A study of IL-6, IL-8, and TNF-α as inflammatory markers in COPD patients
Wafaa S. El-Shimy, Ayman S. El-Dib, Hala M. Nagy, Wael Sabry

**Introduction**
Chronic obstructive pulmonary disease (COPD) is a preventable and treatable disease characterized by airflow limitation that is not fully reversible, usually progressive and is associated with an abnormal inflammatory response of the lungs to noxious particles or gases [1].

Symptoms of COPD range from chronic cough, sputum production and wheezing to more severe symptoms, such as dyspnea, poor exercise tolerance and signs or symptoms of right-sided heart failure [2].

There has been increasing interest in using pulmonary biomarkers to understand and monitor the inflammation in the respiratory tract of patients with COPD; a biomarker refers to any molecule or material (cells, tissue), the measurement of which reflects the disease process. In COPD, several types of biomarkers have been measured and are related to the disease pathophysiology and the inflammatory or destructive process in the lung [3].

Interleukin-6 (IL-6) is synthesized by the airway epithelium, macrophages and other cells at the site of inflammation in response to environmental stress such as smoking; IL-6 has both pro inflammatory and anti-inflammatory properties. Serum IL-6 levels were significantly higher in individuals with COPD when compared with controls [4]. Serum and bronchoalveolar lavage (BAL) levels of IL-6 were more likely to increase during exacerbations of COPD [5,6].

**IL-8** is a multifunctional chemokine involved in inflammation-mediated neutrophil infiltration and chemotaxis. [7] IL-8, also known as CXCL8, is a CXC chemokine that is a potent chemoattractant for neutrophils. In general, monocytes, tissue and alveolar macrophages, pulmonary epithelium, smooth muscles cells of the airway, eosinophils, fibroblasts and endothelial cells are its important sources [8]. IL-8 is frequently increased in patients with COPD; analysis of BAL and sputum samples has also shown increased levels of IL-8 in patients with mild-to-moderate COPD [9].

Tumor necrosis factor-α (TNF-α) is a powerful proinflammatory cytokine primarily produced by activated macrophages; it is thought to play a critical role in the pathogenesis of COPD by promoting and maintaining the expression and the release of various proinflammatory mediators that lead to tissue damage and remodeling [10].

**Aim of the work**
The aim of this work was to assess the diagnostic value of IL-6, IL-8, and TNF-α as inflammatory markers in COPD patients.

**Patients and methods**
This study was carried out on 50 COPD patients and 10 controls at the Chest Department, Tanta University Hospital, from September 2011 to December 2012.

**Conclusion**
Increased serum and/or BAL IL-6, IL-8 and TNF-α can be used as biomarkers of the systemic inflammatory response in COPD patients, and their levels are correlated with the severity of COPD. *Egypt J Broncho* 2014 8:91–99
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**Keywords:** chronic obstructive pulmonary disease, IL-6, IL-8, TNF-α

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They were subdivided into three groups:
(1) Group I included 10 participants who were apparently healthy, nonsmoking volunteers. Their ages ranged from 18 to 68 years.
(2) Group II included 25 patients with mild and moderate COPD. Their ages ranged from 21 to 77 years.
(3) Group III included 25 patients with severe COPD. Their ages ranged from 36 to 76 years.

Inclusion criteria for COPD
(1) Mild and moderate COPD (80% ≥ forced expiratory volume in 1 s (FEV₁) ≥ 50% predicted and FEV₁ > 1.2 l)
(2) Severe COPD (FEV₁ < 50% predicted) (according to Global Initiative for Chronic Obstructive Lung Disease [1]).

COPD can be differentiated from asthma using bronchodilator reversibility testing.

Exclusion criteria
(1) Chest diseases other than COPD.
(2) COPD patients with acute exacerbation (for BAL).
(3) Patients with the following cardiac conditions:
   (a) Unstable ischemic heart disease (recent myocardial infarction < 6 weeks, unstable angina).
   (b) Congestive cardiac failure.
(1) Patients with heart failure. Mechanically ventilated patients with PaO₂ less than 70 mmHg.
(2) Thrombocytopenia with platelets less than 10 000/µl.
(3) Psychological impairment.
(4) Cancer patients and patients receiving immunosuppressive treatment.
(5) Hepatic cirrhosis.
(6) Chronic renal failure.
(7) Autoimmune or connective tissue disorders, taking anti-TNF-α drugs.
(8) Pregnant women.

