

## Airway function and nasal inflammation in seasonal allergic rhinitis and asthma

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### Summary

**Background** Allergic rhinitis (AR) and asthma are frequently associated and characterized by a Th2-dependent inflammation. Nasal and bronchial obstruction may be objectively measured.

**Objective** The aim of this study was to evaluate the relationships among upper and lower airway function and nasal inflammation in subjects with seasonal allergic rhinitis (SAR) and asthma.

**Methods** Twenty out-patients (12 males and eight females, mean age: 23.4 ± 3.6 years) with SAR and asthma were evaluated during the pollen season. All of them showed a moderate–severe grade of nasal obstruction. Total symptom score, rhinomanometry, spirometry, nasal lavage, and nasal scraping were obtained in all subjects. Eosinophils were counted by conventional staining; IL-4 and IFN- $\gamma$  were measured by immunoassay on fluids recovered from nasal lavage.

**Results** Functional parameters, i.e. nasal airflow and forced expiratory volume in 1 s (FEV<sub>1</sub>), were correlated with nasal eosinophils ( $R^2 > 0.83$ ,  $P < 0.001$ ). Inflammatory parameters, i.e. eosinophils were correlated with immunological parameters, i.e. IL-4 and IFN- $\gamma$  levels ( $R^2 = 0.93$ ,  $P < 0.001$ ). Nasal symptoms were correlated with nasal airflow ( $\rho = -0.71$ ,  $P \leq 0.01$ ) and eosinophils ( $\rho = 0.72$ ,  $P < 0.01$ ). Nasal airflow was correlated with FEV<sub>1</sub> ( $r = 0.89$ ,  $P < 0.0001$ ).

**Conclusions** This study demonstrates the close connection between Th2 cytokines and eosinophil infiltration in the nose. There is also clear evidence concerning the relationships between eosinophils infiltration and cytokines levels. Nasal eosinophils can be regarded as the most important predictors of upper and lower airway functions. These findings constitute first evidence of a relationship among nasal Th2-related inflammation and nasal and bronchial airflow in patients with SAR and asthma.

**Keywords** asthma, eosinophils, FEV<sub>1</sub>, nasal airflow, seasonal allergic rhinitis, Th1 and Th2 cytokines  
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### Introduction

A clinical relationship between allergic rhinitis (AR) and asthma has been defined for a long time [1]. Patients very commonly present with both disorders. Thus, an increasing body of evidence suggests the existence of a clear link between upper and lower airways [2]. AR and asthma are connected epidemiologically, anatomically, physiologically, immunopathologically, and therapeutically [3].

Epidemiological link represents strong evidence supporting the connection between AR and asthma. In this regard, we previously reported that 77% of conscripts with respiratory allergy suffered from AR associated with asthma [4].

Anatomical and physiological connections between AR and asthma have been widely reported [5].

The immunopathological feature of AR and asthma is substantially similar: there is an infiltration by inflammatory cells, including T cells, mast cells, and eosinophils, in both disorders [6]. Mediators, such as histamine and leukotrienes,

are demonstrated both at nasal and bronchial levels in rhinitics and asthmatics [7]. The cytokine pattern is typically characterized by a Th2 polarization in both disorders [8]. Th2-derived cytokines account for recruiting and activating eosinophils in airways. Actually, eosinophil infiltration may be considered as a reliable marker of allergic inflammation in both disorders.

However, vascular tissue and smooth muscle, respectively, characterize the final response: nasal congestion and bronchospasm. For these reasons, antihistamines, antileukotrienes, and topical corticosteroids can be prescribed in both diseases, although with different efficacies [9].

Asthma is defined as a chronic inflammation of lower airways [10] as well as an inflammatory infiltration, which has been well documented in AR [11]. However, it is difficult to evaluate bronchial inflammation in the common clinical practice. Bronchoscopy is an invasive method and is not recommended as routine exam. Induced sputum may be more easily performed, but few clinical centres get organized for it still now. On the contrary, nasal investigations, including rhinoscopy, cytology, and lavage, are easy to be performed, economic, and may be repeated many times in the same patient.

In any case, airway inflammation causes airflow obstruction both at nasal and bronchial levels that may be objectively measured by rhinomanometry and spirometry, respectively.

