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Original Article

Obstructive sleep apnea exacerbates airway inflammation in patients with chronic obstructive pulmonary disease

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ABSTRACT

Background: Chronic obstructive pulmonary disease (COPD) concomitant with obstructive sleep apnea (OSA) is known as overlap syndrome. It has an increased rate of hospitalization due to COPD exacerbation which is believed to indicate a worsening of the underlying chronic airway inflammation. Therefore, the aim of this prospective study was to explore whether OSA exacerbates airway inflammation in subjects with COPD by examining the bronchoalveolar lavage (BAL) fluid.

Methods: This prospective study included 47 patients with overlap syndrome and 28 patients with moderate-to-severe stage stable COPD. Twenty-five patients with overlap syndrome adhered to the continuous positive airway pressure (CPAP) treatment; the remaining patients either refused CPAP treatment or discontinued it within 2 weeks owing to adverse effects or other reasons. BAL fluid was collected from all subjects for the evaluation of cell numbers and tumor necrosis factor alpha (TNFα) and interleukin-8 (IL-8) levels.

Results: The BAL fluid of patients with overlap syndrome showed a significantly increased proportion of neutrophils and higher TNFα concentration and IL-8 levels than that of COPD patients; however, the serum CRP levels were not significantly different. An association was found between the percentage of neutrophils and the TNFα concentration and IL-8 levels. Moreover, the TNFα concentration was significantly correlated with the percentage of nighttime spent with oxygen saturation less than 90%. After CPAP treatment, airway inflammation was found to decrease significantly.

Conclusions: OSA exacerbates airway inflammation in COPD patients. CPAP treatment can improve nocturnal hypoxemia and decrease airway inflammation.

1. Introduction

Obstructive sleep apnea (OSA) is characterized by repetitive occlusion of the upper airway during sleep, which is usually accompanied by intermittent hypoxia-/reoxygenation episodes; this can trigger upper airway and systemic inflammation and increase the expression of inflammatory mediators [1,2]. Chronic obstructive pulmonary disease (COPD) is characterized by chronic airway inflammation leading to progressive decline in lung function and is the fourth leading cause of death worldwide; it also affects approximately 13.9% of the adult population in the United States [3].

The coexistence and association of both COPD and OSA in a single patient is defined as "overlap syndrome" [4]. A large epidemiologic study found that the prevalence of overlap syndrome in adult men is approximately 1% [5]. Compared with patients with COPD only, patients with overlap syndrome have an increased risk of hospitalization for COPD exacerbation. Although such patients can be treated with continuous positive airway pressure (CPAP) [6], the mechanisms of OSA leading to higher frequency of COPD exacerbation and improvement after CPAP treatment remain unclear.

Exacerbation of COPD is believed to indicate a worsening of the underlying chronic airway inflammation, as the frequency of COPD exacerbation is associated with disease severity [7] and a further increase in airway inflammation [8]. The disease severity correlates with the number of neutrophils found in biopsy specimens and sputum [9,10], and patients with frequent COPD exacerbation have higher sputum interleukin-6 (IL-6) and interleukin-8 (IL-8) levels both at exacerbation and in the stable state compared with patients with infrequent exacerbation [11]. Increased IL-8 and tumor necrosis factor alpha (TNFα) levels in the lungs of patients with stable COPD have also been found to recruit neutrophils to the airways [12], thus creating a vicious cycle that leads to progressive

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development of the disease. Several studies have also reported airway inflammation in OSA patients [13,14]. Devouassoux et al. [13] found that neutrophilia and IL-8 concentration were higher in the bronchial airways of untreated OSA patients compared with controls and that IL-8 levels in the sputum supernatant were correlated with the apnea–hypopnea index (AHI). Aihara et al. [14] demonstrated that sputum IL-6, IL-8, TNF-α, and vascular endothelial growth factor (VEGF) levels were significantly correlated with the respiratory disturbance index or oxygen desaturation index (ODI) and that sputum IL-6, IL-8, TNFα, and VEGF levels were also significantly related to the sputum neutrophil number.

The above-mentioned studies indicated that both diseases are characterized by local inflammation, but their interaction has not yet been clarified. We hypothesize that OSA may contribute to COPD by exaggerating airway inflammation associated with nocturnal intermittent hypoxemia, and CPAP treatment can favorably improve airway inflammation in cases of overlap syndrome. Therefore, the aim of this prospective study was to explore whether OSA exacerbates airway inflammation in subjects with COPD by examining the bronchoalveolar lavage (BAL) fluid. We also examined the relationship between nocturnal hypoxemia and airway inflammation and the effect of CPAP treatment on airway inflammation. Specifically, we studied the percentage of neutrophils and the levels of the inflammatory mediators TNFα and IL-8 in BAL fluid and the levels of C-reactive protein (CRP) in serum.

2. Methods

2.1. Study patients

For this prospective study, between July 2010 and December 2013, we enrolled 168 consecutive patients who reported habitual snoring and were diagnosed with obstructive airway disease according to physical and spirometry examinations conducted at the Division of Respiratory Diseases at Renmin Hospital of Wuhan University and Xiangyang Hospital of Hubei University of Medicine.