All participants were subjected to the following:
(1) Through history taking.
(2) Full clinical examination.
(3) Plain chest radiograph.
(4) Routine laboratory investigations.
(5) BMI.
(6) Pulmonary function tests (FEV₁, forced vital capacity (FVC), FEV₁%): all parameters were matched for age, sex, and body weight.
(7) Arterial blood gases.
(8) BAL was performed to groups I and II only under local anesthesia using flexible fiberoptic bronchoscopy.

Blood and BAL were examined for the following:
(a) Total and differential cells.
(b) IL-6, IL-8, and TNF-α levels by ELISA.

Results
The mean value and SD of FEV₁, FVC, and FEV₁% were significantly lower in groups III and II when compared with group I; they were also significantly lower in group III than in group II (t-test < 0.001).

The mean value and SD of serum TLC, neutrophil%, lymphocyte%, and monocyte% were significantly higher in group III than in groups II and I; it was also significantly higher in group II when compared with group I (t-test < 0.001).

The mean value and SD of the serum eosinophil% in the three studied groups showed no significant difference (F-test = 0.721, P = 0.661).

The mean value and SD of BAL TLC, macrophage, lymphocyte, and neutrophil were significantly higher in group II when compared with group I (t-test < 0.001). The mean value and SD of BAL eosinophil showed no significant difference between the two groups (t-test = 0.675, P = 0.504) (Figs. 1–10 and Tables 1–10).

Serum and BAL inflammatory markers
The mean value and SD of serum IL-6, IL-8, and TNF-α were significantly higher in group III when compared with groups II and I; they were also significantly higher in group II than in group I (t-test < 0.001).

The mean value and SD of BAL IL-6, IL-8, and TNF-α were significantly higher in group II than in group I (t-test = 4.85, P < 0.001).

| Table 1 FEV₁ value/l in the three studied groups |
| Groups | FEV₁/l | ANOVA |
| Range | Mean ± SD | F | P-value |
| Control | 3.11–4.78 | 3.935 ± 0.511 | 170.631 | <0.001* |
| Mild and moderate | 1.51–3.34 | 2.279 ± 0.459 | 148.010 | <0.001* |
| Severe | 0.24–1.96 | 0.980 ± 0.383 | 0.721 | P = 0.661 |
| Tukey’s test | | | | |
| Control vs. mild and moderate | Mild and moderate vs. severe | Severe vs. control | <0.001* | <0.001* | <0.001* |

ANOVA, analysis of variance; *Significant.

| Table 2 Serum IL-6 value in the three studied groups |
| Groups | Serum IL-6 (pg/ml) | ANOVA |
| Range | Mean ± SD | F | P-value |
| Control | 1.9–5 | 3.030 ± 1.076 | 148.010 | <0.001* |
| Mild and moderate | 6.6–17.8 | 14.016 ± 3.018 | 0.721 | P = 0.383 |
| Severe | 16.1–28.8 | 22.727 ± 3.716 | 0.721 | P = 0.383 |
| Tukey’s test | | | | |
| Control vs. mild and moderate | Mild and moderate vs. severe | Severe vs. control | <0.001* | <0.001* | <0.001* |

ANOVA, analysis of variance; IL, interleukin; *Significant.
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Fig. 1

The mean value of FEV₁ in the three studied groups.

Fig. 2

The mean value of serum interleukin-6 (IL-6) in the three studied groups.

Fig. 3

The mean value of serum interleukin-8 (IL-8) in the three studied groups.

Fig. 4

The mean value of serum tumor necrosis factor-α (TNF-α) in the three studied groups.

Table 3 Serum IL-8 value in the three studied groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Serum IL-8 (pg/ml)</th>
<th>ANOVA</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>Mean ± SD</td>
<td>F</td>
<td>P-value</td>
</tr>
<tr>
<td>Control</td>
<td>25–44</td>
<td>36.2 ± 6.334</td>
<td>36.112</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Mild and moderate</td>
<td>64.8–188</td>
<td>146.432 ± 28.528</td>
<td></td>
<td></td>
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<tr>
<td>Severe</td>
<td>152.8–620</td>
<td>309.28 ± 141.906</td>
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<td></td>
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<tr>
<td>Tukey’s test</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control vs. mild and moderate</td>
<td>severe vs. severe</td>
<td>0.008*</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

ANOVA, analysis of variance; IL, interleukin; *Significant.

Serum correlations

There was a significant negative correlation between serum IL-6, IL-8, TNF-α, and FEV₁%. These data denote that an increase in inflammatory markers in the serum is correlated with the severity of airway obstruction.