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For these reasons, we aimed at investigating the possible relationships among nasal eosinophils, cytokines recovered from nasal lavage, rhinomanometry, and spirometry in patients with seasonal allergic rhinitis (SAR) and asthma.

## Materials and methods

### Patients

Twenty patients with SAR and asthma were consecutively evaluated: 12 males and eight females (mean age:  $23.4 \pm 3.6$  years). A detailed clinical history and a complete physical examination were carried out for each patient. Asthma was diagnosed according to Global Initiative for Asthma (GINA) guidelines [10] by clinical and functional features. The diagnosis of SAR was made, according to international guidelines [12], on the basis of history and skin prick test (SPT) positivity for pollen allergens. In our geographic area, the most important pollen allergens include grasses and *Parietaria officinalis* [4].

### Study design

The study was conducted during the pollen season, when patients were symptomatic. Only patients who met all the following criteria were eligible for inclusion into the study: men and females aged > 18 years, history of SAR and asthma due to seasonal allergen exposure for the previous 2 years, rhinitis and asthma symptoms for the 2 previous weeks, total nasal symptom score (TSS) minimum value > 6 at baseline, particularly with nasal obstruction moderate-severe (see below), forced expiratory volume in 1 s ( $FEV_1$ ) values < 80% of predicted, reversibility of airway obstruction of at least 12% of  $FEV_1$  from baseline, and an informed consent obtained prior to study entry.

Exclusion criteria were: acute and chronic upper respiratory infections within 30 days before the study, anatomic nasal disorders (i.e. septum deviation), nasal polyps, use of antibiotics, intra-nasal, inhaled or oral corticosteroids within the previous 4 weeks, and use of antihistamines, antileukotrienes, and long-acting  $\beta_2$ -agonists within the previous week.

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

The study was approved by the Navy Hospital Review Board.

### Skin prick test (SPT)

Allergy was assessed by the presence of sensitization to the most common classes of aeroallergens by performing SPT. It was performed as stated by the Italian Society of Allergy and Clinical Immunology [13]. The allergen panel consisted of the following: house dust mites (*Dermatophagoides farinae* and *pteronyssinus*), cat, dog, grasses mix, *Compositae* mix, *P. 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, rhinorrhoea, 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). TSS 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 post-nasal 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 millilitres 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.

### Spirometry

It was performed by using a computer-assisted spirometer (COSMED, Eurase, Italy), with optoelectronic whirl flow meter. Spirometry is performed as stated by the European Respiratory Society [14], using the European Community for Steel and Coal reference equations.

As airway obstruction was present as detected by  $FEV_1$  values less than 80% of the predicted, a test of bronchodilatation was performed using a salbutamol metered dose of 200  $\mu$ g. Reversibility was considered if an increase of at least 12% of  $FEV_1$  from baseline was achieved, according to international guidelines [10].

### Nasal cytology

Nasal cytologic specimens were obtained by scraping the head of the inferior turbinate with a cotton swab, as described in previous reports [15]. 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 analysed by 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 [16]. 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  $-20^{\circ}\text{C}$  [16].

### Cytokine assessment

The cytokines were measured with ELISA method (R&D Systems, Charleston, SC, USA). This assay employed the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for each cytokine was pre-coated 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. Results were the means of duplicate samples. The inter- and intra-assay coefficients of reproducibility for each cytokine were as follows: IL-4, 2–5% and 3–6%, IFN- $\gamma$ , 1–3% and 3–5%. The sensitivity of the commercially prepared ELISA was less than 1 pg/mL both for IL-4 and IFN- $\gamma$ .

### Statistical analysis

Relationships between variables were tested by Pearson's correlation. Spearman's test was used to correlate data with scores. When a functional variable correlated with more than one variable by univariate analysis, their simultaneous effects were analysed by multiple linear regression analysis with independent variables selected by a stepwise procedure. Data are presented as means  $\pm$  SD, unless otherwise indicated. Values of  $P < 0.05$  were considered statistically significant.

## Results

### Subjects

Twenty allergic patients with SAR and asthma were studied. All of them were sensitized to grasses and/or *P. officinalis* only. There was a large amount of these pollens in the environment during the study period as it was performed during the spring (data not shown). General physical examination was normal, apart from rhinitis and asthma symptoms, in all patients.

