doi: 10.1111/j.1365-2222.2009.03439.x
Clinical & Experimental Allergy, **40**, 867–874

ORIGINAL ARTICLE **Asthma and Rhinitis**

Nasal eosinophilia: an indicator of eosinophilic inflammation in asthma

M. M. Amorim, A. Araruna, L. B. Caetano, A. C. Cruz, L. L. Santoro and A. L. G. Fernandes
Asthma Research Group Respiratory Division – Federal University of São Paulo-Brazil (UNIFESP), São Paulo, Brazil

Clinical & Experimental Allergy

Correspondence:
Prof A. L. G. Fernandes, Disciplina de Pneumologia, Rua Botucatu 740 3° andar, São Paulo-SP CEP 04023-004, Brazil.
E-mail: analuisa@pneumo.epm.br
Cite this as: M. M. Amorim, A. Araruna, L. B. Caetano, A. C. Cruz, L. L. Santoro and A. L. G. Fernandes, *Clinical & Experimental Allergy*, 2010 (40) 867–874.

Summary

Background It is noteworthy that there is a clear clinical, epidemiological and pathophysiological association between upper and lower airway inflammation in rhinitis and asthma.
Objective The aim of this study was to compare the eosinophil counts in induced sputum and nasal lavage fluids in asthma, checking their association and the accuracy of nasal eosinophilia as a predictor of sputum eosinophilia by a cross-sectional study.
Methods The clinical evaluation, asthma control questionnaire (ACQ), pre- and post-bronchodilator spirometry, nasal and sputum sample was performed. The nasal eosinophilia was analysed by a receiver operating curve and logistic regression model.
Results In 140 adults, the post-bronchodilator forced expiratory volume in 1 s (FEV_1) did not differ between patients with or without sputum eosinophilia (0.18). After adjusted for upper airway symptoms, age, ACQ score and post-bronchodilator FEV_1, sputum eosinophilia was associated with 52 times increase in odds of nasal eosinophilia, whereas each 1% increase in bronchodilator response was associated with 7% increase in odds of nasal eosinophilia.
Conclusion This study brings further evidence that upper airway diseases are an important component of the asthma syndrome. Furthermore, monitoring of nasal eosinophilia by quantitative cytology may be useful as a surrogate of sputum cytology in as a component of composite measurement for determining airway inflammation.

Keywords asthma, eosinophils, induced sputum, nasal lavage
Submitted 30 June 2009; revised 16 November 2009; accepted 19 November 2009

Introduction

Asthma is a variable chronic inflammatory disorder of the airways. Airway inflammation has been demonstrated to represent an important factor underlying asthma clinical expression [1]. In order to achieve optimal control of asthma, many steps should be taken as defined by the GINA and NIH guidelines [2, 3].

The airway inflammation in asthma is persistent even though symptoms are episodic, and the relationship between the severity of the asthma and the intensity of the inflammation is not clearly established [4]. The eosinophil is a cellular maker of a type of inflammation in asthma, and an indicator of response to treatment as well [5, 6]. Sputum eosinophilia has been associated with asthma exacerbations [6]. It has been taken as a marker of airway inflammation that might be adopted for the assessment of asthma control status [7].

It is noteworthy that there is a clear clinical, epidemiological and pathophysiological association between upper and lower airway inflammation in rhinitis and asthma, which suggests that inflammatory cells may play a key and similar role in both of these syndromes [8–11]. However, little information is available concerning the relevance of the cellular patterns of nasal secretions on the assessment of the type and intensity of the lower airways inflammation and asthma control status.

The collection of sputum to obtain eosinophil counts in subjects with asthma requires a lengthy procedure, and not always allows for a proper sample [12]. If one believes that the airway mucosa behaves as a continuum from the nose down to the bronchi, findings from nasal secretions could be a proxy of those obtained in the less assessable lower airways. The proportion of eosinophils in a quantitative nasal cytology could become a quick test to identify eosinophilic airway inflammation. The aim of this study was to compare the eosinophil counts in induced sputum and nasal lavage fluids in patients with asthma, checking their association and the accuracy of nasal eosinophilia as a predictor of sputum eosinophilia.