All patients with moderate-to-severe COPD (GOLD stage II or III), diagnosed in accordance with the GOLD guidelines [15], were studied when they were clinically stable for at least 6 weeks. Ninety-three subjects were eligible for this study according to the following inclusion criteria: age > 40 years, abstinence from smoking for at least 2 years, no history of systemic diseases, and free from oral corticosteroid or other anti-inflammatory drug use for at least 6 months. The exclusion criteria were as follows: presence of pneumonia or other lung diseases, history of asthma or use of theophylline preparations during the 2 weeks before admission, symptoms of gastroesophageal reflux disease (GERD), forced expiratory volume at 1 s (FEV1) <30% of predicted, and administration of CPAP treatment before admission [16–18]. Furthermore, 10 patients did not provide consent to participate in this study, and fiberoptic bronchoscopy could not be completed in eight patients. Finally, 75 patients were successfully enrolled in the study (Fig. 1). The study was conducted with the approval of the Ethics Committee of Renmin Hospital of Wuhan University and Xiangyang Hospital of Hubei University of Medicine, and written informed consent was obtained from each of the subjects prior to participation in the study. All subjects underwent pulmonary function testing, overnight polysomnography, and bronchoscopy.

2.2. Pulmonary function testing

Pulmonary function tests (Master Screen PFT, Erich Jaeger GmbH, Germany) were performed prior to enrollment into the study to further confirm the diagnosis of obstructive lung disease and to exclude cases of restrictive lung disease, asthma, and very-severe stage COPD.

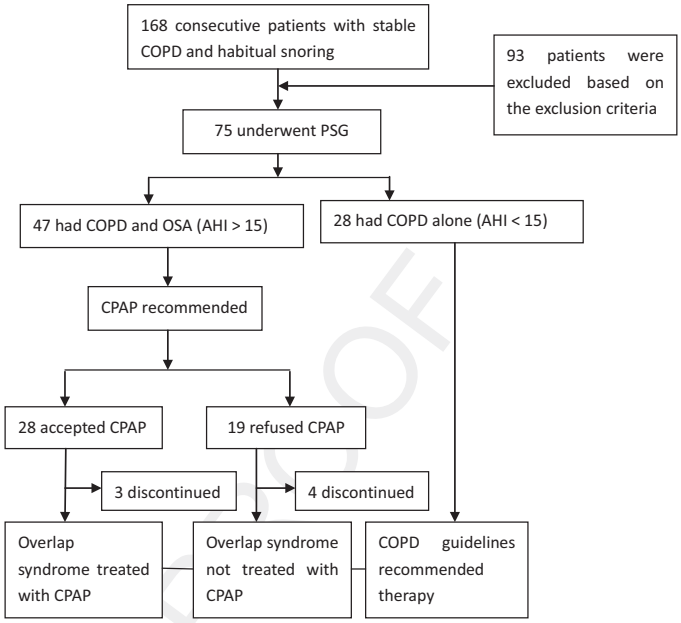

Fig. 1. Selection and treatment of the study cohort. COPD, chronic obstructive pulmonary disease; OSA, obstructive sleep apnea; AHI, apnea–hypopnea index; CPAP, continuous positive airway pressure.

2.3. Polysomnography (PSG)

All subjects underwent full-night PSG using a digital system (Compumedics E-series EEG/PSG recording system, Australia) at the sleep laboratories of Renmin Hospital of Wuhan University and Xiangyang Hospital of Hubei University of Medicine. The parameters recommended by the American Academy of Sleep Medicine (AASM) were monitored simultaneously and continuously: airflow detection via thermocouple and nasal pressure, electroencephalogram, electrooculogram, electrocardiogram, electromyogram (chin and leg muscles), thoracic and abdominal respiratory effort using inductance plethysmography, snoring, body position, oxyhemoglobin saturation (SpO_2), and pulse rate. The findings were scored in accordance with the criteria outlined in the 2007 AASM Manual (v.1) for Scoring Sleep and Associated Events [19]. Hypopneas were defined as events with ≥30% decrease of baseline amplitude in nasal pressure for ≥10 s, accompanied by a ≥4% decrease in SpO_2 from the pre-event baseline.

2.4. Bronchoscopy

As described previously [20], flexible fiberoptic bronchoscopy was performed in the morning at approximately 9:00 a.m. following full-night PSG monitoring to avoid diurnal variations. Briefly, under local anesthesia with topical lidocaine, the BAL fluid was collected from a segment of the middle lobe or the lingula after instillation of 100 mL of sterile warmed saline. The sample was collected in a sterile container, immediately filtered through gauze swabs, and then centrifuged at 400× *g* for 10 min at 4 °C to separate the fluid from cells. The supernatant was collected for later analysis, and the cell pellet was washed in phosphate-buffered saline solution. Total cell counts were obtained with a hemocytometer. Cytospins were prepared using a Shandon cytocentrifuge (Shandon, PA, USA), and the differential cell count was performed using Wright–Giemsa staining by counting 200 nucleated non-squamous cells per sample. Viability was ascertained by the Trypan blue exclusion method.

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Table 1
Clinical characteristics of the patients with COPD alone and those with overlap syndrome.