### Symptoms

All patients were symptomatic with a median (upper–lower quartiles) TSS of 9.5 (11–8).

### Rhinomanometry

It was performed in all patients. A clear decrease of nasal airflow was present in all subjects: the mean of airflow values was  $436 \pm 98.2 \text{ mL/s}$ . Normal values for our laboratory are  $742.8 \pm 128.4 \text{ mL/s}$ .

### Spirometry

All patients had reduced FEV<sub>1</sub> values, ranging from 65% to 79% of predicted values ( $74\% \pm 4$ , mean  $\pm$  SD). All of them showed positive response to bronchodilator.

### Nasal cytology

Eosinophils were present in nasal smears in all patients: the mean was  $10.5 \pm 4.8$ .

### Cytokine levels

The mean value of IFN- $\gamma$  was  $1.7 \pm 0.7 \text{ pg/mL}$  (normal values in our lab:  $32.4 \pm 4.6 \text{ pg/mL}$ ). The mean value of IL-4 was  $9.7 \pm 3.2 \text{ pg/mL}$  (normal values in our lab:  $0.8 \pm 0.4 \text{ pg/mL}$ ).

### Correlations

When functional parameters (Fig. 1) were analysed as dependent variables by multiple regression (Tables 1 and 2), nasal flow (upper panel) and FEV<sub>1</sub> (lower panel) were significantly ( $P < 0.001$ ) inversely correlated ( $r = -0.94$  and  $r = -0.91$ , respectively) with eosinophils, but not with cytokines. However, when inflammatory parameters were analysed as dependent variables by multiple regression, nasal eosinophils were correlated ( $R^2 = 0.93$ ,  $P < 0.001$ ) with IL-4 ( $r = 0.93$ ) and IFN- $\gamma$  ( $r = -0.89$ ) levels (Fig. 2).

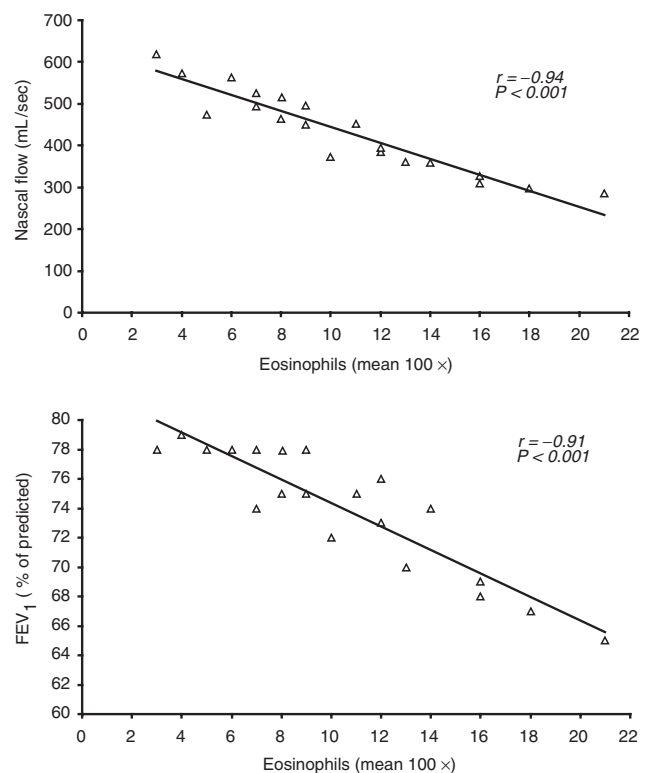


**Fig. 1.** Upper panel: Relationship between the eosinophils' number and the nasal airflow. Lower panel: Relationship between the eosinophils' number and the forced expiratory volume in 1 s (FEV<sub>1</sub>) values.

