



# Airway inflammation in patients affected by obstructive sleep apnea syndrome

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## KEYWORDS

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**Summary** Obstructive sleep apnea syndrome (OSAS) has been shown to be associated to upper airway inflammation. The object of the present study was to establish the presence of bronchial inflammation in OSAS subjects.

In 16 subjects affected by OSAS, and in 14 healthy volunteers, airway inflammation was detected by the cellular analysis of the induced sputum.

OSAS patients, as compared to control subjects, showed a higher percentage of neutrophils ( $66.7 \pm 18.9$  vs.  $25.8 \pm 15.6$ ) ( $P < 0.001$ ) and a lower percentage of macrophages ( $29.4 \pm 18.4$  vs.  $70.8 \pm 15.3$ ) ( $P < 0.001$ ). The percentage of eosinophils and lymphocytes were not significantly different in the two groups.

OSAS subjects show bronchial inflammation characterized by a significant increase in neutrophils.

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## Introduction

Obstructive sleep apnea syndrome (OSAS) is characterized by repetitive episodes of upper airway occlusion during sleep. Nasal inflammation, uvula mucosal congestion and airway hyperresponsiveness has been shown to be associated with OSAS.<sup>1–3</sup> Upper airways and in particular nasal inflammation are believed to be the consequence of the mechanical stress associated to the obstruction to the air passage characteristic of the disease. To the contrary, little or no data is available in literature on the presence of bronchial inflammation in OSAS. Olopade et al.<sup>4</sup> have shown an increase in exhaled pentane, a marker of airway inflammation, in OSAS subjects in the morning suggesting the develop-

ment of airway inflammation during sleep. More recently, Carpagnano et al.<sup>5</sup> have shown in the breath condensate of OSAS patients an increase of two markers of inflammation and oxidative stress, IL6 and 8-isoprostane, suggesting that inflammation and oxidative stress are characteristic in the airways of OSAS patients. However, although these studies provide evidence about the presence of biomarkers of bronchial inflammation, no data on the inflammatory cell profile are available. We, therefore, investigated the bronchial inflammatory cell profile in OSAS patients by utilizing the analysis of the induced sputum.

## Methods

### Subjects

Sixteen consecutive patients (14 males, age  $60 \pm 10$  years, BMI  $37 \pm 9$ ) affected by OSAS and 14 age-

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matched healthy subjects (9 males, age  $56 \pm 4$  years, BMI  $21 \pm 1$ ) chosen as the control group were enrolled. All subjects had no history of asthma, chronic bronchitis, wheezing or allergies or other systemic diseases that might contribute to sleep disorders, were no smokers, and free from steroids or other anti-inflammatory drugs. None of the subjects were using CPAP therapy. Informed consent was obtained from all subjects and Institution Ethic Committee approved the study.

All subjects underwent to the Berlin Questionnaire,<sup>6</sup> a pulmonary function test, a nocturnal oxymetry and induced sputum. Because all healthy subjects presented no risk of OSAS at the Berlin Questionnaire and no alterations of nocturnal oxymetry, only the OSAS subjects underwent to a polysomnography.

### Nocturnal oxymetry

Nocturnal haemoglobin oxygen saturation was evaluated by finger pulse oxymetry (Minolta, Pulsox DP 8, Japan). A pattern of oxymetry tracing positive for OSAS was defined as a cyclical oxygen desaturation defined as a drop in oxygen saturation  $\geq 4\%$ .

### Pulmonary function testing

Pulmonary function tests (Vmax 6200 Autobox, SensorMedics, Yorba Linda, CA) were performed prior to entry into the study in order to exclude an obstructive lung disease defined as a FEV<sub>1</sub>%pred of less than 80 and a FEV<sub>1</sub>/FVC ratio of less than 0.7.

### Polysomnography

The OSAS subjects were evaluated in the sleep laboratory with an overnight polysomnography (Sleep Lab 1000p, Aequitron Medical, MN, USA). Sleep disordered breathing was quantified by RDI (Respiratory Disturbance Index), as the ratio of the number of respiratory events (apneas + hypopneas) per hour of sleep. Apneas were defined as the total cessation of airflow for at least 10 s in duration. Hypopneas were defined as a 50% or greater reduction in tidal volume from baseline value lasting at least 10 s. An RDI larger than 10 was considered diagnostic of OSAS.

### Induced sputum technique

#### Inhalation procedure

After baseline FEV<sub>1</sub> and FVC measurements, salbutamol was given by inhalation (200  $\mu$ g by a

metered-dose inhaler) and subjects inhaled hypertonic (4.5%) saline nebulized for three periods of 5 min each. An ultrasonic nebulizer (De Vilbiss 65, De Vilbiss Corporation, Somerset, PA, USA) nebulized saline solutions.