There was a significant positive correlation between serum IL-6, IL-8, and TNF-α and serum total leucocytic count, lymphocytic%, and neutrophilic %, which increase in association with an increase in serum inflammatory markers.

BAL correlations (in group II)

There was a significant negative correlation between BAL IL-6, IL-8, TNF-α, and FEV₁%. Hence, their levels were correlated with the severity of COPD.

Table 4 Serum TNF-α value in the three studied groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Serum TNF-α (pg/ml)</th>
<th>ANOVA</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>Mean ± SD</td>
<td>F</td>
<td>P-value</td>
</tr>
<tr>
<td>Control</td>
<td>12.2–24.2</td>
<td>18.52 ± 4.028</td>
<td>38.442</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Mild and moderate</td>
<td>30.22–74.4</td>
<td>43.833 ± 10.531</td>
<td></td>
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<tr>
<td>Severe</td>
<td>34.6–87</td>
<td>54.472 ± 12.972</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tukey’s test</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control vs. mild and moderate</td>
<td>severe vs. severe</td>
<td>&lt;0.001*</td>
<td>0.003*</td>
<td>0.003*</td>
</tr>
</tbody>
</table>

ANOVA, analysis of variance; TNF-α, tumor necrosis factor-α; *Significant.
The correlation between serum interleukin-8 (IL-8) and FEV\textsubscript{1}/FVC (FEV\textsubscript{1}%).

The mean value of bronchoalveolar lavage (BAL) interleukin-6 (IL-6) in groups I and II.

The mean value of bronchoalveolar lavage (BAL) tumor necrosis factor-\(\alpha\) (TNF-\(\alpha\)) in groups I and II.

The correlation between serum interleukin-6 (IL-6) and FEV\textsubscript{1}/FVC (FEV\textsubscript{1}%).

The mean value of bronchoalveolar lavage (BAL) interleukin-8 (IL-8) in groups I and II.

The mean value of bronchoalveolar lavage (BAL) tumor necrosis factor-\(\alpha\) (TNF-\(\alpha\)) in groups I and II.

The correlation between serum interleukin-8 (IL-8) and FEV\textsubscript{1}/FVC (FEV\textsubscript{1}%).

The correlation between serum tumor necrosis factor-\(\alpha\) (TNF-\(\alpha\)) and FEV\textsubscript{1}/FVC (FEV\textsubscript{1}%).
### Discussion

**Pulmonary function studies**

In the present study, a significant decrease was found in the pulmonary functions of severe COPD patients when compared with mild and moderate COPD patients and controls; the findings of the present work are consistent with those of many authors who found that pulmonary function data (FEV₁, FVC, FEV₁%, and PEFR) were significantly lower in COPD patients when compared with controls [11–14].

The extent of inflammation, fibrosis and luminal exudates in the small airways is correlated with the reduction in FEV₁ and FEV₁% [15]. The airflow limitation in COPD patients is due to the increase in the resistance to airflow, which is caused by smooth muscle hypertrophy, goblet cell metaplasia, degeneration of the airway cartilage and mucous hypersecretion [16].

### Serum IL-6

In the present study, serum IL-6 was significantly higher in severe than in mild and moderate COPD patients and Control participants. The results of this work are consistent with the study of Seemungal et al. [17], Arschang et al. [18], and Eickhoff et al. [19], who found that serum IL-6 increases during COPD exacerbation compared with stable COPD patients and healthy controls.

Also, the results of this study agree with Attaran et al. [20], Abd El-Maksoud et al. [21], and Garcia-Rio et al. [22], who found that the concentrations of circulating serum IL-6 were significantly higher in patients with COPD in comparison with control participants, and their levels increased according to the stage of the disease.

Our results also agree with the study of Celli et al. [23], which included 2164 COPD patients and 245 healthy controls who had been followed for 3 years, and they found that the circulating IL-6 levels were significantly higher in individuals with COPD when compared with controls.

### BAL IL-6

In the present study, it was significantly higher in mild and moderate COPD patients than in control participants. The results of the present work are consistent with the study of Soler et al. [24], who found that BAL IL-6 levels increased virtually linearly from nonsmoking controls through smoking controls and mild and moderate COPD to severe COPD patients.

Also, Weidong et al. [25] studied seven nonsmoking apparently healthy individuals and 21 patients with
COPD, and they found higher concentrations of IL-6 in BAL of the COPD group than in control individuals.