**Table 1.** Regression coefficients by multiple linear regression when nasal flow was selected as dependent variable ( $R^2 = 0.88$ ,  $P < 0.0001$ )

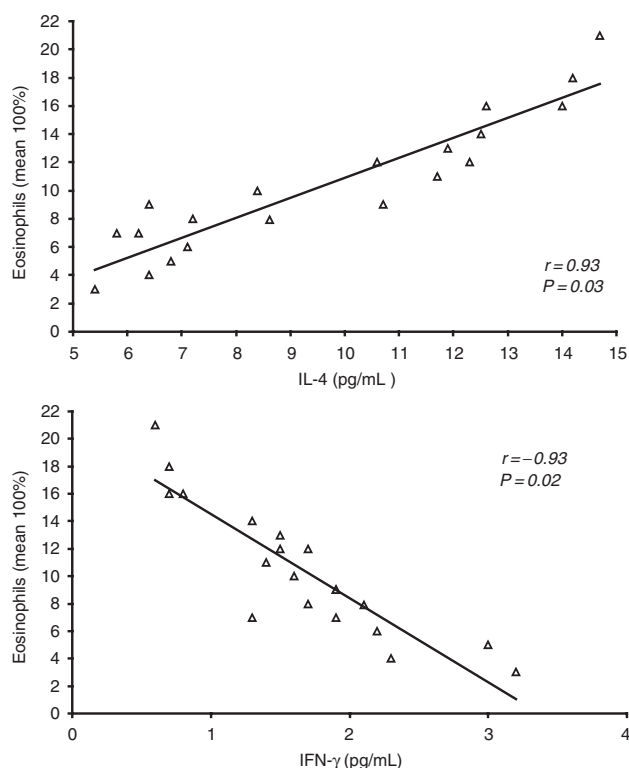
|               | Coefficient | Std error | Std coeff | t-Value | P-value |
|---------------|-------------|-----------|-----------|---------|---------|
| Intercept     | 636.86      | 83.75     | 636.86    | 7.60    | <0.001  |
| Eos           | -15.78      | 6.48      | -0.77     | -2.43   | 0.02    |
| IL-4          | -4.49       | 7.26      | -0.14     | -0.61   | 0.54    |
| IFN- $\gamma$ | 4.54        | 27.45     | 0.03      | 0.16    | 0.87    |

Std error, standard error; Std coeff, standard coefficient; Eos, eosinophil.

**Table 2.** Regression coefficients by multiple linear regression when FEV<sub>1</sub>% was selected as dependent variable ( $R^2 = 0.83$ ,  $P < 0.0001$ )

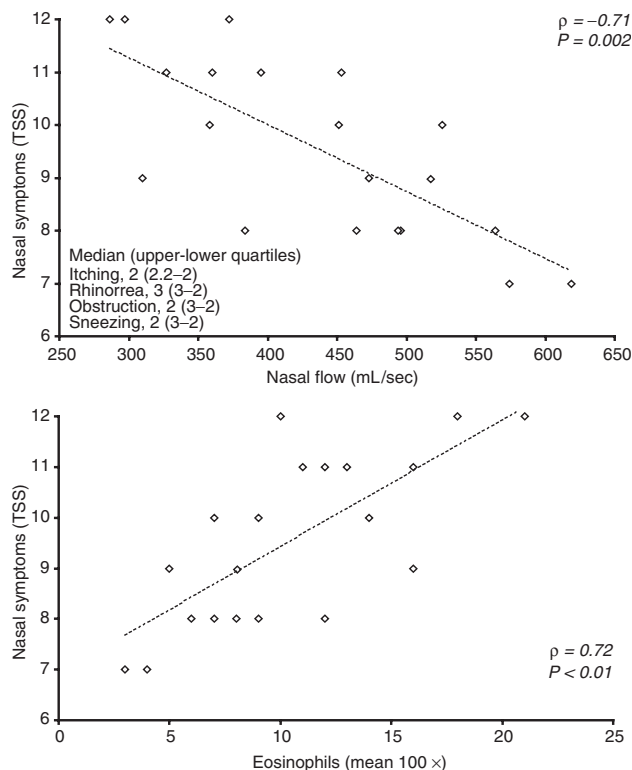
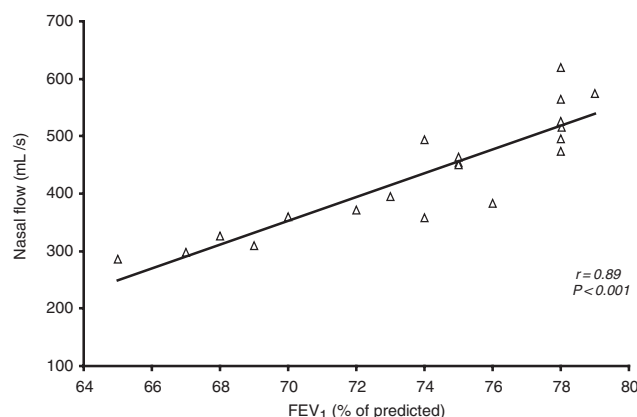
|               | Coefficient | Std error | Std coeff | t-Value | P-value |
|---------------|-------------|-----------|-----------|---------|---------|
| Intercept     | 79.42       | 4.341     | 79.429    | 18.29   | <0.001  |
| Eos           | -0.81       | 0.336     | -0.91     | -2.40   | 0.03    |
| IL-4          | -0.18       | 0.37      | 0.13      | 0.48    | 0.63    |
| IFN- $\gamma$ | 0.75        | 1.423     | 0.12      | 0.532   | 0.60    |