Materials and methods

This is a cross-sectional study including patients >18 years old, non-smokers or ex-smokers (<5 pack/years), with documented clinical history of moderate–severe stable asthma. All patients have been regularly treated with combined therapy of budesonide plus LABA (400+12 μg b.i.d.) for the previous 6 months (Step 4 of GINA) [13]. Nasal corticoid was prescribed for those patients who have referred allergic nasal symptoms. All subjects had documented bronchodilator response defined as increase in post-bronchodilator forced expiratory volume in 1 s (FEV_1) more than 12% and 200 mL related to pre-bronchodilator value, at a screening visit [14, 15]. Exclusion criteria: patients with upper or lower respiratory tract infections in previous 4 weeks; other respiratory diseases or any relevant co-morbidity that might interfere with study requirements, procedures or outcomes. Furthermore, the use of nasal and inhaled medications had been discontinued 48 h before the samples were obtained. All eligible patients were recruited from an asthma clinic within Hospital São Paulo/UNIFESP and invited to participate in this study. All the patients had not received any other treatments, such as anti-IgE, anti-IL-5, or any immunosuppression. All subjects signed an informed consent and the study was approved by the institutional review board.

Protocol

Screening visit: Each patient was evaluated to determine the current level of asthma control according to GINA Guidelines, to identify the presence of upper airway symptoms by structured questionnaire, to measure the significant bronchodilator response and the expertise to filling up the diary card.

Inclusion visit: Review of the diary card filled out for day and night symptoms, the use of rescue bronchodilator, measurement of peak expiratory flow over the last 7 days and upper airway symptoms according to a structured questionnaire including presence or absence of headache, facial pain, nasal obstruction, purulent post-nasal discharge or purulent rhinorrhoea and hyposmia or anosmia, fetid odours, dental pain, ear pain and cough. Asthma control was defined by the following criteria: day time symptoms twice a week or less, no limitations of activities, no awakening, short-bronchodilator usage less than twice a week, FEV_1 of more than 80% of predict or the best personal value. Pre- and post-β-agonist bronchodilator spirometry were performed. They underwent clinical evaluation, skin prick test to common aeroallergens, nasal lavage followed by induction of sputum, and answered the asthma control questionnaire (ACQ) [16]. All subjects were recommended to discontinue all topical nasal medication for 48 h and bronchodilator for 6 h before the evaluation of nasal and bronchial secretions (Fig. 1).

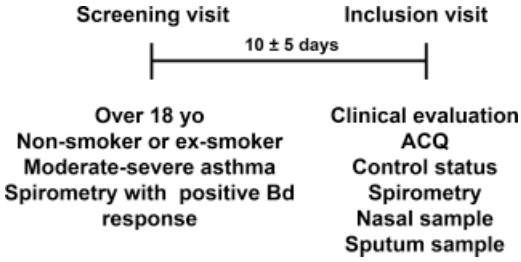

Fig. 1. Study protocol.

Procedures

The atopic status of the subjects was evaluated using ASAC pharma® skin prick test system (São Paulo, Brazil) with 10 common environmental aeroallergens: *Dermatophagoides farinae, Dermatophagoides pteronyssimus*, *Blomia tropicallis, Periplanata americana, Blatalla germanica*, dog, cat, mix pollen, mix moulds and *Aspergillus fumigatus*. Histamine hydrochloride (10mg/mL) and sterile saline solution 0.9% were used as positive and negative controls. Atopy was defined as at least one positive reaction with a diameter of ⩾3 mm of negative control [17].

Spirometry was performed according to American Thoracic Society criteria and the Brazilian reference values [14, 15]. Reversibility of airflow limitation (ΔFEV_1) was measured after administration of 400 μg salbutamol and expressed as changes in FEV_1 as percentage of predict value.

Nasal lavage was performed before induced sputum to avoid the hypertonic saline nasal epithelium challenge or the induction of chemotactic factor releasing. It was processed as previously described by instilling 10 mL of 0.9% sterile saline solution (22–28 °C), 5 mL in each nostril, with the subject's head tilted 30° backwards [9]. Subjects were instructed neither to breathe nor swallow for approximately 10 s during a Valsalva maneuver, and then to bend the head forward in order to collect the mixture of saline and nasal secretions. A sample of at least 7 mL was collected in a sterile container. The liquid obtained was vigorously shaken and subsequently centrifuged (2500 g at 4 °C for 15 min). The cell pellet was resuspended in 0.5 mL of PBS and shaken again. Total cell counts were obtained in a Neubauer's haemocytometer (DBC Medical Inc., WACO, TX, USA). Two cell smears were obtained by low-speed cytocentrifugation (48.3 g for 6 min) with a Cytospin 4 Shandon with 200 μL of resuspended material. The smears were stained with Panotic® (Pfizer Inc., Exton, PA, USA) method, a commercial kit for the Wright's stain, and the differential cell count of 200 cells was then obtained. Cells were identified according to their structure as epithelial, neutrophil, eosinophil and lymphomononuclear cells. For nasal lavage, the percentage of eosinophil cells was obtained among a total of 200 cells count due to the minimal cell concentration of final standard sample dilution is 500 cells/μL [18].