It has been established that stable COPD is associated with low-grade systemic inflammation, besides an increase in airway inflammation; COPD exacerbations are associated with more increase in systemic inflammation as demonstrated by an increase in blood leukocytes, acute-phase proteins, C-reactive protein and fibrinogen, and inflammatory cytokines. During acute exacerbations of COPD, higher levels of IL-6 have been demonstrated, which decrease again during recovery [26].

Biologically, IL-6 is the primary cytokine regulator of both C-reactive protein and fibrinogen in the liver. It also plays a critical role in hematopoiesis, causing thrombocytosis and leukocytosis with its overexpression [27].

**Serum IL-8**

In the present study, it was significantly higher in severe COPD patients than in mild and moderate COPD patients and control participants.

The results of the present work are consistent with the study of Daldegan et al. [28] who found that serum IL-8 concentrations were higher in COPD patients than in patients with asthma or in healthy control individuals.

Also, our results agree with Xie et al. [29], who found that serum IL-8 was statistically higher during COPD exacerbation compared with patients with stable COPD and healthy controls.

Also, Garcia-Rio et al. [22] found that COPD patients showed higher levels of IL-8 compared with controls, and serum concentrations were related to the severity of COPD.

Also, Demirci et al. (2013), studied 23 COPD patients (Stage I), 15 (Stage II) and 12 (Stage III–IV). Ten healthy nonsmoking as control group. They found that as the stage of COPD increased, the levels of IL-8 increased [30].

**BAL IL-8**

In the present study, it was significantly higher in mild and moderate COPD patients than in control participants.

The present findings are consistence with Riise et al. [31], who studied 42 patients with chronic bronchitis and 13 healthy controls. They found that BAL IL-8 levels were higher in patient with COPD compared with control participants [31].

Pesci et al. [32] studied 20 COPD patients and 10 normal control participants. They found that levels of IL-8 were higher in COPD patients compared with control participants [32].

Also, Soler et al. [24] and Rutgers et al. [33] found that there was a trend for BAL IL-8 to be higher in smoking controls and COPD patients as compared with controls.

Drost et al. [34] and Cheng et al. [35] found that IL-8 levels in the BAL fluid were significantly higher in patients with COPD than in controls.

**Serum TNF-α**

In the present study, serum TNF-α was significantly higher in severe than in mild and moderate COPD patients and control participants.

The results of this work are consistent with those of Takabatake et al. [36], Bolton et al. [37], and Itoh et al. [38], who found that the serum level of TNF-α was higher in COPD patients than in control participants.

Our results agree with Abd El-Maksoud et al. [21], Garcia-Rio et al. [22], Xie et al. [29], Ibrahim et al. [39], and Abd El Aziz et al. [40], who found that the concentrations of circulating TNF-α were significantly higher in patients with COPD in comparison with the control group, and their levels increased according to the stage of the disease.

In contrast to our results, Yende et al. [41], Shin et al. [42], and Piehl-Aulin et al. [43] found that there were no significant difference between serum levels of TNF-α in COPD patients and controls.

El-Adl et al. [44] studied 60 COPD patients (divided into three groups: group I: 20 AECOPD patients without malnutrition; group II: 20 stable patients without malnutrition; and group III: 20 stable patients with malnutrition) and 10 healthy control individuals; they found that there was no statistically significant difference in serum TNF-α levels between the groups.

Also, Amer et al. [45] and Bruno et al. [46] studied 90 individuals [subdivided into three equal groups: group I (control), group II (patients with COPD), and group III (patients with COPD and cardiovascular complications)], and they found no significant difference between the groups.

Yende et al. [41] and Amer et al. [45] explain the decrease in serum TNF-α in COPD patients by the relatively short serum half-life of TNF-α and by the wide range of disease progression in each group.
BAL TNF-α
In the present study, it was significantly higher in mild and moderate COPD patients than in control participants.

The present findings are consistent those of with Soler et al. [24], who found that the BAL concentration of TNF-α was higher in smoking controls and COPD patients than in healthy nonsmoking control individuals.

Cheng et al. [35] found that the levels of BAL TNF-α were also significantly higher in patients with COPD than in controls.

Abd El Aziz et al. [40] found that BAL TNF-α was highly significantly elevated in COPD patients than in the control group.

In contrast to our results, Drost et al. [34] found that TNF-α levels detected in airway secretions and the BAL fluid were generally low and were not significantly different between COPD patients and control participants; they explain the decrease in TNF-α in BAL by the action of cytokines mainly in peripheral lung tissues.