FEV<sub>1</sub>, forced expiratory volume in 1 s; Std error, standard error; Std coeff, standard coefficient; Eos, eosinophil.

**Fig. 2.** Upper panel: Relationship between the eosinophils' number and the concentration of IL-4 in nasal lavages. Lower panel: Relationship between the eosinophils' number and the concentration of IFN- $\gamma$  in nasal lavages.

Nasal symptoms expressed as TSS (Fig. 3) were significantly correlated with nasal flow ( $\rho = -0.71$ ,  $P < 0.01$ , upper panel) and eosinophils ( $\rho = 0.72$ ,  $P < 0.01$ , lower panel).

Finally, positive correlation was shown between nasal airflow and FEV<sub>1</sub> values ( $r = 0.89$ ,  $P < 0.0001$ ) (Fig. 4).

**Fig. 3.** Upper panel: Relationship between the nasal total symptom score (TSS) and the nasal airflow. Lower panel: Relationship between the nasal TSS and the eosinophils' number.**Fig. 4.** Relationship between the nasal airflow and the forced expiratory volume in 1 s (FEV<sub>1</sub>) values.

## Discussion

The present study aimed at investigating possible relationships among nasal eosinophils, IL-4, and IFN- $\gamma$  collected from nasal lavage, nasal airflow, and FEV<sub>1</sub> in patients with SAR associated with asthma.

AR and asthma are characterized by a Th2 polarization. Th2-derived cytokines have been previously investigated in patients both with AR and asthma, particularly concerning the role of IL-4 [17]. IL-4 is a pivotal cytokine that orchestrates allergic inflammation as it is the most important

signal to induce Th2 polarization in allergic subjects [18]. IL-4 is produced by a variety of cells, including Th2 lymphocytes, eosinophils, and mast cells. IL-4 promotes IgE synthesis, up-regulates adhesion molecules selective for eosinophil recruitment, and causes increased mucus production [18]. Thus, there is agreement about the role exerted by IL-4 as main pathogenetic factor in inducing, maintaining, and amplifying inflammatory allergic inflammation.

Otherwise, there is an accumulating evidence that Th1-related cytokines, such as IFN- $\gamma$  and IL-12, may suppress and counteract this Th2 response and vice versa as there is a clear functional dichotomy between Th1 and Th2 cells [19]. The suppressive effects of IFN- $\gamma$  are mediated by various mechanisms, such as inhibiting the production of Th2-derived cytokines and skewing the differentiation of naïve T cells toward Th1 subtype preference [20]. In addition, exogenous IFN- $\gamma$  protein could reverse the ongoing reactions [21]. Thus, IFN- $\gamma$  may play a regulatory role in the extent of airway responses to allergen. Some of the mechanisms that may be relevant include: suppressing the release of Th2-type cytokines from activated T cells [20], facilitating apoptosis of T cells and of eosinophils [16], and inhibiting local recruitment of eosinophils [22].

However, there is evidence that Th1 cells may be themselves pro-inflammatory as it has been demonstrated that the development of a Th1-associated inflammatory response could exacerbate asthma [23]. Furthermore, the increase in prevalence of atopic diseases over the past 20 years has been accompanied by an increase in the prevalence of autoimmune, Th1-driven, diseases, also in the same subject [24]. Thus, forms of immunity other than pure Th1 responses must be effective in regulating the allergic inflammation: such as regulatory T cells [25]. Anyway, Th2-derived cytokines cover an important role in regulating allergic inflammation, mainly concerning eosinophil recruitment and activation. In this regard, allergic inflammation accounts for occurring respiratory symptoms, especially regarding airflow obstruction.

