Role of Osteopontin and NGAL in Differential Diagnosis of Acute Exacerbations of COPD and Pneumonia

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ABSTRACT

Objective: Chronic Obstructive Pulmonary Disease (COPD) is an inflammatory lung disease that progresses with attacks. Pneumonia is an infectious lung disease that progresses with lung infiltrations. Osteopontin (OP) is a cytokine which participates in inflammation. Neutrophil Gelatinase Associated Lipocalin (NGAL) is an antimicrobial peptide with neutrophil activation and antibacterial properties. In this study, serum OP and NGAL levels were assayed in COPD exacerbation, stable COPD and pneumonia. The aim of our study is to assess the importance of NGAL and OP levels as biomarkers in the differential diagnosis of COPD and pneumonia.

Material and Methods: One hundred twenty consecutive patients who were admitted to our department between May 2011 and August 2013 were included in the study. Serum OP and NGAL levels were measured with ELISA method within 24 hours following the determination of diagnosis. COPD acute exacerbation (AE-COPD) group was comprised of 95 patients (87 male and mean age 69.0±10.6), and the pneumonia group was comprised of 25 patients (16 male and mean age 57.5±22.9). Serum OP and NGAL levels of the patients in the AE-COPD group were re-measured within 30-45 days following acute exacerbation in stable period.

Results: Serum OP levels were higher in the pneumonia group compared to the AE-COPD group (93.47 ng/ml vs 53.10 ng/ml; p<0.001). Multivariate regression analyses indicated that OP levels to be >84 ng/ml is an independent predictor that increases risk for pneumonia more than 8-fold (95% CI, 2.43-26.59). Sensitivity and specificity of OP in the differentiation of pneumonias from AE-COPD were determined to 80% and 92%, respectively. Serum NGAL levels also increased as COPD severity increased and was found to be statistically significant (p: 0.032).

Conclusion: It has been indicated that serum osteopontin level can be an independent predictor in differentiating COPD exacerbation from pneumonia. Additionally, as COPD severity (stage) increases, serum NGAL levels also increase, which may be helpful in assessing the severity of COPD.

Keywords: COPD acute exacerbation, NGAL, osteopontin, pneumonia

INTRODUCTION

Chronic Obstructive Pulmonary Disease (COPD) attacks and pneumonia are common diseases caused by inflammation in the lungs. Differential diagnosis of these diseases can sometimes be difficult. In our study, we investigated whether using biomarkers such as OP and NGAL may aid in the differential diagnosis.

COPD is an inflammatory lung disease and one of the major causes of mortality and morbidity all over the world. COPD is a prevalent respiratory condition characterized by progressive and irreversible airflow limitation. Factors such as infection, air pollution, and pulmonary embolism may cause a COPD attack (1).

According to the World Health Organization, COPD is estimated to affect over 330 million individuals globally and is projected to become the third leading cause of death by 2030 (2).
There is typically a persistent inflammatory response within the small airways and pulmonary tissues often accompanied by signs of enhanced systemic inflammation (1,3). Spirometry is important in the diagnosis of COPD and is used to classify the severity of the disease (1) (Table 1)

**Table 1. GOLD Grades and Severity of Airflow Obstruction in COPD**

<table>
<thead>
<tr>
<th>Grade</th>
<th>Severity</th>
<th>FEV1 % Predicted</th>
</tr>
</thead>
<tbody>
<tr>
<td>GOLD 1</td>
<td>Mild</td>
<td>FEV1≥80% predicted</td>
</tr>
<tr>
<td>GOLD 2</td>
<td>Moderate</td>
<td>50%≤FEV1&lt;80% predicted</td>
</tr>
<tr>
<td>GOLD 3</td>
<td>Severe</td>
<td>30%≤FEV1&lt;50% predicted</td>
</tr>
<tr>
<td>GOLD 4</td>
<td>Very Severe</td>
<td>FEV1&lt;30% predicted</td>
</tr>
</tbody>
</table>

COPD courses commonly with exacerbations, and the reason for symptoms to get worse is commonly accompanying co-infections. Patients with COPD typically complain of dyspnea, activity limitation and/or cough with or without sputum production and may experience acute respiratory events characterized by increased respiratory symptoms called exacerbations that require specific preventive and therapeutic measures (1). One of the causes of acute exacerbations of COPD is pneumonias, and risk for pneumonia in COPD cases is high (4, 5).

