Comparison of Induced Sputum Cell Counts in COPD and Asthma

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Abstract

Introduction : Airway obstruction and inflammation are characteristic features of asthma and COPD. Induced sputum may provide an alternative method in the investigation of airway inflammation.

Aim: The aim of this study was to demonstrate and compare the relative proportion of the cells in induced sputum samples in patients with asthma and COPD.

Materials and Methods: A group of 30 patients with mild to moderate asthma and a group of 20 patients moderate to severe COPD were studied. Spirometry with assessment of reversibility were recorded. Sputum was induced with inhalation of 3% hypertonic saline solution. Total and differential cell counts of sputum samples were determined.

Results: Neutrophils were the predominant cells in the induced sputum samples of COPD patients and eosinophils were predominant in the samples from asthmatics. Induced sputum lymphocyte and macrophage counts were significantly higher in asthma than COPD.

Conclusions: Increase in sputum neutrophils is characteristic of COPD patients, while an increase in eosinophils is found in asthma. Induced sputum procedure is a noninvasive, safe method for the determination of predominant cells of airway inflammation.

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Introduction

Airway obstruction and inflammation are characteristic features of asthma and chronic obstructive pulmonary disease (COPD). However, important differences exist between these two conditions with respect to both the obstruction and inflammation processes. Airway obstruction is usually reversible in asthma while no short term improvement to bronchodilators is seen in COPD (1). Predominantly eosinophilic inflammation has been reported in asthma and neutrophilic inflammation in COPD (2,3).

BAL, biopsy and induced sputum are several direct methods to assess airway inflammation (4,5). Sputum induction was recently proposed as a noninvasive method for obtaining airway secretions. It is possible to obtain samples from the lower airways with minimal discomfort to the patient by sputum induction (6). Comparative studies on inflammatory markers in induced sputum samples of COPD and asthmatic patients have been reported. Most of these studies have shown, as expected, eosinophils in induced sputum samples were higher in asthma

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than COPD (7,8). However, publications reporting opposite findings in these diseases also exist (9,10), suggesting that an increase in sputum eosinophils in asthma and neutrophils in COPD is not a universal finding which applies to all cases. In this respect, assessment of airway inflammation is important, since treatment methods are different in asthmatic and COPD patients.

In this study, our aim was to compare the total and differential cell counts of inflammatory cells in induced sputum samples in patients with asthma and COPD.

Materials and Methods

Twenty COPD patients and 30 patients with asthma presenting to the Outpatient Department of the Kocaeli University Hospital were included in the study. A postbronchodilator FEV_1<80% of the predicted, a FEV_1/FVC ratio lower than 70%, and a smoking history of at least 10 pack-years were the nclusion criteria for the COPD patients. To be a nonsmoker with a stable asthmatic condition and to show a reversibility greater than 12% in predicted FEV_1 following inhaled salbutamol were the inclusion criteria for the asthma patients.

Subjects who had suffered from a respiratory tract infection or exacerbation of airway diseases within the previous 8 weeks, those who had taken inhaled or oral steroids within the past 4 weeks and those who had inadequate sputum despite three induction procedures on separate days were not included in the study.

The study was approved by the local ethics committee and all patients gave informed consent.

All patients were receiving treatment with combined bronchodilator drugs before the study. The study was conducted in two consecutive days. On day 1, the history was reviewed, clinical examination and spirometry were performed. Reversibility was also assessed by spirometry and recorded. On day 2, sputum induction procedure was performed. In patients who were not able to produce a sufficient amount of sputum, the procedure was repeated three times on separate days. Patients who had inadequate sputum despite three induction procedures were excluded from the study.

All subjects were first premedicated with 200 mg salbutamol administered by a metered dose inhaler that was adapted to a spacer. The sputum was induced by using the method of Pin and coworkers (11). For the induction process, a Pulmo-Aide ultrasonic nebulizer with an output of 0.35 ml/min and particle size of 5 mm was used. Nebulizaton was done using a 3% hypertonic saline solution. Nebulization time consisted of 5 minute intervals until a maximum nebulization time of 30 minutes was reached. After each period of inhalation PEF was measured. To minimize contamination with saliva and with postnasal drip, the subjects were asked to rinse their mouths with water, to swallow the water and to blow their noses. Then they were encouraged to expectorate their sputum into a sterile container. The procedure was continued until either a sufficient amount (±1 ml) of sputum was obtained or maximum nebulization time of 30 minutes was reached (11,12).