The pathogenesis of nasal obstruction is very complex and consists of three main events: inflammatory mucosal oedema, vascular congestion, and mucus hypersecretion [26]. On the other hand, bronchial obstruction is sustained by the spasm of smooth muscle, the inflammatory oedema, and the mucus hyperproduction [8]. All these pathogenetic events are generated by mediators, including leukotrienes and histamine, and cytokines, mainly concerning IL-4, released by activated mast cells, eosinophils, and T cells [18]. It is noteworthy to highlight that similar pathophysiologic pathways occur both at nasal and bronchial level thus airflow obstruction may appear. In this regard, airway obstruction constitutes the symptom more representative of allergic inflammation.

On the basis of these issues, the present study suggests some considerations concerning the link between upper and lower airways.

Firstly, there is a significant positive relationship between nasal eosinophil infiltration and IL-4 levels, confirming previous findings [10]. This aspect highlights the pathogenetic role exerted by Th2 cytokines in accumulating eosinophils.

Secondly, negative relationship between nasal eosinophils and IFN- $\gamma$  has been demonstrated, indirectly confirming the 'protective' role of Th1-derived cytokines. As a proof of this finding, a close relationship between IL-4 and IFN- $\gamma$  levels is

provided by our results. That confirms the close dichotomy existing between Th1 and Th2 subpopulations.

Thirdly, 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.

All these aspects underline the close association regarding the relationship between the structure and the function of the nose. In other words, as nasal mucosa is more inflamed, nasal airflow is more impaired. In addition, these findings seal the strict Th1–Th2 functional dichotomy.

Fourthly, we provided evidence concerning the relationship between nasal allergic inflammation, sustained by eosinophil infiltration, and bronchial obstruction as provided by FEV<sub>1</sub> values. Results by multivariate regression clearly indicate a strong relationship of upper and lower airway functions with nasal inflammation, but not with immunological parameters. Therefore, nasal eosinophils may be considered as important predictors of airway function.

This issue probably represents the most important outcome of the present study as it documents the close association between allergic inflammation at nasal level and bronchial obstruction. Moreover, this finding indirectly confirms our previous remark observed in asthmatic children with rhinosinusitis [27].

This finding constitutes the formal and objective proof concerning the close link between nasal and bronchial functions.

On the other hand, our results are conflicting with previous studies showing poor correlation between nasal symptoms and rhinomanometry assessment [28, 29]. Particularly, it has been reported that peak inspiratory flow rate is more sensitive than rhinomanometry [30]. However, our primary aim was to investigate the relationship between nasal inflammation and airflow and not to compare directly the nasal symptoms with rhinomanometry as patients were evaluated only once.

Furthermore, present findings have to be added to our previous survey in AR. Very recently, we demonstrated in a group of 100 patients with perennial AR alone that five patients showed impaired FEV<sub>1</sub> values (<80% of predicted), without any perceived lower respiratory symptoms and 48 patients showed reduced forced expiratory flow from the first to third quarters (FEF<sub>25–75</sub>) values [31]. Moreover, 72 patients showed positive methacholine challenge, and there was a significant relationship between bronchial hyper-responsiveness (BHR) degree and FEV<sub>1</sub> and FEF<sub>25–75</sub> values [31].

In conclusion, these results, taken together, are consistent with the concept of the link between AR and asthma, based on the so-called nasobronchial cross-talk [32–34]. There is a bidirectional relationship between nasal and bronchial inflammation: bronchial challenge of rhinitis patients leads to nasal inflammation and vice versa [35–38]. Moreover, nasal inflammation has been demonstrated to be closely associated with BHR [39, 40]. Anyway, the present study provides first evidence of the relationships among nasal eosinophil infiltration, Th1- and Th2-derived cytokines, nasal airflow impairment and FEV<sub>1</sub> values in patients with SAR and asthma. Therefore, we retain that these data may be considered convincing proof of the close link existing between AR and asthma.

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