The nasal lavage was considered to present eosinophilia when the relative cell count exceeded 5% [9, 18]. Samples were examined in a blinded fashion by the same observer.

Sputum was induced and processed according to a validated technique [19, 20]. The method was performed by inhaling increasing concentrations of saline (3%, 4% and 5%) for 7 min each, through a mouthpiece without a valve or nose clip. After each inhalation period, a FEV_1 value was measured for safety. The sample of bronchial secretions was collected into a container and processed within 1 h. In sputum, according to validated technique, squamous cell contamination up to 5% was accepted for analyses whereas those with more than 5% of squamous cells were excluded from the study [19]. Cell viability was determined by trypan blue exclusion method. Total non-squamous cell count was performed in a haemocytometer and expressed as million per milligram of selected induced sputum. The cells were identified according to their structure as neutrophils, eosinophils, lymphocytes and macrophages. For induced sputum, the percentage of cells was obtained among a total of 400 cells count due to the minimal cell concentration of final standard sample dilution was 1000 cells/μL [21]. These differences in the number of cells in the sample dilution may be related to the extension of eosinophilic inflammation of the reticular basement membrane thickness and the epithelium shedding which is greater in bronchial than in nasal mucosa of asthmatic patients with perennial rhinitis [22]. Eosinophils are expressed as absolute and relative values. The sputum was considered eosinophilic when the relative cell count exceeded 3% [21, 23, 24].

Statistical analysis

Values were expressed as means with standard deviations. Mann–Whitney test was used to compare continuous variables for which a normal distribution was not recognized. Spearman's coefficient was used to assess correlation between nasal eosinophil count or FEV_1 and sputum eosinophil count. Receiver operating curve (ROC) was used to express the sensitivity and specificity of the presence of nasal eosinophils in predicting the presence of eosinophils in the induced sputum as well as to the presence of nasal neutrophils in predicting the presence of neutrophils in the induced sputum. The comparison between subjects with asthma presenting $>5\%$ eosinophils in their nasal secretion with those with $\leqslant 5\%$ eosinophils in their nasal secretion was conducted by a logistic regression analysis model. The variables included in the model were age, FEV_1 post-bronchodilator, percentage of bronchodilator response, presence of upper airway symptoms (reference: absence of upper airway symptoms), ACQ score and presence of sputum eosinophilia $\geqslant 3\%$ (reference: sputum non-eosinophilia). A P-value of <0.05 was taken as significant. Statistical analyses were performed using the SPSS 13.0 software.

Results

Out of 186 patients screened, 140 were enrolled in the study. Cases were excluded for respiratory infection in the previous 30 days ($n = 12$; 6.5%), systemic corticoid usage in the previous 30 days ($n = 15$; 8.3%), partly controlled asthma status ($n = 8$; 4.4%), inability to fill up the diary card ($n = 5$; 2.8%) and refusing to participate of the study ($n = 6$; 3.3%). The sputum induction was successful in 131 (93.6%), the sputum was unsuitable for analysis in nine samples because of insufficient processable material in four, excessive squamous cells contamination (over 20%) in three and excessive cell degeneration and low viability ($<20\%$) in two samples. The nasal lavage was obtained from 135 (96.4%), the nasal lavage was unsuitable for analysis in five samples due to patients swallowed the nasal instillation. The majority of those included were female, had a positive skin prick test at the inclusion visit (Table 1).

Table 1. Subjects characteristics at the inclusion visit

Demographic variables	Total
n	140
Age (mean±SD)	46.8±13.1
Male gender % (*n*)	27.0 (38.0)
Body mass index	25.8±6.4
Clinical variables	
Inhaled corticosteroids+LABA % (*n*)	100 (140)
%Nasal corticosteroids (*n*)	21.4 (30)
%FEV_1 pre-BD (mean±SD)	72.9±16.9
%FEV_1 post-BD (mean±SD)	79.6±15.9
Bronchodilator response % (mean±SD)	10.9±10.3
ACQ (mean±SD)	0.6±0.3
Atopy % (*n*)	88.0 (123)
Nasal symptoms % (*n*)	
Any symptoms	25.0 (35)
Itching	0.7 (1)
Sneezing	21.4 (30)
Rhinorrhoea	17.1 (24)
Nasal obstruction	17.1 (24)
Cytological variables	
Sputum (*n*)	131
%Viability (mean±SD)	62.7±14.0
%Eosinophil median (min/max)	2.0 (0/62)
%Neutrophil median (min/max)	68.8 (5/97)
Nasal (*n*)	135
%Eosinophil median (min/max)	1.5 (0/86)
%Neutrophil median (min/max)	36.0 (0/98)

Subjects discontinued the use of LABA for 48 h before spirometry test.
n, number of patients; SD, standard deviation; LABA, long-action bronchodilator; %pre-FEV_1, pre-bronchodilator forced expiratory volume in 1 s; % post FEV_1, post-bronchodilator forced expiratory volume in 1 s; ACQ: asthma control questionnaire.