Characteristic	COPD ($N = 28$)	Overlap syndrome ($N = 47$)	p-value
Age (years)	58.6 ± 5.9	60.5 ± 5.2	0.153
Sex (male:female)	26:2	43:4	0.601
COPD exacerbation (events/24 min)	1.0 (0, 2.0)	2.0 (1.0, 2.0)	0.009
FEV1 (L)	1.40 ± 0.41	1.35 ± 0.30	0.529
FEV1 % predicted	49.0 (40.5, 63.0)	46.4 (41.4, 54.4)	0.360
FEV1/FVC ratio (%)	62.1 (48.5, 67.4)	58.3 (54.1, 62.9)	0.283
PaO_2 (mmHg)	86.6 (82.5, 88.6)	83.8 (77.2, 90.8)	0.641
$PaCO_2$ (mmHg)	39.0 (37.5, 40.7)	40.5 (37.3, 42.8)	0.156
BMI (kg/m^2)	30.4 ± 2.1	31.4 ± 2.1	0.051
AHI (events/h)	1.7 (0.8, 2.7)	34.0 (24.4, 43.7)	<0.001
$T_{SpO_2<90\%}$ (%)	0.19 (0, 0.85)	3.70 (2.41, 8.53)	<0.001
Mean SpO_2 (%)	93.8 ± 2.5	91.5 ± 2.6	<0.001
Nadir SpO_2 (%)	88.1 ± 2.4	80.3 ± 5.2	<0.001
ODI (events/h)	2.6 (1.4, 3.0)	34.5 (26.4, 43.7)	<0.001
Sleep time (min)	411.4 ± 39.6	398.0 ± 38.4	0.153
Sleep efficiency (%)	82.4 ± 6.1	80.3 ± 5.7	0.136
Serum CRP (mg/L)	8.1 ± 0.8	8.4 ± 0.7	0.074
BAL mononuclear (%)	80.8 ± 6.6	71.8 ± 8.9	0.002
BAL neutrophils (%)	16.5 ± 5.9	25.7 ± 8.6	<0.001
BAL TNFα (pg/mL)	40.0 (32.0, 49.3)	60.0 (50.5, 78.0)	<0.001
BAL IL8 (pg/mL)	57.9 ± 22.8	88.1 ± 30.1	<0.001

COPD, chronic obstructive pulmonary disease; overlap syndrome, patients with obstructive sleep apnea and COPD; FEV1, forced expiratory volume at 1 s; FEV1/FVC ratio, the FEV1 and the forced vital capacity (FVC) ratio; BMI, body mass index; AHI, apnea–hypopnea index; Mean SpO_2, mean nocturnal oxyhemoglobin saturation; TNFα, tumor necrosis factor alpha; IL8, interleukin 8; $T_{SpO_2<90\%}$, percent of nighttime spent with oxygen saturation < 90%; ODI, oxygen desaturation index; CRP, C-reactive protein.

2.5. CRP, IL-8, and TNFα

Venous blood samples were obtained from all of the subjects in the morning of PSG (at 7:00–8:00 a.m.) to measure the CRP level in mg/L using a Beckman Coulter Inc. Immage 800 (USA) device. Quantitative sandwich enzyme immunoassay kits (R&D Systems) were used to measure TNFα and IL-8 concentrations in BAL fluid. Cytokine concentrations were measured in pg/mL. The investigators who performed the cytokine assays were blinded to the sequence of the specimens.

2.6. CPAP treatment and follow-up

All patients with overlap syndrome were suggested to undergo treatment with CPAP [21] along with inhaled corticosteroids and long-acting β agonists as recommended by the COPD guidelines [15]. Noninvasive ventilation titration was performed according to the recommended guidelines [22]. According to AASM standards, CPAP titration during the next night of PSG is started at 4 cmH_2O and then increased after two obstructive apneas, three hypopneas, or five RERAs and no more than every 5 min to achieve AHI <5 events/h and $SaO_2 > 90\%$. If the patient is intolerant of high pressures or obstructive respiratory events continue at 15 cmH_2O of CPAP during the titration study, the patient may be switched to bilevel positive airway pressure (PAP) [22]. In the present study, supplemental oxygen was provided by an adapted nasal mask with a flow of 1.5 L/min to attain $SaO_2 > 90\%$ in some patients with persistent SaO_2 <88%. Twenty-five patients completed the CPAP treatment. No patients were on auto-titrating PAP. No patients used bilevel PAP or required supplemental oxygen during the study period. All patients were treated with expiratory pressure relief (C-Flex). The mean CPAP level was 12.4 ± 1.7 cmH_2O. After 2 weeks of CPAP treatment, PSG and bronchoscopy were repeated in the overlap syndrome patients.

2.7. Statistical analysis

All of the analyses were conducted using SPSS software for Windows, version 19.0 (SPSS, China). The differences between the overlap group and COPD patients were compared using the chi-square test for categorical variables, unpaired t-test for normally distributed continuous variables, and Wilcoxon rank sum test for non-normally distributed continuous variables. The differences between pre-treatment and post-treatment overlap syndrome were evaluated using the paired t-test. Correlations between different parameters were tested using Spearman's rank test. The relationship of the indices of airway inflammation with other variables was assessed by multivariate linear regression analysis. The normally distributed variables were represented as mean ± SD, and the non-normally distributed variables were represented as the median (25th and 75th percentiles), as shown in Table 1. A value of $p < 0.05$ was considered statistically significant.