### Sputum processing

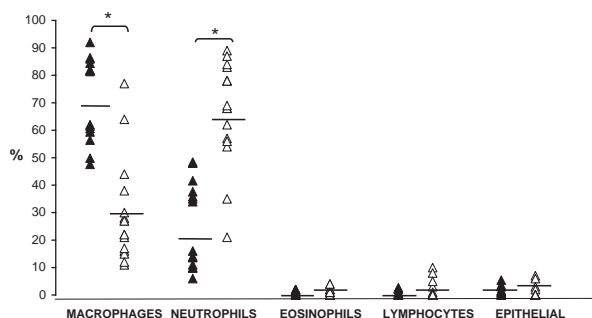
The collected sputum samples were examined within 2 h. Selected portions of the sputum sample originating from the lower respiratory tract were analyzed. Dithiothreitol (DTT, Sputolysin, Calbiochem Corp, San Diego, CA, USA), freshly prepared in a dilution of one in 10 with distilled water, was added in a volume (in  $\mu$ l) equal to 4 times the weight of the sputum portion (in mg). Selected sputum was placed in a shaking water bath at 37°C for 20 min and homogenized. It was further diluted with Phosphate Buffered Saline (PBS) in a volume equal to the sputum plus DTT. The suspension was filtered through gauze to remove mucus and was centrifuged at 1000 rpm for 5 min. The cell pellet was resuspended in a volume of PBS equal to that of the sputum plus DTT and PBS as above. Total cell count (TCC) and viability (Trypan blue exclusion method) were determined using a Burkert's chamber haemocytometer. The cell suspension was placed in a Shandon 3 cytocentrifuge (Shandon Southern Instruments, Sewickley, PA, USA) and cytopins were prepared at 450 rpm for 6 min. Cytospin slides were fixed by methanol and stained by May Grunwald Giemsa for an overall differential cell count on 500 nucleated nonsquamous cells. Only samples with cell viability  $> 50\%$  and squamous cell contamination  $< 20\%$  were considered adequate.

### Statistical analysis

Mann-Whitney *U*-test was used to analyze the differences between the two groups. Spearman Rank test was used to analyze the effect of BMI on the % cells. Values are reported as mean  $\pm$  SD. Significance was defined as a *P* value  $< 0.05$ .

## Results

All subjects had a normal pulmonary function test; FVC%pred was  $109 \pm 15$  vs.  $102 \pm 9$ , and FEV<sub>1</sub>%pred was  $109 \pm 15$  vs.  $103 \pm 11$  in the control and OSAS group respectively. BMI was significantly higher in the OSAS subjects ( $P < 0.001$ ). RDI in the OSAS group was  $44 \pm 25$  (range 13–95). Figure 1 shows the cellular profile of the induced sputum in the control and OSAS group. A significantly higher percentage of neutrophils ( $66.7 \pm 18.9$  vs.  $25.8 \pm 15.6$ )



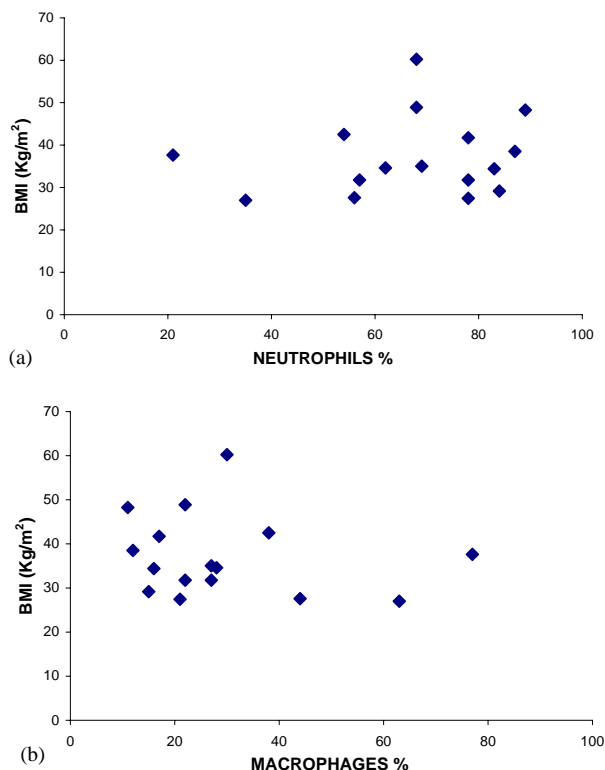
**Figure 1** Induced sputum cellular profile in the OSAS group (empty triangles) and in the control group (filled triangles) as % of the total cell count. \*,  $P < 0.001$  vs. control.