Table 2. Characteristics of patients according to sputum eosinophilia level and nasal corticosteroids usage

Clinical variables	Eosinophilia sputum	Non-eosinophilia sputum	*P*
n	57	74	
Pre-FEV_1% (%±SD)	72.90±16.6	73.46±18.40	0.35
Post-FEV_1% (%±SD)	80.82±16.96	79.42±15.42	0.18
Bronchodilator response (%±SD)	11.64±9.88	11.00±10.62	0.90
ACQ (mean±SD)	0.54±0.28	0.56±0.30	0.93
Nasal eosinophil %median (min/max)	7.75 (0/86)	0.25 (0/49)	<0.001*
Nasal neutrophil % median (min/max)	30.75 (0/89)	53.50 (2/98)	0.02*
Sputum neutrophil % median (min/max)	54.00 (10/91)	77.13 (5/97)	<0.001*

Clinical variables	Nasal corticosteroids	Without nasal corticosteroids	*P*
n	30	106	
Pre-FEV_1% (%±SD)	70.37±15.30	73.80±17.46	0.48
Post-FEV_1% (%±SD)	77.63±14.76	80.36±16.24	0.73
Bronchodilator response (%±SD)	11.36±10.53	10.84±10.43	0.97
ACQ (mean±SD)	0.59±0.29	0.54±0.28	0.54
Nasal eosinophil % median (min/max)	8.23 (0/58)	6.46 (0/86)	0.53
Nasal neutrophil % median (min/max)	48.60 (0/96)	42.74 (2/98)	0.45

*Statistically significant.

Mann–Whitney test.

Pre-FEV_1%, pre-bronchodilator forced expiratory volume in 1 s; post-FEV_1%, post-bronchodilator forced expiratory volume in 1 s; ACQ, score of asthma control questionnaire; min, minimum value; max, maximum value. []Bronchodilator used: albutamol pMDI HFA 400 μg.

The mean time from collection to cell count result for nasal lavage procedure took 50 min, whereas for induced sputum it was around 120 min.

The skin prick test showed positive values for perennial common aeroallergens: *D. farinae* (53%), *D. pteronyssimus* (57%), *B. tropicallis* (46%), *P. americana* (21%), cat (24%), Fungi sp. (1%).

FEV_1 pre- and post-bronchodilator, response to bronchodilator and ACQ scores did not differ between patients with or without eosinophilia in the sputum. There was no correlation between sputum eosinophil count and FEV_1 ($r = 0.04$; $P = 0.63$). The median of the nasal eosinophil count was significantly higher whereas the median of the nasal neutrophil count was significantly lower in the eosinophilic sputum group (Table 2).

Figure 2a showed the ROC curve, depicting a range of sensitivities and specificities of nasal eosinophilia of different cut-off to assess the discriminative power of nasal eosinophil on the prediction of eosinophilic sputum. The area under the curve is 84%, indicating the accuracy of the predictor ($P < 0.001$). The correlation between sputum and nasal eosinophilia was significant, $r = 0.67$ ($P < 0.001$) and Fig. 2b showed the plots of nasal and sputum cell counts. Figure 2c showed the ROC curve illustrated nasal neutrophilia of different cut-off to assess the discriminative power of nasal neutrophil on the prediction of neutrophilic sputum. The area under the curve is 61%, indicating the accuracy of the predictor ($P < 0.04$). At Fig. 2d the sputum and nasal neutrophilia had also a weak correlation, although it reached a statistical significance, $r = 0.26$ ($P < 0.013$).

The nasal lavage and sputum cell count distribution did not differ according to the nasal corticosteroid usage (Fig. 3).

The false-negative nasal lavage (low nasal eosinophils and eosinophilic sputum) did not differ from true-positive test (nasal and sputum eosinophilia) regard to pulmonary function and ACQ. The patients with false-negative nasal lavage had significant lower values of sputum eosinophilia (median 0.5; min/max 3/8) compared with the true-positive tests (median 7; min/max 3/62) (Table 3).

A logistic regression model was built to measure the independent variables associated to nasal eosinophilia. After adjusted for presence of upper airway symptoms, age, ACQ score and FEV_1 post-bronchodilator, the presence of sputum eosinophilia (reference do not have sputum eosinophilia) was associated with 52 times increase in odds of nasal eosinophilia, whereas each one percent increase in bronchodilator response was associated with 7% increase in odds of nasal eosinophilia (Table 4).

