.

#### Single Allergen Monosensitization

To describe this population more thoroughly, details on the single allergen and their relationship to cell populations and nasal resistances were collected. Patients were monosensitized to cypress ( $n = 17$ ), grasses ( $n = 20$ ), olive tree ( $n = 6$ ), *P. judaica* ( $n = 11$ ), or *Dermatophagoides* HDM ( $n = 23$ ). The levels of cells were compared between the single allergens in an exploratory analysis, by means of the nonparametric analysis of variance. Significant differences were observed for the following cell populations (fig. 2): eosinophils (Kruskal-Wallis test,  $p = 0.004$ ; fig. 2a), where median concentrations were highest for grasses ( $p = 0.006$  vs. cypress;  $p < 0.001$  vs. *Dermatophagoides*;  $p = 0.012$  vs. olive tree); degranulated eosinophils (Kruskal-Wallis test,  $p = 0.002$ ; fig. 2b), where median concentrations were highest for grasses ( $p = 0.039$  vs. cypress;  $p = 0.017$  vs. *Dermatophagoides*;  $p = 0.006$  vs. olive tree) and lowest for *Dermatophagoides* ( $p = 0.017$  vs. cypress), and differences were also observed between *Parietaria* and olive tree ( $p = 0.046$ ); mast cells (Kruskal-Wallis test,  $p = 0.006$ ; fig. 2c), where differences were observed between grasses and cypress ( $p = 0.031$ ), *Dermatophagoides* ( $p = 0.002$ ) and olive tree ( $p = 0.001$ ), between *Dermatophagoides* and *Parietaria* ( $p = 0.026$ ), and between *Parietaria* and olive tree ( $p = 0.028$ ); degranulated mast cells (Kruskal-Wallis test,  $p < 0.001$ ; fig. 2d), where differences were again observed between grasses and cypress ( $p = 0.001$ ), *Dermatophagoides* ( $p = 0.001$ ) and olive tree ( $p = 0.005$ ), and between *Dermatophagoides* and *Parietaria* ( $p = 0.049$ ).

Nasal resistance values were also significantly different between allergens (Kruskal-Wallis test,  $p = 0.023$ ; fig. 2e); specifically, the lowest values were recorded for *Dermatophagoides*. Differences were observed between *Dermatophagoides* and cypress ( $p = 0.007$ ), grasses ( $p < 0.001$ ) and *Parietaria* ( $p = 0.011$ ).

#### Discussion

Allergic rhinitis is characterized by Th2-dependent inflammation that accounts for the occurring nasal symptoms, mainly nasal obstruction [9, 10].

This study demonstrated that subjects with HDM and pollen monosensitization elicit a significantly different

pattern regarding both the inflammatory cell concentrations and the nasal resistance measures. In particular, pollen-sensitized subjects showed higher levels of inflammatory infiltrate and augmented nasal resistance, while HDM-sensitized patients showed a higher number of goblet cells which might be attributed to chronic allergic inflammation. This finding confirms our previous reports indicating that SAR subjects show a more pronounced inflammation, with higher levels of inflammatory cells and cytokines, and impaired nasal airflow, compared with PAR patients [14, 15]. It is well known that SAR patients are different from PAR patients [16, 17], but no study investigated the relationship between allergic inflammation and nasal airflow resistance. Therefore, SAR and PAR, according to the traditional classification, may be considered as two separate entities as they are characterized by a substantially different inflammatory and functional response. On the other hand, persistent rhinitis has recently been recognized as a different disease in comparison with intermittent rhinitis, at least from a clinical point of view [18].

Another interesting finding of our study is the observation of higher nasal cell concentrations with increased levels of nasal airflow resistance (after controlling for the

confounding effect of pollen/HDM sensitization in a multivariate analysis), although a statistical significance is reached only once.

Finally, we observed different concentrations of eosinophils, degranulated eosinophils, mast cells and degranulated mast cells between the single allergens. The major differences were observed between grasses or *Parietaria* and the other allergens, though due to the sparseness of data, the analysis should be considered exploratory and the conclusions drawn should be considered with caution. However, it is noteworthy that it is not easy to enroll a large number of monosensitized patients [6], and it is well known that each pollen has a particular calendar of pollination [19, 20]. The duration and the intensity of pollen concentration deeply influence the persistence of allergic inflammation [21]. Moreover, each pollen has specific biological properties that are reflected in sensitization prevalence and the severity of symptoms [22]. Therefore, it is clearly conceivable that each allergen may elicit different immunologic and functional responses.

