

Spirometric parameters and levels of interferon gamma and IL-5 in induced sputum from patients with allergic rhinitis or asthma

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ABSTRACT

Background: This study was designed to analyze spirometric parameters and cytokine (interferon [IFN] gamma and IL-5) levels in induced sputum from patients with asthma or allergic rhinitis (AR) and nonatopic subjects.

Methods: Thirty-three subjects aged 18–60 years were enrolled in the present study. Eight patients had asthma without AR symptoms, 16 had AR without asthma symptoms or history, and both groups had positive skin-prick test (SPT) to aeroallergens. The nine remaining subjects were healthy nonatopic subjects with negative SPT to aeroallergens. Spirometry was performed by evaluating the forced vital capacity (FVC), forced expiratory volume in the 1st second (FEV₁), and forced expiratory flow between 25 and 75% (FEF_{25–75}) of FVC before and after bronchodilator use. Induced sputum samples were also collected for measuring cytokine (IFN-gamma and IL-5) levels by enzyme-linked immunosorbent assay.

Results: Significant pre- and postbronchodilator change was observed only for FEV₁ with higher variation values in asthmatic subjects compared with patients with AR ($p < 0.05$) and nonatopic subjects ($p < 0.01$). There was no significant difference in pre- and postbronchodilator spirometric parameters among the three groups, although asthmatic patients showed lower prebronchodilator FEF_{25–75} values ($p = 0.065$). IL-5 levels were higher in induced sputum from patients with asthma and AR compared with nonatopic subjects ($p = 0.020$ and $p = 0.032$, respectively), but IFN-gamma levels showed no significant difference between the groups.

Conclusion: Spirometric measurements were not able to show any occurrence of bronchial constriction in patients with AR, but predominant IL-5 levels in induced sputum from these patients reinforce the role of Th2-type immune response in lower respiratory airways that could contribute to the concept of “one airway, one disease.”

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Allergic rhinitis (AR) and asthma are chronic inflammatory conditions of the airways characterized by typical symptoms induced by an IgE-mediated response and associated with the predominance of Th2-type immune response at a local level. The alterations promote an augment of cytokines related with this profile, mainly IL-4, IL-5, and IL-13, and an eosinophilic infiltrate in the nasal and bronchial mucosa. This altered response promotes an inflammatory process responsible for the disease symptoms.^{1–4}

IL-5 is one of the key cytokines responsible for eosinopoiesis in the bone marrow, along with recruitment and survival of eosinophils in the tissues.⁵ The presence of IL-5 in the inflammatory sites in asthmatic patients, including the ones with recent onset asthma, mild asthma, or even asymptomatic asthmatic patients, suggests that this cytokine may be considered a good marker for airway inflammation observed in asthma.^{4,6}

Although there are differences between AR and asthma symptoms, it seems that the upper and lower airways are affected by a common inflammatory process, which can be maintained and enhanced by interrelated mechanisms.¹ In this context, epidemiological studies have consistently shown that asthma and AR frequently coexist in the

same patient worldwide.⁷ AR is present in up to 80% of subjects suffering asthma^{1,8} while the prevalence of asthma in subjects with AR varies from 10 to 40%.^{9,10} AR has been associated with more severe asthma, higher difficulty to control asthma, and considerable impairment in quality of life,¹¹ and it has been shown to be a strong risk factor for the onset of asthma in adults.²

Asthma is characterized by a reversible lower airway obstruction and forced expiratory volume in the 1st second (FEV₁) is considered the gold standard with physiological evidence to support clinical diagnosis of asthma. Other alterations related to asthma include the relation of FEV₁/forced vital capacity (FVC) below 75% in adults and 86% in children and the obstruction of the airflow, which disappears or improves significantly after the usage of a β_2 -agonist.¹² On the other hand, asthma pathogenesis is associated with the involvement of small airways,¹³ and although there is no a direct parameter capable of assessing small airways, the forced expiratory flow between 25 and 75% (FEF_{25–75}) of FVC has been considered a more sensitive spirometric parameter to evaluate obstruction in small airways than FEV₁.¹⁴

Considering the close relation between AR and asthma in addition to prevalence and severity of asthma in AR patients, studies are encouraged to establish parameters for helping in early diagnosis of asthma, reinforcing the theory of “one airway, one disease.” Therefore, this study aimed to investigate the spirometric parameters and levels of IL-5 and interferon (IFN) γ cytokines in induced sputum from patients with asthma or AR.