Discussion

The eosinophil counts in nasal lavage fluids are good predictors of sputum eosinophilia in our sample of subjects with asthma. Thus, they may be useful in assessing airway inflammation among subjects with controlled

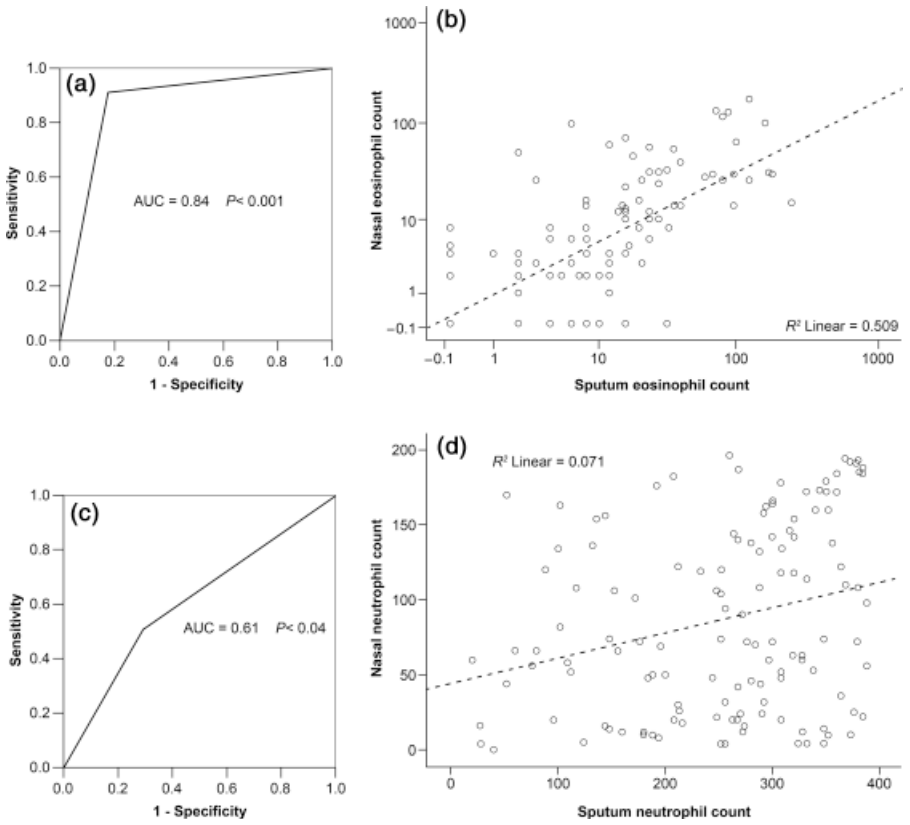

Fig. 2. (a) Receiver operating curve (ROC) nasal eosinophil for predict eosinophil sputum. (b) Correlation between nasal and sputum eosinophil cell count (values log transformed). (c) ROC nasal neutrophil for predict neutrophil sputum. (d) Correlation between nasal and sputum neutrophil cell count.

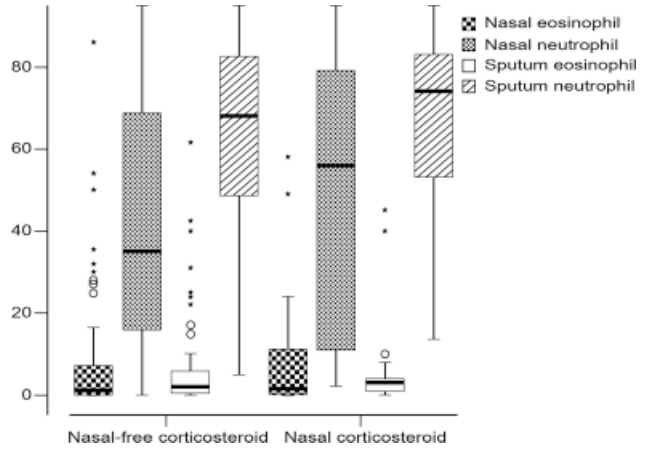

Fig. 3. Cytology of nasal lavage and induced sputum according to nasal steroid usage. No significant difference was identified between groups (Mann–Whitney test).

moderate–severe asthma. The results of our objective and quantitative survey of airway inflammation by cytology of the upper and lower airways allowed us to compare patients with and without eosinophilia in nasal secretions and in induced sputum. It is noteworthy the strong correlation observed between eosinophilia in the nose and the bronchi. The logistic regression model shows that the observation of eosinophilia in one of the compartments of the airway increases markedly the odds of having eosinophilia in the other site.