Sputum samples were processed within 2 hours after the collection, according to a protocol validated by Popov *et al* with modifications (13). The volume of induced sputum was measured and mixed with an equal volume of 1% sputalysin (Dithiothreitol, Sigma, Italy) diluted to 0.1% by the addition of distilled water just before the procedure. The mixture was incubated at room temperature for 20 min, and during this time vortexed every 5 min to ensure homogenization and maximize cell dispersion. To stop the effect of DTT (dithiothreitol) on the cell suspension, an equal volume of PBS (phosphate buffered saline) was added. The mixture was then centrifugated at 1500 rpm for 10 minutes. Supernatants were aspirated and the cell pellets were resuspended with PBS to obtain a final volume 2-5 ml, then filtered through a gauze (pore size approximately 1 mm) to remove mucus and cell debris.

Total cell counts were performed in a hemocytometer (Thoma). The cell suspension was adjusted to $1x10^6$ cells/ml and cytospin slides were prepared using 50 ml of the cell suspension (Model 3 cytospin; Shandon Scientific, Sewickley, PA). The slides were air-dried and stained by Giemsa and 200-400 nonsquamous cells were counted by the blinded investigator (cytopathologist). If > 80% of the cells consisted of squamous cells, the quality of the sputum sample was judged unsatisfactory and excluded from the analysis.

Spirometry was performed using a Sensormedics Vmax 20C. FEV_1, FVC, FEV_1/FVC and VC were measured.

The data were expressed as means and standard deviaton values. The Kruskal Wallis test was used to estimate the differences among groups with regard to various parameters. In case of a significant difference between groups, nonparametric Mann-Whitney U test was used for intergroup comparisons. A p value less than 0.05 was considered significant.

Results

The demographic features of 30 asthmatic and 20 COPD patients are shown in Table 1. Mean age was significantly higher in the COPD group than asthmatics ($p<0.05$).

Table 1. Demographic characteristics and respiratory function in COPD and asthma patients

	COPD	Asthma
N	20	30
Sex (M/F)	16/4	7/23
Age, years (mean SD)	62.8±3.30	41.9±9.8
Smoking history (pack-years)	64±7	-
FEV_1,% predicted	45.4±10	95.3±12.8
FVC,% predicted	55±66.6	108.3±13.5
FEV_1/FVC,%	58.3±11.1	75.2±6.08

Approximately 80% of subjects were male in the COPD group while 80% of the asthmatics were female.

Induced sputum total cell counts were higher in the COPD group compared to the asthmatics, but the difference did not reach statistical significance ($p>0.05$). Sputum differential cell counts showed a predominance of neutrophils in COPD patients while eosinophils, lymphocytes and macrophages were more frequently seen in asthma patients. All these differences between the two groups were statistically significant (Table 2, Figure 1).

Discussion

All patients produced a sufficient sputum sample without significant decrease on PEF values or any unpleasant symptomatology. All patients with COPD were able to produce sputum in the first attempt while three induction procedures were required in some patients with asthma. We conclude that induced sputum is a safe, noninvasive, simple method to obtain airway secretions.

Previous studies have used induced sputum to determine airway inflammation (8,11), to compare the inflammatory markers with other direct methods (14) and to assess

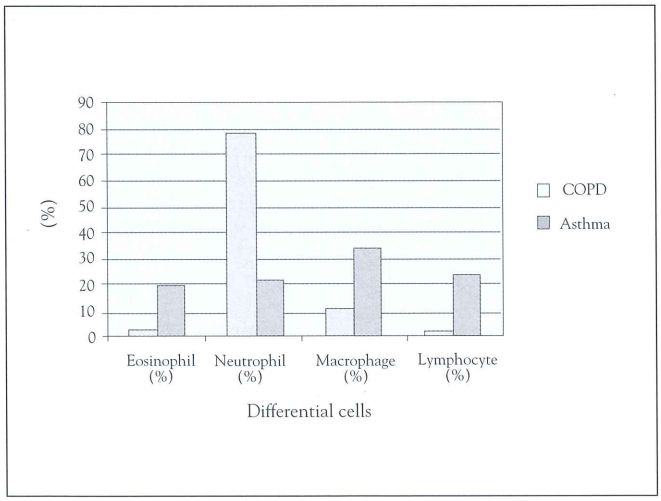

Figure 1. *Differential cell counts of induced sputum.*

Table 2. Total and differential cell counts of induced sputum in COPD and asthma patients