3. Results

3.1. Subjects

A flow chart showing the selection and enrollment of patients is presented in Fig. 1. Of the 168 patients with COPD and habitual snoring, 75 were eligible to participate in this study. Of these patients, 47 had overlap syndrome and 28 had moderate-to-severe COPD alone. In the overlap syndrome group, 28 patients were treated with CPAP and standard COPD treatment. Three of these patients were withdrawn from the overlap group within 1 week of treatment initiation due to non-adherence to CPAP therapy. The remaining 19 patients in the overlap syndrome group declined CPAP therapy because of the cost involved (the expense is not covered by medical insurance) or other reasons. All patients in the COPD group were treated using the standard COPD treatment regimen (inhaled corticosteroids and long-acting β agonists); four patients were withdrawn from this study group because of non-adherence or acute exacerbation.

3.2. Baseline characteristics

The characteristics of the subjects at the time of enrollment into the study are shown in Table 1. No statistical differences were found with regard to age, sex, body mass index (BMI), pulmonary function, and arterial blood gas between the two groups. The frequency of confirmed COPD exacerbation requiring emergency department visits or hospitalization during the 24 months before entry

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into the study was higher in the overlap syndrome group than in the control group ($p = 0.009$, Table 1). Based on the study design, the AHI and ODI were significantly different between the overlap and COPD patients ($p < 0.001$). No significant differences were found between the two groups regarding sleep time and sleep efficiency. The nocturnal mean SpO_2 (91.5 ± 2.6% vs 93.8 ± 2.5%, $p < 0.001$) and percentage of nighttime spent with oxygen saturation less than 90% ($T_{SpO_2<90\%}$) [3.70% (2.41, 8.53) vs 0.19% (0, 0.85), $p < 0.001$] were significantly different between the overlap and COPD groups. The nadir SpO_2 in overlap syndrome was significantly lower than that in COPD patients (80.3 ± 5.2% vs 88.1 ± 2.4%, $p < 0.001$). No control subjects had continuous hypoxemia and no overlap syndrome patients had daytime hypoxemia.

3.3. BAL fluid cell counts

A significantly higher percentage of neutrophils (24.2 ± 9.4% vs 16.5 ± 5.9%, $p < 0.001$) and a significantly lower percentage of mononuclear cells (74.0 ± 9.6% vs 80.8 ± 6.6%, $p < 0.001$) were detected in the BAL fluid of the overlap group compared to that of the control group (Table 1). Moreover, the percentage of neutrophils showed a linear relationship with the $T_{SpO_2<90\%}$ ($r = 0.553$, $N = 75$, $p < 0.001$; Fig. 2), mean SpO_2 ($r = –0.417$, $N = 75$, $p < 0.001$; Fig. 3), nadir SpO_2 ($r = –0.548$, $N = 75$, $p < 0.001$), ODI ($r = 0.450$, $N = 75$, $p < 0.001$), percentage of mononuclear cells ($r = –0.972$, $N = 75$, $p < 0.001$), AHI ($r = –0.485$, $N = 75$, $p < 0.001$), TNFα levels ($r = 0.461$, $N = 75$, $p < 0.001$) and IL-8 levels ($r = 0.696$, $N = 75$, $p < 0.001$). The percentage of mononuclear cells also had a linear relationship with the above indices, but an inverse relationship to the percentage of neutrophils, as the reduction in the fraction of mononuclear cells may be a consequence of the increase in neutrophils. Multivariate linear regression analysis showed a significant relationship between the percentage of neutrophils and the level of TNFα (unstandardized coefficients, 0.036, 95% CI, 0.013–0.059; $p = 0.002$) and percentage of mononuclear cells (unstandardized coefficients, –0.894, 95% CI, –0.954 to –0.835; $p < 0.001$).

3.4. Inflammatory mediators

Although the serum CRP levels were higher in the overlap syndrome patients than in patients with COPD only, no significant differences were detected between the two groups (Table 1). TNFα and IL-8 levels in the BAL fluid were significantly higher in the overlap syndrome patients than in the simple COPD patients (Table 1) and showed a linear relationship with the percentage of neutrophils in BAL fluid. The TNFα level showed a linear relationship with the FEV1/forced vital capacity (FVC) ratio ($r = –0.268$, $N = 75$, $p = 0.02$), percentage of mononuclear cells ($r = –0.386$, $N = 75$, $p < 0.001$), percentage of neutrophils ($r = 0.461$, $N = 75$, $p < 0.001$), AHI ($r = 0.592$, $N = 75$, $p < 0.001$), $T_{SpO_2<90\%}$ ($r = 0.855$, $N = 75$, $p < 0.001$; Fig. 4), mean SpO_2 ($r = –0.664$, $N = 75$, $p < 0.001$), nadir SpO_2 ($r = –0.697$, $N = 75$, $p < 0.001$), ODI ($r = 0.575$, $N = 75$, $p < 0.001$), and IL8 levels ($r = 0.356$, $N = 75$, $p = 0.002$). Multivariate linear regression analysis revealed a significant association between the level of TNFα and $T_{SpO_2<90\%}$ (unstandardized coefficients, 4.886, 95% CI, 4.320–5.452; $p < 0.001$). The IL-8 level showed a linear relationship with the percentage of mononuclear cells ($r = –0.673$, $N = 75$, $p < 0.001$), percentage of neutrophils ($r = 0.696$, $N = 75$, $p < 0.001$), AHI ($r = 0.336$, $N = 75$, $p = 0.003$), $T_{SpO_2<90\%}$ ($r = 0.453$, $N = 75$, $p < 0.001$), mean SpO_2 ($r = –0.303$, $N = 75$, $p = 0.008$), nadir SpO_2 ($r = –0.477$, $N = 75$, $p < 0.001$), ODI ($r = 0.307$, $N = 75$, $p = 0.007$), and TNFα ($r = 0.356$, $N = 75$, $p = 0.002$). Multivariate linear regression analysis revealed a significant association between the IL-8 level and percentage of neutrophils (unstandardized coefficients, 2.437, 95% CI, 1.850–3.025; $p < 0.001$).