Table 3. Characteristics of patients nasal eosinophil false-negative (who had low nasal eosinophils but eosinophilic sputum) compared with true-positive patients (who had nasal and sputum eosinophils)

Clinical variables	False negative	True positive	*P*
n	15	41	
%Pre-FEV_1 (%±SD)	68.86±16.46	74.71±19.08	0.31
%Post-FEV_1 (%±SD)	75.80±15.23	82.39±17.51	0.20
Bronchodilator response (%±SD)	11.09±8.98	11.98±10.34	0.78
ACQ (mean±SD)	0.62±0.31	0.52±0.27	0.27
%Nasal eosinophil median (min/max)	0.25 (0/4)	14 (5/86)	<0.001*
%Sputum eosinophil median (min/max)	3.5 (3/8)	7 (3/62)	<0.001*

*Statistically significant.
Mann–Whitney test.
%Pre-FEV_1. pre-bronchodilator forced expiratory volume in 1 s; %post-FEV_1, post-bronchodilator forced expiratory volume in 1 s; ACQ, score of asthma control questionnaire; min, minimum value; max, maximum value.
Bronchodilator used: albuterol MDI HFA 400 μg.

Table 4. Logistic regression model to characterize the nasal eosinophilia group adjusting for age, FEV_1, bronchodilator response, ACQ, upper airway symptoms and sputum eosinophilia

Variable	Odds ratio	95% CI	*P*
%Post-FEV_1 bronchodilator	1.00	0.94–1.06	0.96
% BD response	1.07	1.00–1.13	0.03*
ACQ	0.27	0.006–12.84	0.51
Upper airway symptoms	1.10	0.30–3.99	0.88
Age	0.97	0.93–1.01	0.19
%Eosinophilic sputum	53.45	14.70–194.25	<0.001*

*Statistically significant.

CI, confidence interval; %post-FEV_1, post-bronchodilator forced expiratory volume in 1 s (albuterol 400 μg); % BD response, difference between FEV_1 post- and FEV_1 pre-bronchodilator/FEV_1 pre-bronchodilator; ACQ: score of asthma control questionnaire.

Chronic rhinitis has been known for a long time to be associated with asthma. There is a close inter-relation between upper and lower airway diseases. This study was not designed to contribute to the understanding of the pathophysiologic interdependence mechanisms [8, 10], but only to make observations on the potential value of exploring the assessable nose instead of the tricky bronchi in measuring and characterizing the type of airway inflammation.

It is also important to point out that the asthmatics studied are under good asthma control (ACQ < 1.5). All of them were using inhaled corticosteroid and 25% were using nasal steroids as well. The results of this study are really only applicable to patients under regular drug therapy.

Both allergic rhinitis and asthma are often related to eosinophilic airway inflammation, therefore, using cytology to identify the inflammation of the upper and/or lower airway would be useful for differential diagnosis eosinophilic and non-eosinophilic in rhinitis/asthma. Monitoring the proportion and the total number of eosinophils in nasal lavage fluids may also be useful for research purposes, as their numbers reflect the intensity of upper airway inflammation [25].

As the regular use of corticoid influences the nasal and sputum cytology, in order to avoid this effect in our study, not only were patients included taking the same dose of inhaled corticoid, but also this medication was withdrawn 48 h before the samples were obtained.

A significant correlation between nasal and sputum inflammatory cells offers relevant information about asthma control in patients that are in an apparently controlled clinical condition. However, the sputum collection is hard to obtain, while the nasal lavage is an easier and faster sample collection technique. Moreover, no standardization exists for various nasal cytology techniques used in clinical practice (swab smear, blown secretions and scrapings), which are merely qualitative methods. Comparison of differential counts between regular swab slides and cytospin slides, has demonstrated that this cytology nasal technique is more sensitive to detect eosinophils in different pathologies [18] and a low cost non-invasive method. In general, the nasal cytology is cheaper than induced sputum because of the former has fewer technique steps. For the nasal procedure, neither a special nebulizer to induce the collection nor a spirometer for safety reasons are necessary. Furthermore, the sputum procedure requires a more expensive trained technician.

Current asthma guidelines propose the use of quantitative composite measures for assessment and follow-up of asthma control status [2]. Our results confirm the existence of lower airway inflammation even among asymptomatic patients with asthma, being consistent with a limited correlation between asthma symptoms and airway inflammation. Then, a total cell count in nasal lavage, in analogy to leukocyte counts in urine examination, could be considered as a marker.