Community-acquired pneumonia is defined by acute symptoms and the presence of signs of lower respiratory tract infection without another obvious cause, whereas a new pulmonary infiltrate on chest radiograph is needed for a definite diagnosis. The most common signs and symptoms are dyspnea, cough, fever, and new focal chest signs (6).

Sometimes, clinical differentiation of acute exacerbations and pneumonia in COPD cases can be troublesome. Symptoms and clinical presentation may resemble in both cases. It is also hard to radiologically recognize pneumonia because of emphysematous areas, bullae and blebs, air cysts and relatively normal pulmonary parenchymal areas, which are constricted accordingly, atelectasis and bronchiectasis and sequela lesions due to diseases such as tuberculosis.

Osteopontin (OP) is a cytokine that participates in inflammation and causes proliferation, especially in vascular smooth muscle. OP may interact with various cell surface receptors, including a part binding arginine, glycine and aspartic acid. It protects monocytes, macrophages and T-cells from the effects of inflammations and empowers them. Inflammatory markers such as TNF-α and IL1β potentlie stimulate the release of OP (7, 8). Plasma levels of OP have been found to be related to the severity and mortality of idiopathic pulmonary arterial hypertension (iPAH) (8). Emerging evidence suggests that OP also plays a critical role in the pathogenesis of respiratory diseases. OP can be produced and secreted by various cell types in the lungs, and overexpression of OPN has been found in acute lung injury/acute respiratory distress syndrome (ALI/ARDS), pulmonary fibrosis diseases, lung cancer, lung infection, COPD and asthma (9).

Neutrophil Gelatinase Associated Lipocalin (NGAL) is an antimicrobial peptide that causes collagen degradation through Matrix metalloproteinase-9. It has a strong affinity for bacterial-secreting siderophores that remove iron from the circulation. By binding to these siderophores, it reduces the free iron required for bacterial growth. NGAL has neutrophil activation, and antibacterial and matrix degradation properties (10).

In this study, the potential role of OP in differential diagnosis of both pathologies was investigated based on the fact that Serum Osteopontin and NGAL levels can be different in pneumonia and acute exacerbations of COPD. Also we investigated the serum NGAL and OP levels as the severity of COPD increased and aimed to evaluate the possibility of this being a parameter.

**MATERIAL and METHODS**

**Study Design**

Patients who were followed up or treated with a diagnosis of acute exacerbation of COPD and pneumonia in the outpatient clinic or pulmonary diseases ward of our hospital between May 2011 and August 2013 and signed consent forms were included in this study. COPD was diagnosed according to 2013 GOLD Guideline, whereas the diagnosis of pneumonia was established according to IDSA/ATS Guideline (4). Patients diagnosed with acute exacerbation of COPD were re-evaluated within 30-45 days following treatment of exacerbation in the stable period (4). Patients with FEV1/FVC<70% in the respiratory function test, over 40 years of age, and with a history of smoking were included in the study.

Patients who were diagnosed with cancer within last 5 years, a known active inflammatory disease or history of COPD exacerbation within last 4 weeks were excluded. Patients in the pneumonia group were not diagnosed with COPD. There is no infectious focus other than pneumonia in the COPD exacerbations included in the study. Patients with a history of COPD and increased respiratory complaints, at least two of the following complaints: increased sputum amount, sputum purulence, shortness of breath, and infiltration on chest X-ray or lung tomography, were evaluated as COPD infectious exacerbation. No further examination of pulmonary arterial hypertension was performed in pneumonia patients. However, patients without clinical findings suggestive of pulmonary arterial hypertension were included in the study.

**Records and Analyses**

Demographical data, general health statuses, complaints for admission, smoking, environmental and occupational exposures, co-morbidities, examination findings, chest x-ray findings, respiratory function tests, mMRC dispnea scales, BODE, scores, methods of treatment, durations of hospital stay, results of serum biochemical analyses (hemogram, erythrocyte sedimentation rate, CRP, procalcitonin, arterial blood gas analysis) of all patients included in the study were recorded.