MATERIALS AND METHODS

Patients and Study Design

This is an observational transversal analytic study that was conducted in the Laboratory of Allergy and Clinical Immunology, and Laboratory of Pulmonary Function of the University Hospital, Federal University of Uberlândia, Brazil, between May and November 2006. Twenty-four patients (18–60 years old) were recruited by the

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Table 1 Demographic and clinical characteristics of patients enrolled in the study

Characteristics	Groups		
	Asthma	Rhinitis	Control
Number (n)	8	16	9
Age (yr) Median (minimum–maximum)	39.5 (20.0–60.0)	24.5 (20.0–45.0)	30.2 (21.0–38.0)
Gender (M/F)	1/7	5/11	4/5
Allergy family history	3	6	1

Department of Nosology of the University Hospital, with clinical diagnosis of mild-to-moderate asthma without AR or mild-to-moderate persistent AR without asthma. As inclusion criteria, patients should have a positive skin-prick test (SPT) to mite (*Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, or *Blomia tropicalis*) aeroallergens associated or isolated. The exclusion criteria included patients with a diagnosis of associated pulmonary diseases or infectious intercurrents, smokers, usage of oral or inhaled corticosteroids, cromones or leukotriene receptor antagonists in the last 3 weeks, usage of antihistaminic drugs in the last week, and pregnant women or women in puerperal period up to 6 months.

As control group, nine nonatopic healthy volunteers without symptoms or clinical history of allergic diseases and with negative SPT to all tested aeroallergens were selected among age- and socioeconomic status-matched individuals. Patients and volunteers that fulfilled the inclusion criteria were evaluated by performing spirometry and induced sputum. This study was approved by the Ethics Committee in Human Research of the institution and written informed consent was obtained from all participants.

Skin-Prick Test

SPT was performed as described elsewhere,¹⁵ using a panel of standardized allergenic extracts, such as house-dust mites (*D. pteronyssinus*, *D. farinae*, and *B. tropicalis*), pet danders (*Canis familiaris* and *Felis domesticus*), cockroaches (*Blattella germanica* and *Periplaneta americana*), and mold (*Cladosporium* spp; IPI/ASAC, São Paulo, Brazil). A wheal mean of 3 mm in diameter larger than the negative control was considered to be positive.

Spirometry

Spirometry was performed using a computer-assisted spirometer (Multispiro SX/PC; Win DX Spirometry, San Clemente, CA) applying biosecurity filters for the Multispiro and following the guidelines for lung function tests.¹⁶ Spirometric parameters included the FVC, FEV₁, and FEF_{25–75} of FVC before and after use of bronchodilators (400 µg of salbutamol; Glaxo Wellcome S.A., Burgos, Spain).

Sputum Induction

The induced sputum was obtained as previously described,¹⁷ with some modifications. All volunteers inhaled previously 400 µg of salbutamol (Glaxo Wellcome S.A.) to prevent bronchial constriction. The induction was done by aerosol with an inhalation rate of 1 mL/min and particle sizes of 0.5–10 µm, produced in an ultrasonic nebulizer (Respira Max Ultra-Sonic Inhalator; NS, São Paulo, Brazil), using hypertonic saline solution (3%) for 7 minutes. When necessary for obtaining a sufficient amount of sputum samples, 4% saline solution was used during the same period. Sputum samples were collected in sterile Petri dishes (Biosystems Brasil, São Jose dos Pinhais, Brazil) and immediately transferred to sterile tubes on ice until being processed. After the induction, FEV₁ was measured to detect possible bronchial constriction induced by the hypertonic saline solution.

Sputum Processing

The collected induced sputum samples were promptly processed according to protocols described elsewhere,¹⁷ with minor modifica-

tions. Sputum samples were added (v/v) in sterile cold 0.01 M of phosphate-buffered saline solution, pH 7.2, containing 0.1% dithiothreitol (1% Sputolysin; Calbiochem Corp., San Diego, CA), followed by homogenizing 10 times with Pasteur pipette and orbital shaking for 20 minutes at room temperature. Next, samples were filtered in 40-µm nylon cell strainers (BD Biosciences Discovery Labware, Bedford, MA) and centrifuged at 900 × g for 10 minutes at 4°C. Supernatants were collected and stored at –70°C until cytokine analysis.