3.5. CPAP treatment

All of the complete CPAP treatment participants used CPAP for >5.0 h/night, with a mean 5.6 ± 0.4 h/night. CPAP treatment decreased sleep time but did not affect sleep efficiency. The residual AHI during the therapy period was 3.9 ± 1.1 events/h. After CPAP treatment, the nadir SpO_2 increased and $T_{SpO_2<90\%}$ decreased significantly. The CPAP treatment produced a significant decrease in the

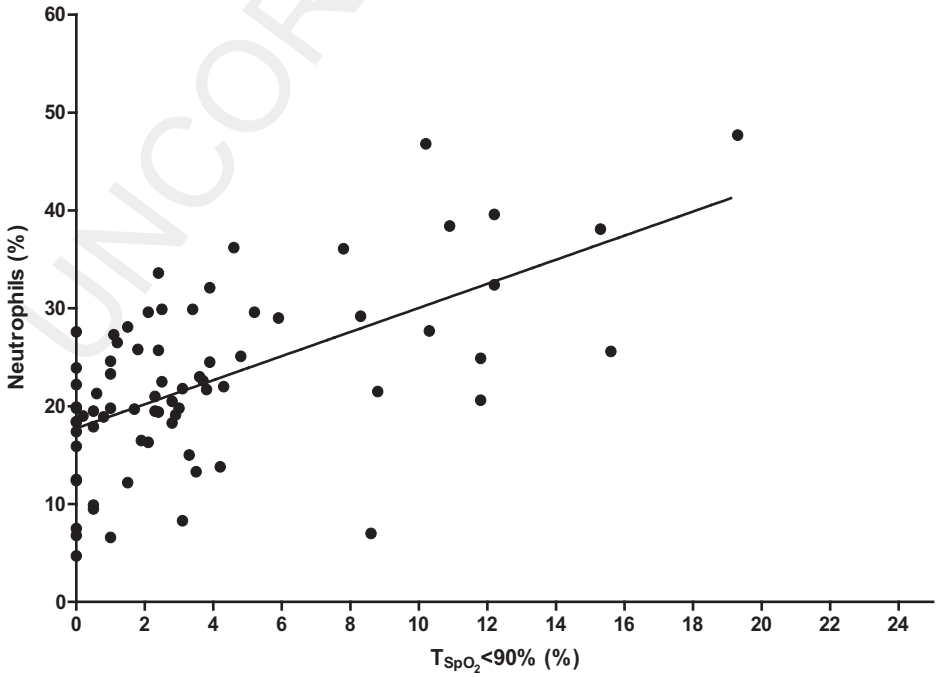

Fig. 2. Relationship between the percentage of neutrophils in bronchoalveolar lavage (BAL) fluid and the T_{SpO_2} ($r = 0.598$, $N = 75$, $p < 0.01$).

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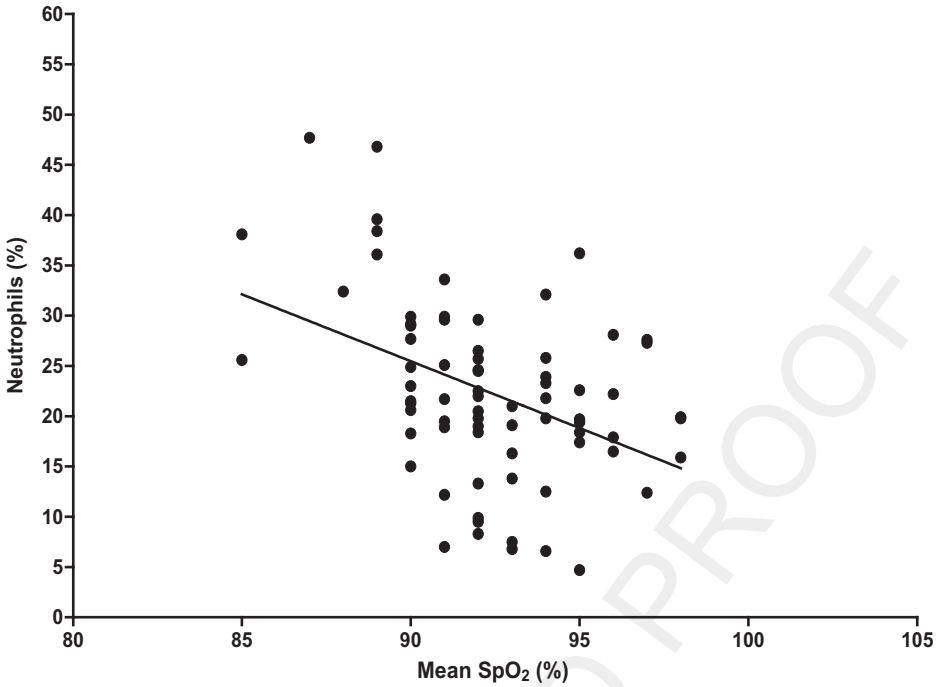