**Biochemical analysis**

Serum Osteopontin and NGAL levels were measured within 24 hours following the determination of diagnosis. In patients in the COPD group, serum osteopontin and NGAL levels were re-measured within 30-45 days following acute exacerbation in a stable period. After 4 cc of venous blood, which was collected for this purpose, was centrifuged in a biochemistry tube, it was stored at -80 degrees Celsius in the biochemistry laboratory of our hospital.
At the end of the study, human osteopontin levels were measured from these materials by using eBioscience brand commercial ELISA kits with reference number BMS2066 in accordance with kit procedure. NGAL levels were measured using commercial ELISA kits with BioVendor brand reference number RD19102200R and per the kit procedure.

Spectrophotometric measurements of assayed ELISA kits were performed with ELISA microplate reader device of the brand Versamax of the company Molecular Devices.

**Statistical analysis**

Statistical analysis was performed using IBM SPSS V20. The Kolmogorov-Smirnov test was used to assess the distribution of the data. Categorical variables were compared using Fisher’s exact test, whereas continuous data were compared using the Student’s t-test. Multivariable logistic regression analysis was used as a stepwise descending method from prognostic factors with a P value significance of <0.05 in the univariate analysis. The difference between the baseline and follow-up serum OP was analyzed using the paired sample t-test. A p-value <0.05 was used as statistically significant in all analyses.

**RESULTS**

Totally 120 patients, 95 being from COPD group and 25 being from pneumonia group, were included in the study, general characteristics of the patients were given comparatively in Table 2. Mean age, male gender ratio and ratio of cases which were smoking or not were significantly higher in the COPD group.

Complaints of sputum purulence and fever were more in the pneumonia group (80%, 56% vs. 56,8% and 56,8% vs. 56,8% and 10,5%; p<0.05); whereas shortness of breath was more in the COPD exacerbation group (86,3% vs. 40%; p<0.001). Causes of COPD exacerbations were as follows in order of frequency: Infection (65,3%), air pollution (15,8%), Pulmonary Embolism (5,3%) Arrhythmia (1,1%) and Others (12,6%).

On chest x-rays of patients with COPD exacerbation, infiltrative shadows 42.1% (n=40), flattening of diaphragms 24.2% (n=23) and hyperinflation 22.1% (n=21) were most commonly observed. Of patients with COPD exacerbation; 9.5% (n=9) were bound to invasive mechanical ventilator, 30.5% (n=29) received treatment with non-invasive mechanical ventilation. 20 (21.1%) of the patients with COPD became exitus during acute exacerbation period.

Table 2. General characteristics of the study population

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>COPD exacerbation</th>
<th>Pneumonia</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender, male</td>
<td>87 (91.6)</td>
<td>16 (64.0)</td>
<td>0.002</td>
</tr>
<tr>
<td>Age, years</td>
<td>69.4 ± 10.5</td>
<td>57.5 ± 22.9</td>
<td>0.018</td>
</tr>
<tr>
<td>Smoking</td>
<td>95 (100)</td>
<td>16 (64.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Duration of hospital stay, days</td>
<td>7.8 ± 6.0</td>
<td>8.7 ± 5.4</td>
<td>0.498</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>120.00 (75-200)</td>
<td>110.00 (90-160)</td>
<td>NS</td>
</tr>
<tr>
<td>Pulse, min</td>
<td>24.00 (14-46)</td>
<td>24.00 (18-40)</td>
<td>NS</td>
</tr>
<tr>
<td>WBC, µL</td>
<td>7.00 (4.00-20.00)</td>
<td>14.00 (4.00-30.00)</td>
<td>NS</td>
</tr>
<tr>
<td>CRP, mg/Dl</td>
<td>3.0 (0.5-49.0)</td>
<td>14 (0.5-33.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PCT, µg/L</td>
<td>0.92 (0.4-49.0)</td>
<td>0.25 (0.3-73.0)</td>
<td>0.011</td>
</tr>
<tr>
<td>OP, ng/ml</td>
<td>53.15</td>
<td>93.47</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>NGAL, ng/ml</td>
<td>90.0 ± 14.6</td>
<td>106.3 ± 18.9</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values are given as interquartile range and standard deviation.

CRP: C-Reactive protein, PCT: Procalcitonin, SBP: Systolic blood pressure

Serum osteopontin levels were higher in the pneumonia group than in the COPD group (p < 0.001). However, there was no difference between measurements in acute exacerbation and stable periods (mean ± SD; 53.1 ± 22.4 and 52.4 ± 23.3; p=0.797) (fig-1).