Measurement of IFN-γ and IL-5 in Induced Sputum

IFN-γ and IL-5 levels were measured in supernatants from induced sputum samples by a sandwich enzyme-linked immunosorbent assay according to manufacturer instructions (R&D Systems, Minneapolis, MN) in comparison with a standard curve built with the respective recombinant human cytokines. The sensitivity of each assay was 7.8 pg/mL for IFN-γ and 11.7 pg/mL for IL-5. The intra- and interassay coefficients of variations for these cytokines were below 10 and 15%, respectively.

Statistical Analysis

Statistical analysis was performed using the software GraphPad Prism 4.0 (GraphPad Software, Inc., San Diego, CA). Because the data did not show a normal distribution, nonparametric tests such as the Kruskal-Wallis and Dunn's multiple comparison tests were used for comparison among spirometric parameters or cytokine levels in the different groups. The Fisher's exact test was applied for comparison between proportions. Values of $p < 0.05$ were considered statistically significant.

RESULTS

The demographic and clinical characteristics of the study subjects are shown in Table 1. There was no significant difference in age ($p = 0.143$), gender ($p = 0.357$), and familiar history of allergy ($p = 0.339$) among the groups of patients with asthma and AR and nonatopic individuals (control group).

As shown in Table 2, the analyzed prebronchodilator spirometric parameters showed no significant difference among the patient groups, although asthmatic patients exhibited the lowest mean value for FEF_{25–75} (48.8; CI 95%, 12.5–85.2) with borderline significance level ($p = 0.065$). In the postbronchodilator evaluation, no significant difference was observed among the groups for all of the analyzed spirometric parameters. When the variation between the pre- and postbronchodilator parameters was compared, only FEV₁ showed significantly higher variation (Δ FEV₁) in the group of asthmatic patients in relation to AR patients ($p < 0.05$) and control nonatopic subjects ($p < 0.01$).

IL-5 levels detected in induced sputum samples from patients with asthma or AR were significantly higher when compared with control nonatopic subjects ($p = 0.020$ and $p = 0.032$, respectively). However, no significant difference was found between the groups of atopic patients with asthma and AR ($p = 0.560$; Fig. 1). IFN-γ levels were below the assay sensitivity in all induced sputum samples from patients with asthma, AR, and control nonatopic subjects.

Table 2 Spirometric parameters in patients with asthma, allergic rhinitis, and nonatopic subjects (controls)

Parameters	Groups		
	Asthma	Rhinitis	Controls
Prebronchodilator (% mean, CI 95%)			
FVC	92.0 (78.4–105.7)	96.9 (91.1–102.8)	93.3 (83.9–102.8)
FEV ₁	72.4 (49.5–95.4)	93.7 (85.6–101.8)	92.3 (83.1–101.6)
FEF _{25–75}	48.8 (12.5–85.2)	88.1 (71.2–105.0)	86.8 (69.1–104.6)
Posbronchodilator (% mean, CI 95%)			
FVC	91.7 (78.6–104.7)	97.1 (91.2–102.9)	94.5 (84.0–105.1)
FEV ₁	77.7 (54.9–100.5)	95.5 (87.8–103.2)	93.2 (84.6–101.8)
FEF _{25–75}	59.5 (20.5–98.5)	93.2 (78.3–108.1)	88.2 (73.8–102.6)
Pre- and posbronchodilator variation (% mean, CI 95%)			
ΔFVC	−0.35 (6.95–6.25)	0.13 (−1.00–1.26)	1.22 (−1.08–3.51)
ΔFEV ₁ *#	5.25 (2.98–7.51) ^a	1.87 (0.23–3.50) ^b	0.84 (−0.81–2.49) ^b
ΔFEF _{25–75}	10.66 (1.72–19.59)	5.08 (−1.37–11.53)	1.41 (−6.94–9.75)

^{a,b}Different letters indicate significant differences as determined by the Kruskal-Wallis and Dunn's multiple comparison tests.

* $p < 0.05$ between asthma and rhinitis groups; # $p < 0.01$ between asthma and controls groups.

FEF_{25–75} = forced expiratory flow between 25 and 75%; FEV₁ = forced expiratory volume in the 1st s; FVC = forced vital capacity.