	COPD	Asthma	P
Total cell count	3.47±1.07	2.03±1.22	0.002*
($x10^6$ cells/g)			
Eosinophils (%)	3±1	20±6	0.002*
Neutrophils (%)	78±11	22±9	0.002*
Macrophages (%)	11±4	34±14	0.002*
Lymphocytes (%)	1.3±1.1	24±14	0.002*

* $p<0.05$ statistically significant

response to anti-inflammatory therapy (15). Despite its safety and repeatability, induced sputum is not yet accepted as a routine method for assessment of airway inflammation. This might be related to difficulty of the sputum processing method requiring specially trained personnel for the conduction of the process and evaluation of the induced sputum samples. Development of faster and easier methods for sputum processing might be helpful to incorporate the sputum method into routine clinical practice.

In this study, we determined total and differential cell counts in induced sputum samples. As reported previously, we found that in COPD, neutrophils were the predominant sputum cells (3,16). On the other hand, some authors reported airway eosinophilia in patients with COPD (10), and eosinophilic inflammation in these patients was found especially in exacerbation periods (17). Our subjects with COPD were stable patients who had moderate to severe disease and none of them showed reversibility after inhalation of salbutamol. It is possible that patients with less severe disease and reversible airway obstruction may have higher eosinophil ratios in the sputum samples than the more severe cases of COPD with irreversible airway obstruction.

Previous studies have reported significantly higher percentages of eosinophils in asthmatic patients as compared to healthy controls (2,10,18). Reported eosinophil ratios in induced sputum samples of asthmatic patients varies from 10% to 29% depending on asthma severity (9,19,20). In our study, a mean value of 20% was found for the percentage of eosinophils in the differential cell counts performed on sputum samples collected from this group of mild to moderate persistent asthmatics. This figure is slightly higher than previously reported values in this subgroup of asthmatics. We did not include mild intermittent and severe persistent asthmatics in the study and the number of mild persistent and moderate persistent asthmatics is not enough to suggest an association between sputum eosinophil counts and asthma severity. Louis *et al.* reported a mean percentage value of 5% for sputum eosinophils in mild intermittant asthmatics while this ratio was 28.7% in severe persistent

asthmatic patients (9). These authors also reported a significant increase in sputum neutrophil counts in severe persistent asthma. They found sputum neutrophil counts of 33.6% in severe persistent asthma and of 27% in mild to moderate asthma. Our findings are similar to these results. Green *et al.* reported neutrophil counts >65% in induced sputum samples of some asthmatic patients and suggested that sputum neutrophilia is associated with poor response to corticosteroid therapy (21). They also found that these neutrophilic patients were older and more likely to be non-atopic. None of our asthmatic patients showed neutrophil predominance in induced sputum samples. However, we believe that the findings reported by Green *et al.* are important and may be of help in identifying patients with poor response to anti-inflammatory therapy. Further studies are needed to determine whether irreversible airway obstruction in patients with chronic asthma is associated with increased percentages of neutrophils in the airways.

Previous studies comparing induced sputum inflammatory markers in asthma and COPD, have shown some differences between these two groups of patients (1,7,10). Keatings and Barnes found high concentrations of neutrophils and neutrophil activation markers in induced sputum samples of patients with COPD (22). They also found that despite a lower ratio of eosinophils in COPD patients compared to the asthmatics, eosinophil activation markers were increased in induced sputum samples from both groups. The authors suggested that the eosinophils present were highly activated in COPD.

Together, these data reveal that there are subgroups of patients among asthmatics in whom the neutrophils predominate the inflammation and subgroups of patients among COPD cases in whom the eosinophils predominate the inflammation. It is important to identify these groups for adequate management of asthma and COPD. Obtaining sputum by induction is a simple, safe and noninvasive method. This method may be used to determine the predominant cells in airway inflammation and possibly may be of use to predict the response to anti-inflammatory therapy.

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