Fig. 3. Relationship between the percentage of neutrophils in bronchoalveolar lavage (BAL) fluid and mean SpO_2 ($r = -0.417$, $N = 75$, $p < 0.001$).

percentage of neutrophils and the levels of the inflammatory mediators CRP (serum), TNFα, and IL-8 (BAL fluid) in patients with overlap syndrome (Table 2). On comparing the treated with CPAP group and the not-treated with CPAP overlap group, significant differences were found between the percentage of neutrophils (18.2 ± 6.8% vs 22.8 ± 5.2%, $p = 0.029$) and inflammatory mediators CRP (6.0 ± 1.3 mg/L vs 8.1 ± 0.7 mg/L, $p < 0.001$) in the serum and TNFα (48.2 ± 14.0 pg/mL vs 60.2 ± 20.5 pg/mL, $p = 0.034$) and IL8 (63.1 ± 23.4 pg/mL vs 80.1 ± 26.9 pg/mL, $p = 0.042$) levels in the BAL fluid (Table 2).

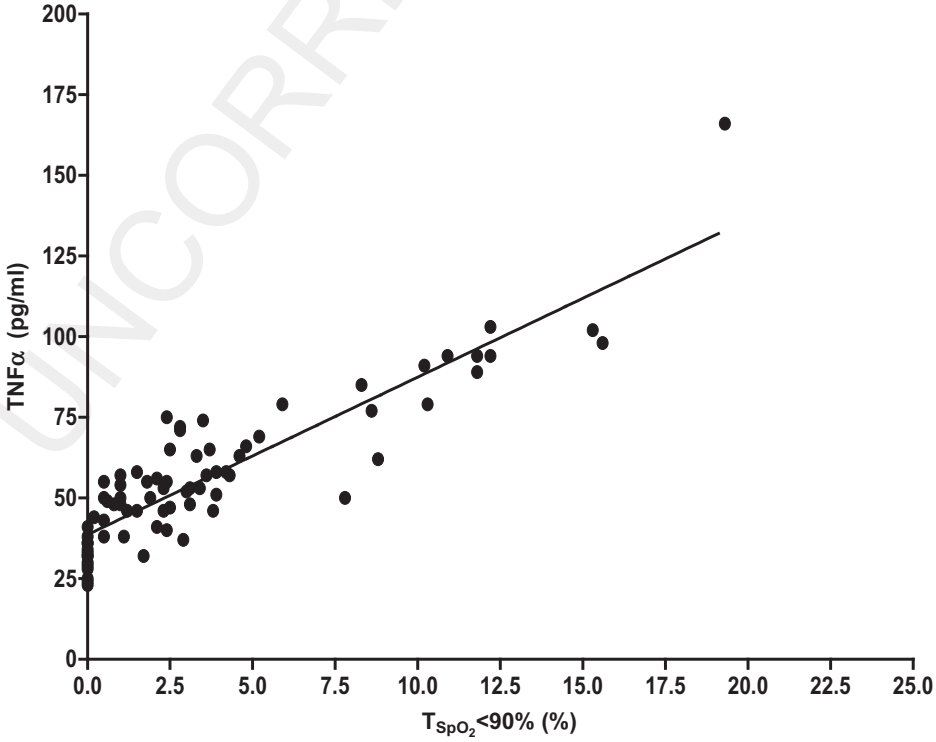

Fig. 4. Relationship between the tumor necrosis factor-α (TNFα) levels and the $T_{SpO_2<90\%}$ ($r = 0.855$, $N = 75$, $p < 0.001$).

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Table 2
Clinical characteristics of overlap syndrome patients treated or not treated with CPAP.