DISCUSSION

There is a close association between AR and asthma, because the nasal and bronchial mucosa show similarities and one of the most important concepts regarding nose–lung interactions is the functional complementarities.^{18,19} On the other hand, most asthmatic patients present rhinitis whereas only a fraction of rhinitis patients present clinically obvious asthma, even though a great number of patients have nonspecific bronchial hyperreactivity.²⁰ Therefore, there should be important differences between nasal and bronchial mucosa.²¹

The present study investigated two parameters to evaluate the relationship between upper and lower airways. First, the presence of spirometric alterations in patients with AR without symptoms of asthma that could be prognostic clinical markers for alterations in lower airways, commonly found in asthmatic patients, was investigated and compared with asthmatic patients and nonatopic controls. Contrasting with other studies that showed altered values mainly for FEF_{25–75} in AR patients,^{22–25} we did not find a significant difference between the AR group and controls in all spirometric parameters considered. However, asthmatic patients exhibited the lowest FEF_{25–75} value in prebronchodilator analyses, even though presenting borderline significance level. These data support the role and strength of this parameter as a marker of bronchial involvement in asthmatic patients as previously suggested by other authors.^{14,23,25} It is important to emphasize that differences may be minimal because of sample size.

When the variation between the pre- and postbronchodilator parameters was compared, only FEV₁ showed significantly higher variation (ΔFEV₁) in the group of asthmatic patients in relation to AR patients ($p < 0.05$) and control nonatopic subjects ($p < 0.01$), showing the reversible obstruction that characterized the lower airway of asthmatic patients, distinct of other groups, in according with American Thoracic Society and European Respiratory Society guidelines.²⁶

Second, the potential occurrence of alterations in cytokine profiles in a local level of lower airways was evaluated in induced sputum from asthmatic or AR patients and nonatopic subjects. One of the recent advances in the investigation of the airway inflammation has been the procedure of sputum induction by inhalation of an aerosol containing hypertonic saline solution to obtain secretions directly from the lower airways in various pathologies of the respiratory system.¹⁷ This noninvasive method to achieve secretions may allow a better knowledge of the inflammation profile and the opportunity for follow-up of asthma management by evaluating inflammatory markers in the local level. The analysis of the profile of Th1 and Th2 proinflammatory cytokines in the induced sputum from allergic pa-

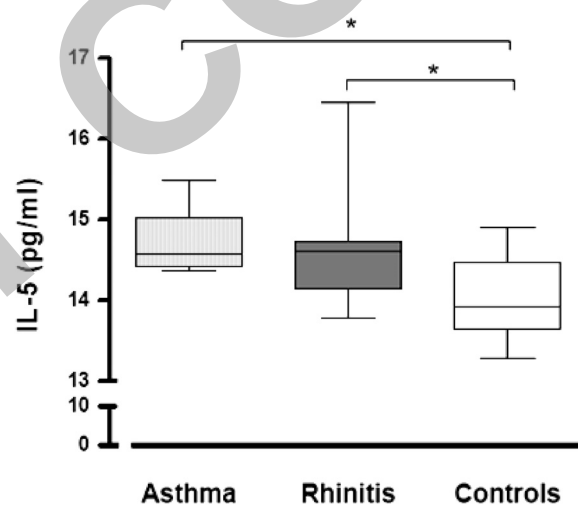


Figure 1. IL-5 levels in induced sputum samples from patients with asthma, allergic rhinitis, and control nonatopic individuals. Data are expressed in a box plot with median and 25–75% quartiles. * $p < 0.05$ as determined by the Kruskal-Wallis and Dunn multiple comparison tests.

tients has allowed establishing determinant factors for the early diagnosis of asthma.¹

In the present study, we used this noninvasive method to obtain the sputum with safety in AR and asthmatic patients as well as in control nonatopic subjects for investigating probable differences in cytokine profile between the diseases. IFN- γ levels in induced sputum were below the assay sensitivity in all study groups. Interestingly, IL-5 levels were significantly higher in induced sputum from atopic (AR and asthma) patients in relation to control nonatopic subjects, but not between asthmatic and AR patients. This finding suggests the induction of a Th2-type immune response in the local level, the lower airways from both AR and asthma patients. Our results corroborate the findings of a recent study²⁷ that detected higher levels of IL-5 and vascular endothelial growth factor mRNA in induced sputum from AR patients with airway hyperresponsiveness compared with AR patients without airway hyperresponsiveness, but lower levels of these cytokine mRNA when compared with asthmatic patients. Likewise, no significant difference in levels of IFN- γ mRNA was found between the groups.²⁷ In conclusion, this study highlights the close relationship between upper and lower airways, showing increased

IL-5 levels in induced sputum of AR patients without asthma symptoms and reinforcing the concept of "one airway, one disease."

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