The weak correlation demonstrated between reduction in lung function and eosinophilic airway inflammation found among our patients might be explained by the exclusive enrollment of clinically controlled patients by inclusion criteria. On the other hand, it creates the opportunity to identify airway inflammation among apparently controlled subjects that did not have clear abnormalities in functional parameters. A relationship between the proportion of sputum eosinophils and reduction in FEV_1 has been found previously [26]. A strong association between sputum eosinophils and nasal eosinophils has been demonstrated in our study. However, we could not identify a correlation between the proportion of eosinophils, neither nasal nor from the sputum and FEV_1% pre- or post-bronchodilator, % of FEV_1 response or the scores of the ACQ.

We have no clear explanation for this, but it might be due, in part to the controlled population selected in our study.

We could not identify any significant associations between ACQ scores and either nasal or sputum eosinophil counts. This may indicate a lack of sensitivity of the ACQ for such minor degree of inflammation found in the controlled patients. Nevertheless, the cross-sectional nature of our study limits the interpretation of results since exacerbations and other temporal variations could not be addressed.

One mechanism that could explain the interaction between eosinophilic inflammation in the nose and the lower airways in patients with allergic rhinitis is a bronchial aspiration of nasal inflammatory cells or mediators [27]. The possibility of a contamination of bronchial airways with nasal secretion through aspiration cannot be excluded with the present study. In our patients, the nasal lavage was performed before induced sputum because

the hypertonic saline, used in sputum collection, could represent a challenge of the nasal epithelium or induce the production of chemotactic factors.

The nasal lavages neutrophilia and sputum neutrophilia had also a weak correlation. The presence of sputum mixed granulocytic inflammation has been reported in stable persistent asthma [28], although the presence of sputum neutrophilia is not directly related to uncontrolled disease.

This is a novel surrogate approach to measure airway inflammation. There are not many studies about the relationship between nasal cytology and others markers of asthma control. Hence, this is among the first studies to assess the potential contribution of identification of eosinophils by quantitative nasal cytology as a proxy of sputum eosinophilia in the role of an indicator of asthma control. This simple nasal procedure could be adopted as a useful tool to identify different patterns of inflammation associated with airway disease, in epidemiological studies as well as in clinical trials to measure asthma control status in children, elderly or any special population who have difficulties in sputum collection.

In conclusion, this study brings further evidence that upper airway diseases are an important component of the asthma syndrome. Furthermore, monitoring of nasal eosinophilia by quantitative cytology in asthma may be useful as it complements composite measurements for determining the extent and type of airway inflammation. Our report of nasal eosinophilia as a strong predictor of sputum eosinophilia should be explored in future studies, as it brings prospects of marked simplification of the objective measurements of airway inflammation with remarkable accuracy.

Acknowledgements

The authors thank the research Grant # 2005/04714-1 from Fundação do Amparo a Pesquisa do Estado de São Paulo (Fapesp).

The authors declare the role of each author in preparing the manuscript: *Maria Marta Amorim, Alexandre Araruna and Lilian B. Caetano*: Acquisition of data, analysis and interpretation of data, and drafting the article; *Alvaro C. Cruz and Ilka L. Santoro*: Analysis and interpretation of data, and revising it critically; *Ana Luisa Godoy Fernandes*: Conception and design, analysis and interpretation of data, revising it critically and final approval. The authors thank FAPESP (Governmental Research Foundation of São Paulo) by the grant support. We also would like to acknowledge the Methods in Epidemiologic, Clinical and Operations Research (MECOR) Program.

Financial disclosure: Prof Ana Luisa Godoy Fernandes had a supported research project from Novartis® as an investigator during the previous calendar year. The other authors do not have any disclose financial to declare.