Characteristic	Baseline			Follow-up	
	CPAP treatment ($N = 25$)	Not treated with CPAP ($N = 15$)	p-value	CPAP treatment ($N = 25$)	Not treated with CPAP ($N = 15$)
Age (years)	61.5 ± 4.4	59.6 ± 4.9	0.214		
Sex (male:female)	22:3	14:1	0.516		
FEV1 (L)	1.34 ± 0.32	1.35 ± 0.26	0.916		
FEV1 % predicted	48.9 ± 9.2	47.9 ± 9.9	0.761		
FEV1/FVC ratio (%)	56.6 ± 7.3	59.0 ± 7.4	0.321		
PaO_2 (mmHg)	88.0 (76.5, 91.5)	83.0 (82.0, 88.0)	0.576		
$PaCO_2$ (mmHg)	39.6 ± 4.3	40.2 ± 3.2	0.644		
BMI (kg/m^2)	32.0 ± 1.9	31.3 ± 2.1	0.275		
AHI (events/h)	36.9 ± 11.6	31.7 ± 10.9	0.175	3.9 ± 1.1†	31.4 ± 9.6§
Mean SpO_2 (%)	91.6 ± 3.0	91.7 ± 2.4	0.943	93.7 ± 1.5†	91.5 ± 1.8§
$T_{SpO_2<90\%}$ (%)	5.49 ± 4.89	5.35 ± 4.26	0.930	0.79 ± 0.55†	4.98 ± 3.80§,¶
Nadir SpO_2 (%)	79.5 ± 5.7	81.9 ± 4.4	0.163	87.6 ± 2.3†	83.1 ± 3.3§
ODI (events/h)	37.7 ± 11.3	34.6 ± 10.2	0.393	3.7 ± 0.9†	33.9 ± 10.2§
Sleep time (min)	399.3 ± 40.0	403.5 ± 40.1	0.751	384.9 ± 34.8*	404.3 ± 29.0
Sleep efficiency (%)	80.7 ± 5.7	80.3 ± 6.2	0.852	79.5 ± 4.1	81.3 ± 4.9
Serum CRP (mg/L)	8.4 ± 0.8	8.4 ± 0.5	0.889	6.0 ± 1.3†	8.1 ± 0.7§,¶
Mononuclear (%)	70.8 ± 10.4	71.6 ± 6.4	0.797	77.4 ± 7.8*	74.4 ± 5.8
Neutrophils (%)	27.4 ± 9.9	24.5 ± 6.3	0.305	18.2 ± 6.8*	22.8 ± 5.2‡
TNFα (pg/mL)	71.6 ± 25.8	59.1 ± 22.8	0.127	48.2 ± 14.0*	60.2 ± 20.5‡
IL8 (pg/mL)	93.7 ± 30.9	81.3 ± 29.5	0.221	63.1 ± 23.4*	80.1 ± 26.9‡

* $p < 0.05$, $^{\dagger}p < 0.001$, CPAP treatment group compared before and after CPAP treatment.
‡ $p < 0.05$, $^{\S}p < 0.001$, compared between groups after follow up.
¶ $p < 0.05$, $\varphi < 0.001$, not treated with CPAP group compared between baseline and after follow-up.

4. Discussion

The aim of this study was to investigate whether OSA exacerbates airway inflammation in COPD patients. The important findings of this study are as follows. (1) The percentage of neutrophils and levels of cytokines TNFα and IL-8 were significantly increased in the BAL fluid of patients with overlap syndrome compared to COPD patients, independently of the levels of the systemic inflammatory cytokine CRP. (2) A significant correlation existed between the airway inflammatory markers and the nocturnal mean SpO_2, $T_{SpO_2<90\%}$, and nadir SpO_2. Moreover, multivariate linear regression analysis revealed a significant correlation between the percentage of neutrophils and the levels of TNFα and IL-8 in the BAL fluid and a significant correlation between the levels of TNFα and $T_{SpO_2<90\%}$. (3) CPAP treatment significantly decreased the levels of these markers of airway inflammation in BAL fluid. These findings suggest that OSA can exacerbate airway inflammation in patients with COPD. Thus, whereas previous studies have shown that COPD is characterized by neutrophil airway inflammation, the present study is the first to show that OSA was one of the risk factors leading to exacerbation of airway inflammation in patients with COPD, which may contribute to the overlap-related increase in exacerbation frequency and mortality [6].

Several studies have shown that OSA patients develop airway inflammation [14,15,23] and that CPAP treatment can correct the local upper airway inflammation present in OSA patients [23]. However, the relationship between local and systemic inflammation remains to be elucidated. Although it has been hypothesized that local inflammation is a direct consequence of systemic inflammation, an alternative hypothesis is that airway inflammation is a local consequence of mechanical stress, airway fat deposition and intermittent hypoxia. In our study, we found no significant differences between overlap syndrome patients and patients with COPD only with regard to systemic inflammation, as previously reported by Nural et al. [24] and Shiina et al. [25]. Furthermore, Philippe et al. [26] exposed airway epithelial cells (AECs) and bronchial smooth muscle cells (BSMCs) to intermittent hypoxia (IH) or normoxic conditions for 24 h in vitro to evaluate local effects of IH, and found an significant increase in the levels of IL-8, platelet-derived growth factor (PDGF)-AA, and vascular endothelial growth factor (VEGF) secreted by AECs, in addition to VEGF expressed by BSMCs. They also found that neutrophil chemotaxis and BSMC migration were enhanced by IH and supernatants of IH-exposed AECs and that the enhanced BSMC migration could be abolished by an antibody blocking PDGF-AA. It was thus speculated that the specific inflammatory response of airway cells to IH was independent of systemic events. The serum CRP level did not accordingly increase with local inflammation, rather, our study demonstrated that the serum CRP level decreased after the COPD standard regimen with or without CPAP treatment, and that these results may further support our hypothesis that OSA may exacerbate COPD airway inflammation independent of systemic inflammation.