In conclusion, this study provides the first evidence of differing nasal responses when considering the type of allergen sensitization.

## References

- Settipane GA: Allergic rhinitis – update. *Otolaryngol Head Neck Surg* 1986;94:470–475.
- The International Study of Asthma and Allergies in Childhood (ISAAC) Steering Committee: Worldwide variation in prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and atopic eczema: ISSAC. *Lancet* 1998;351:1225–1232.
- Ciprandi G, Vizzaccaro A, Cirillo I, et al: Increase of asthma and allergic rhinitis prevalence in young Italian men. *Int Arch Allergy Immunol* 1996;111:279–283.
- Dykewicz MS, Fineman S: Executive summary of joint task force practice parameters on diagnosis and management of rhinitis. *Ann Allergy Asthma Immunol* 1998;81:463–468.
- Bousquet J, van Cauwenberge P, Khaltaev N, ARIA Workshop Group, World Health Organization: Allergic rhinitis and its impact on asthma: ARIA. *J Allergy Clin Immunol* 2001;108:S1–S315.
- Ciprandi G, Cirillo I, Vizzaccaro A, et al: Seasonal and perennial allergic rhinitis: is this classification adherent to real life? A population-based study. *Allergy* 2005;60:882–887.
- Christodoulopoulos P, Cameron L, Durham S, Hamid Q: Molecular pathology of allergic disease II: upper airway disease. *J Allergy Clin Immunol* 2000;105:211–223.
- Ciprandi G, Cirillo I, Vizzaccaro A, Milanese M, Tosca MA: Nasal obstruction in patients with seasonal allergic rhinitis: relationships between allergic inflammation and nasal airflow. *Int Arch Allergy Immunol* 2004;134:34–40.
- Ciprandi G, Marseglia GL, Klersy C, Tosca MA: Relationships between allergic inflammation and nasal airflow in children with persistent allergic rhinitis due to mite sensitization. *Allergy* 2005;60:957–960.
- Ciprandi G, Cirillo I, Vizzaccaro A, Milanese M, Tosca MA: Airway function and nasal inflammation in seasonal allergic rhinitis and asthma. *Clin Exp Allergy* 2004;34:891–896.
- Dreborg S, EAACI Subcommittee on Skin Tests: Skin tests used in type I allergy testing. Position paper. *Allergy* 1989;44(suppl 10):22–31.
- Gelardi M: Atlas of Nasal Cytology. Torino, Centro Scientifico Editore, 2004.
- Kern EB: Committee report on standardization of rhinomanometry. *Rhinology* 1981;19:231–236.
- Ciprandi G, Cirillo I, Vizzaccaro A, Palestini E, Tosca MA: Decongestion test in patients with allergic rhinitis: functional evaluation of nasal airflow. *Am J Rhinol*, in press.
- Ciprandi G, Cirillo I, Klersy C, Vizzaccaro A, Tosca MA, Marseglia GL: The response to decongestion test is associated with sensitizations and allergic inflammation. *Ann Allergy Asthma Immunol*, in press.
- Wilson SJ, Lau L, Howarth PH: Inflammatory mediators in naturally occurring rhinitis. *Clin Exp Allergy* 1998;28:220–227.
- Foresi A, Leone C, Pilucchi A, et al: Eosinophils, mast cells, and basophils in induced sputum from patients with seasonal allergic rhinitis and perennial asthma: relationship to methacholine responsiveness. *J Allergy Clin Immunol* 1997;100:58–64.
- Bauchau V, Durham SR: Epidemiological characterization of the intermittent and persistent types of allergic rhinitis. *Allergy* 2005;60:350–353.
- Negrini AC, Ariano R, Delbono G, Ebbli A, Quaglia A, Arobba D: Incidence of sensitization to the pollens of Urticaceae, Poaceae and Oleaceae and pollen rain in Liguria (Italy). *Aerobiologia* 1992;8:355–358.
- Negrini AC, Arobba D: Allergic pollens and pollinosis in Italy: recent advances. *Allergy* 1992;47:371–379.
- Ricca V, Landi M, Ferrero P, Bairo A, Tazzer C, Ciprandi G: Minimal persistent inflammation is present also in patients with seasonal allergic rhinitis. *J Allergy Clin Immunol* 2000;105:54–57.
- Fasce L, Tosca MA, Olcese R, Milanese M, Erba D, Ciprandi G: The natural history of allergy: the development of new sensitizations in asthmatic children. *Immunol Lett* 2004;93:45–50.