Correlation
Serum IL-6, IL-8, and TNF-α in comparison with pulmonary functions
In the present study, there were a significant negative correlation between serum IL-6, IL-8, and TNF-α and FEV₁, FEV₁/FVC (FEV₁%, and FEF25–75%.

The data of our work are consistent with those of Soler et al., [24] Pinto-Plata et al. [47], Abd El-Maksoud et al. [21], Attaran et al. [20], and Ramadan et al. [48], who found a significant negative correlation between IL-6 levels and FEV₁.

In contrast to our results, Akbulut et al.[7] found no correlation between the IL-6 value and FEV₁ and FEV₁/FVC values.

Also, Kanazawa et al. [49], Soler et al. [24], Zhang et al. [50], and Demirici et al. [30] found a negative correlation between IL-8 and FEV₁.

In contrast to our results, Pinto-Plata et al. [51] and Akbulut et al. [7] found no correlation between the IL-8 value and FEV₁, and FEV₁/FVC values.

In contrast, Pinto-Plata et al. [47] and Amer et al. [46] found a significant negative correlation between TNF-α levels and FEV₁. In contrast to our results, Abd El-Maksoud et al. [21]found no significant correlation between TNF-α and FEV₁.

From the previous correlations, it is clear that the increase in serum inflammatory markers has a direct correlation with the severity of COPD.

Serum IL-6, serum IL-8, and TNF-α in comparison with the total and the differential cell count%
There were a significant positive correlation between serum IL-6, IL-8, and TNF-α and the serum total leucocytic count, lymphocyte%, and neutrophil%.

Our results are consistent with the study of Bathoorn et al. [51] and Moermans et al. [52], who found a significant positive correlation between serum IL-6 and neutrophil%.

Also, Daldegan et al. [28] and Demirici et al. [30] found a positive correlation between the number of neutrophils and serum IL-8 and TNF-α.

Our data denote that the increase in the serum total cell count, lymphocyte%, and neutrophil % is associated with an increase in inflammatory markers IL-6, IL-8, and TNF-α.

BAL IL-6, IL-8, and TNF-α in comparison with pulmonary functions
There were a significant negative correlation between BAL IL-6, IL-8, and TNF-α and both FEV₁/FVC and FEF25–75%.

The present results match with the study of Soler et al. [24] and Weidong et al. [25], who found a significant negative correlation between BAL IL-6 and IL-8 and FEV₁%.

Also, Drost et al. [34] found a significant negative correlation between BAL IL-8 and the FEV₁/FVC ratio.

The present results matched with the study of Cheng et al. [35], who found a significant negative correlation between BAL TNF-α and FEV₁%.

BAL IL-6, IL-8, and TNF-α in comparison with the BAL total and differential cell count %
There was a significant positive correlation between BAL IL-6, IL-8, and TNF-α and both neutrophil % and macrophage %.

Our results are consistent with the study of Rouhani et al. [53] and Moermans et al. [52], who found a significant positive correlation between BAL IL-6 and IL-8 and both neutrophil and macrophage %.

In contrast to our results, Drost et al. [34] found no correlation between BAL IL-8 and neutrophil%. 

Also Kwon et al. [54] and Soler et al. [24] found a significant positive correlation between BAL TNF-α and both neutrophil and macrophage %.

COPD is characterized by progressive obstructive airflow limitation resulting from an abnormal inflammatory response to noxious particles or gases [55]. The initial inflammatory response to damage from noxious particles or gases is characterized by increased neutrophils, macrophages, T-lymphocytes, and increased cytokines including IL-6, IL-8, and TNF-α. The inflammation that develops is not limited to the lungs. Studies have shown increased systemic levels of IL-6, IL-8, and TNF-α in patients with COPD. This may be due to an ‘overspill’ of mediators from the lungs. The increase in these proinflammatory cytokines is correlated to the severity of COPD and is believed to contribute to the systemic comorbidities associated with COPD, Hacievliyagil et al. [56] observed that higher concentrations of inflammatory cytokines, including IL-6, IL-8 and TNF-α, are reported in patients with more severe COPD compared with those with less severe COPD. Because of the previous association, cytokine inhibitors are tried in treatment of COPD; TNF-α-blocking antibodies, such as infliximab, have been studied as a treatment for COPD. Unfortunately, they have not been able to show any differences in inflammatory markers. There is evidence, however, that etanercept, another TNF-α antagonist, decreases COPD hospitalizations [57].

Tocilizumab, a potent inhibitor of IL-6, is yet to be tested in COPD patients. These drugs are still in the development phase [58].

Acknowledgements
Conflicts of interest
None declared.

References
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