( $P < 0.001$ ), and a significantly lower percentage of macrophages ( $29.4 \pm 18.4$  vs.  $70.8 \pm 15.3$ ) ( $P < 0.001$ ) were detected in the induced sputum of the OSAS group as compared to the control group. The relative percentage of eosinophils ( $1.1 \pm 1.4$  vs.  $0.8 \pm 0.8$ ), lymphocytes ( $1.8 \pm 3.2$  vs.  $0.8 \pm 1.0$ ) and epithelial cells ( $1.1 \pm 2.3$  vs.  $1.8 \pm 1.7$ ) in the OSAS and control group, respectively, were not statistically different. The relative percentage of lymphocytes was somewhat higher in the OSAS population for the presence of two subjects with a 10% and 8% of lymphocytes. There was no significant correlation between BMI and any of the cellular components of the induced sputum within the OSAS group (Fig. 2).

## Discussion

In the present study we have shown that patients affected by OSAS present a variable degree of neutrophilic bronchial inflammation.

There is evidence in the literature that patients affected by OSAS develop upper airways inflammation. Rubinstein et al.<sup>1</sup> have shown in the nasal mucosa of patients affected by OSAS, an increase in the percentage of PMN (Polymorphonuclear Leukocyte), as well as an increase in other mediators of inflammation such as bradykinin and VIP (Vasoactive Intestinal Peptide), suggesting a pathogenetic role of the nasal inflammation in the upper airway obstruction characteristic of the OSAS. In addition, Sekosan et al.<sup>2</sup> have shown, in OSAS patients, that the uvula is thicker than normal and that in the lamina propria there is an increase in the number of leukocytes suggesting, again, that soft palate inflammation contributes to the upper airway occlusion observed during sleep. The reports on the presence of bronchial inflammation in the OSAS



**Figure 2** Percentage of neutrophils and macrophages vs. BMI in the OSAS subjects. The correlation between the two cellular components and BMI was not significant (neutrophils vs. BMI:  $r = 0.14$ ; macrophages vs. BMI:  $r = -0.19$ ).

patients are less conclusive. Even though markers of airway inflammation and oxidative stress have been shown in previous studies<sup>4,5</sup> in OSAS patients, the technique used to sample the markers could not differentiate the upper airways from the bronchial compartment.

In the present study in order to investigate the bronchial inflammatory profile of the OSAS population we have utilized the analysis of induced sputum, a standardized and widely used technique.<sup>7</sup> The OSAS patients showed a variable degree of neutrophilic airway inflammation. Despite the study was on a limited number of subjects, and a larger population study is desirable, the difference in the percentage of neutrophils and macrophages in the sputum between the two groups was large enough to make the statistic highly significant. A possible bias of this study is the lack of polysomnography in the control group. However, we feel confident that the nocturnal oxymetry in association with the Berlin Questionnaire were able to screen out the OSAS cases by the control group.

There are a number of reasons why bronchial inflammation may be present in OSAS. The mechanical stress exerted on the mucosa of the respiratory

system by the snoring, proposed as responsible of the upper airway inflammation observed in the OSAS population, may also be responsible of the bronchial inflammation. We speculate that the pressure gradient caused by the intermittent obstruction is likely transmitted to all the respiratory system. As theorized for the uvula and nasal inflammation present in patients with OSAS,<sup>8</sup> the snoring-related mechanical trauma may not be the sole mechanism underlying the bronchial inflammation we have found in the present study. Bronchial inflammation may be caused by the activation of the neural receptors by proinflammatory neuropeptides and peptides causing bronchial edema. Hypoxemia and obesity, alone and in combination, are also theoretical potential causes of bronchial inflammation and their independent role merit further investigation. In the present study the potential effect of obesity on bronchial inflammation has not been studied. To our knowledge, no data are available on a positive correlation between obesity and bronchial inflammation. Carpagnano et al.<sup>5</sup> did not find a clear correlation between obesity alone and biomarkers of inflammation in the breath condensate. In addition, in the present study no correlation was found when BMI was plotted vs. % of neutrophils.

In conclusion obese patients affected by OSAS display neutrophilic airway inflammation. Airway inflammation may be the consequence of the mechanical stress on the mucosa because of the intermittent obstruction of the upper airways

typical of the OSAS. Bronchial inflammation, however induced, may contribute to the pathogenesis of the disease by determining further reduction of the airway caliber. Further studies are necessary in order to assess the effect of therapeutic interventions such as the CPAP and anti-inflammatory agents on the degree of the OSAS-induced bronchial inflammation.

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