Lacedonia et al. [17] reported that patients with overlap syndrome present a high percentage of neutrophils in induced sputum; however, this finding was similar to that in patients with COPD or OSA alone. It is known that COPD along with OSA produces greater sleep disturbance and oxygen desaturation than COPD alone. In the present study, the mean PaO_2 in the COPD group was 73.64 ± 14.92 mmHg, indicating that some of the COPD only patients had nocturnal hypoxemia. It is known that systemic hypoxemia contributes to TNFα elevation in COPD [27]; increased TNFα and related cytokines could induce migration of neutrophils into the airways [12].

Several studies have reported a significant elevation of serum or sputum TNFα in patients with OSA [2,15,28–31], and CPAP treatment has been found to improve the levels of TNFα [31–33]. Our study showed that effect of overlap of OSA on neutrophil numbers in the airways of COPD may be associated with the increased levels of TNFα and IL-8 in BAL fluid. Multivariate linear regression analysis showed a significant correlation between the levels of TNFα and $T_{SpO_2<90\%}$, independent of the AHI, FEV1 % predicted, and FEV1/FVC. The increased TNFα levels may be associated with the migration of neutrophils from the general circulation to the lung parenchyma in the overlap patients. First, TNFα is a potential pro-inflammatory cytokine and can impair macrophage uptake of apoptotic neutrophil cells [34,35]. Borges et al. [36] showed that administration of TNFα impaired the removal of apoptotic cells

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from the lungs in a mouse model of neutrophilic inflammation. Consequently, the apoptotic cells underwent secondary necrosis, releasing proinflammatory components into the air spaces [36] and damaging the surrounding tissue, which in turn delayed the resolution of inflammation. Moreover, TNFα can activate transcription factor nuclear factor-κ-B, which is up-regulated in macrophages and epithelial cells [37]. Second, IL-8 can induce neutrophils to release myeloperoxidase and recruit inflammatory cells to help sustain inflammation. Moreover, exposure to IL-8 can cause rapid mobilization of macrophage antigen 1 (Mac-1) to the neutrophil surface [38]. Mac-1, which showed increased neutrophil surface expression in patients with COPD, can facilitate neutrophil adhesion via intercellular adhesion molecule 1 [39]. Thus, a combination of failure of clearance and enhanced recruitment may contribute to the increased accumulation of neutrophils in the lungs and airways of patients with overlap syndrome.

This is the first study to report a beneficial effect of CPAP on reduction of airway inflammation in patients with COPD associated with OSA. At baseline, treated and untreated patients with overlap syndrome reported similar levels of airway inflammation. Nevertheless, during follow-up, a significant decrease in airway inflammation was observed in the CPAP-treated group compared with overlap patients not treated with CPAP. Thus far, several studies have shown that the overlap syndrome is associated with an increased risk of death and hospitalization because of exacerbation, and that CPAP treatment could improve survival, decrease hospitalizations [6,40–42], and improve walking capacity [43]. Several potential mechanisms that may account for the observed higher mortality in overlap patients have been reviewed previously [44]. However, the mechanism underlying the effect of CPAP treatment on delaying the exacerbation and decreasing hospitalization in patients with overlap syndrome has not been elucidated. This study reveals that CPAP treatment may decrease COPD exacerbation by decreasing airway inflammation as nocturnal hypoxemia improves.

This study has several limitations. First, a major limitation is the small size of the study group. Second, we excluded GERD only through symptoms assessment and could not monitor the 24-h esophageal pH to completely exclude GERD; thus the airway inflammation may have resulted from GERD and silent aspiration [18]. However, the airway inflammation decreased significantly after CPAP treatment; we speculate that the possibility of airway inflammation caused by GERD can be excluded. Third, the overlap syndrome patients may have had nocturnal continuous hypoxemia, and our study could not differentiate between the effects of continuous hypoxemia and intermittent hypoxemia on airway inflammation. The AHI and ODI in the overlap patients were almost identical and the $T_{SpO_2<90\%}$ in this group was only 3.70% (median); therefore, we could infer that the overlap group had no nocturnal continuous hypoxemia. Moreover, the patients with COPD only may have had nocturnal hypoxemia. However, we selected only moderate-to-severe stable COPD patients to take part in this study, the mean SpO_2 and ODI in the COPD group were 93.8 ± 2.5%, 2.6 (median) events/h, respectively. Thus, the patients did not exhibit nocturnal hypoxemia. Furthermore, there is no exact definition of intermittent hypoxia and continuous hypoxia, and no specific markers in BAL are known. Fourth, this was an observational study; the patients with overlap syndrome who refused CPAP treatment may have also been nonadherent to other therapies for their COPD and CPAP was not allocated randomly, and these factors could have thus resulted in bias based on factors related to subsequent airway inflammation, although the baseline characteristics were similar between those who accepted and those who refused CPAP treatment (Table 2). Thus, further studies using a larger study group are warranted to support our hypothesis.

5. Conclusion

In conclusion, these data show that in patients with COPD, the coexistence of OSA is associated with increased airway inflammation. However, treatment with CPAP was associated with improved airway inflammation. Patients with COPD should be screened for OSA because, if present, its treatment with CPAP is associated with improved pathophysiological changes.

Conflict of interest

The authors have declared that there are no conflicts of interest.

The ICMJE Uniform Disclosure Form for Potential Conflicts of Interest associated with this article can be viewed by clicking on the following link: http://dx.doi.org/10.1016/j.sleep.2015.04.019.

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