References

1 Romagnolim , Vachier I, Tarodo de la Fuente P *et al*. Eosinophilic inflammation in sputum of poorly controlled asthmatics. *Eur Respir J* 2002; **20**:1370–7.
2 National Asthma Education and Prevention Program. Expert panel report 3 (EPR-3): Guidelines for the diagnosis and management of asthma – summary report 2007. *J Allergy Clin Immunol* 2007; **120** (Suppl. 5):S94–138.
3 Bousquet J, Clark TJ, Hurd S *et al*. GINA guidelines on asthma and beyonds. *Allergy* 2007; **62**:102–12.
4 Taylor B, Dinh M, Kwok R, Dinh D, Chu M, Tang E. Electronic interface for emergency department management of asthma: a randomized control trial of clinician performance. *Emerg Med Aust* 2008; **20**:38–44.
5 Djukanovic R, Homeyard S, Gratziou C *et al*. The effect of treatment with oral corticosteroids on asthma symptoms and airway inflammation. *Am J Respir Crit Care Med* 1997; **155**:826–32.
6 Holgate ST. Pathogenesis of asthma. *Clin Exp Allergy* 2008; **38**:872–97.
7 Green RH, Brightling CE, McKenna S *et al*. Asthma exacerbations and sputum eosinophil counts: a randomised controlled trial. *Lancet* 2002; **360**:1715–21.
8 Ciprandi G, Vizzaccaro A, Cirillo I, Tosca M, Massolo A, Passalacqua G. Nasal eosinophils display the best correlation with symptoms, pulmonary function and inflammation in allergic rhinitis. *Int Arch Allergy Immunol* 2005; **136**:266–72.
9 Cruz AA, Naclerio RM, Proud D, Togias A. Epithelial shedding is associated with nasal reactions to cold, dry air. *J Allergy Clin Immunol* 2006; **117**:1351–8.
10 Leynaert B, Neukirch C, Kony S *et al*. Association between asthma and rhinitis according to atopic sensitization in a population-based study. *J Allergy Clin Immunol* 2004; **113**:86–93.
11 van Cauwenberge P, Bachert C, Passalacqua G *et al*. Consensus statement on the treatment of allergic rhinitis. European academy of allergology and clinical immunology. *Allergy* 2000; **55**:116–34.
12 Moritz P, Steidle LJ, Felisbino MB, Kleveston T, Pizzichini MM, Pizzichini E. Determination of the inflammatory component of airway diseases by induced sputum cell counts: use in clinical practice. *J Bras Pneumol* 2008; **34**:913–21.
13 Bateman ED, Hurd SS, Barnes PJ *et al*. Global strategy for asthma management and prevention: GINA executive summary. *Eur Respir J* 2008; **31**:143–78.
14 Gardner RM, Hankinson JL. Standardization of spirometry – 1987 ATS update (American Thoracic Society). *J Occup Med* 1988; **30**:272–3.
15 Pereira C, Neder JA. Diretrizes para teste de função pulmonar. *J Pneumol* 2002; **28**:S2–115.
16 Juniper EF, O'Byrne PM, Guyatt GH, Ferrie PJ, King DR. Development and validation of a questionnaire to measure asthma control. *Eur Respir J* 1999; **14**:902–7.
17 Dreborg S. Skin testing. The safety of skin tests and the information obtained from using different methods and concentrations of allergen. *Allergy* 1993; **48**:473–5.
18 Cruz AA, Carvalho E. Citologia nasal quantitativa simplificada. *Rev Bras Alerg Imunopatol* 1997; **20**:56–74.
19 Hargreave FE, Pizzichini E, Pizzichini M. Induced sputum examination. *J Allergy Clin Immunol* 1998; **101** (4, Part 1):569–70.
20 Pizzichini E, Pizzichini MM, Kidney JC *et al*. Induced sputum, bronchoalveolar lavage and blood from mild asthmatics:

inflammatory cells, lymphocyte subsets and soluble markers compared. *Eur Respir J* 1998; **11**:828–34.

21 Pizzichini E, Pizzichini MM, Efthimiadis A *et al*. Indices of airway inflammation in induced sputum: reproducibility and validity of cell and fluid-phase measurements. *Am J Respir Crit Care Med* 1996; **154** (2, Part 1):308–17.

22 Chanez P, Vignola AM, Vic P *et al*. Comparison between nasal and bronchial inflammation in asthmatic and control subjects. *Am J Respir Crit Care Med* 1999; **159**:588–95.

23 Berlyne GS, Efthimiadis A, Hussack P, Groves D, Dolovich J, Hargreave FE. Sputum in asthma: color versus cell counts. *J Allergy Clin Immunol* 2000; **105** (1, Part 1):182–3.

24 Pizzichini MM, Pizzichini E, Efthimiadis A *et al*. Asthma and natural colds. Inflammatory indices in induced sputum: a feasibility study. *Am J Respir Crit Care Med* 1998; **158**:1178–84.

25 Roponen M, Kiviranta J, Seuri M, Tukiainen H, Myllykangas-Luosujarvi R, Hirvonen MR. Inflammatory mediators in nasal lavage, induced sputum and serum of employees with rheumatic and respiratory disorders. *Eur Respir J* 2001; **18**: 542–8.

26 Pizzichini MM, Pizzichini E, Clelland L *et al*. Prednisone-dependent asthma: inflammatory indices in induced sputum. *Eur Respir J* 1999; **13**:15–21.

27 Prat J, Xaubet A, Mullol J *et al*. Immunocytologic analysis of nasal cells obtained by nasal lavage: a comparative study with a standard method of cell identification. *Allergy* 1993; **48**: 587–91.

28 Simpson JL, Scott R, Boyle MJ, Gibson PG. Inflammatory subtypes in asthma: assessment and identification using induced sputum. *Respirology* 2006; **11**:54–61.