Although serum OP levels were increased as the severity (stage) of COPD increased, this relationship was not found to be statistically significant (p = 0.270) (fig-2).

In the COPD group, Serum osteopontin levels did not differ between infectious and non-infectious exacerbations (56.7 ± 22.1 and 47.0 ± 22.0; p=0.071). Furthermore, serum OP level in patients who had infection-induced COPD exacerbation did not differ from Serum OP level in stable period (56.8±22.1 vs. 53.1±2.5; p=0.323)

When COPD attack and pneumonia groups were compared, serum NGAL level was found to be significantly higher in the pneumonia group (p<0.001) (fig-3). In the pneumonia group, the mean serum NGAL value was determined as 106.3 ±18.9 ng/mL (Table 3)

When we look at the NGAL level according to COPD Stage, the NGAL level is increased as the severity of COPD increased (p=0.032) (fig-4).

Serum CRP, Procalcitonin (PCT) and Osteopontin values were statistically significantly higher in the pneumonia group compared to the COPD exacerbation group (Table 3).

ROC analysis was used to determine diagnostic values of markers claimed in differentiation between COPD exacerbation and pneumonia. In ROC analysis, AUC value of OP for diagnosis of pneumonia was determined to be 0.847 (% 95 CI: 0.733 – 0.962; p<0.01). AUC value for NGAL was determined to be 0.744 (% 95 CI: 0.616 – 0.872; p<0.001) (fig-3). At osteopontin values > 84 ng/mL, sensitivity was determined to be 80%, and specificity was determined to be 92% for diagnosis of pneumonia (fig-5).

In the univariate regression analysis conducted to assess the diagnostic values of markers in differentiating between COPD exacerbation and pneumonia, OP, NGAL, CRP, and PCT were found to be significant (p < 0.05). Accordingly, Multivariate regression analyses indicated that Osteopontin levels >84 ng/mL is an independent predictor that increases diagnosis of pneumonia more than 8-fold (Table 4).
Table 3. Serum CRP, PRC, OP and NGAL values in COPD exacerbation and pneumonia

<table>
<thead>
<tr>
<th>Parameters</th>
<th>COPD exacerbation (n: 95) (median value)</th>
<th>Pneumonia (n: 25) (median value)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP (mg/dL)</td>
<td>2.96</td>
<td>13.98</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PCT (ng/mL)</td>
<td>0.09</td>
<td>0.24</td>
<td>0.011</td>
</tr>
<tr>
<td>OP (ng/mL)</td>
<td>53.15</td>
<td>93.47</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>NGAL (ng/mL)</td>
<td>88.12</td>
<td>106.30</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Table 4. Role of biomarkers in the differentiation of COPD exacerbation and pneumonia in multivariate regression analysis

<table>
<thead>
<tr>
<th>OR</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Osteopontin&gt;84 (ng/mL)</td>
<td>8.03</td>
<td>2.428 - 26.59</td>
</tr>
<tr>
<td>NGAL ≥ 94 (ng/mL)</td>
<td>3.23</td>
<td>0.735-14.20</td>
</tr>
<tr>
<td>CRP ≥ 10 mg/DL</td>
<td>5.14</td>
<td>1.653 - 16.01</td>
</tr>
<tr>
<td>Procalcitonin ≥ 0.09 ng/mL</td>
<td>0.80</td>
<td>0.209 - 3.14</td>
</tr>
</tbody>
</table>

Figure 1. Comparison of Serum OP values in COPD exacerbation, stable COPD and Pneumonia Groups

Figure 2. Comparison of Serum OP values according to COPD stages

(Stage 1: GOLD 1: A, Stage 2: GOLD 2: B, Stage 3: GOLD 3: C, Stage 4: GOLD 4: D)
DISCUSSION

The most important outcomes of this study are these: The first one is that serum OP level is an independent marker that increases the risk for pneumonia more than 8-fold in differentiation between pneumonia and COPD exacerbation. The second one is that there is no difference between COPD exacerbation period and the stable period in terms of serum OP levels. The third one is that serum NGAL level is correlated with the stage of COPD.

OP levels in sputum supernatant were compared among healthy individuals who smoke or not in patients with COPD. The highest values were determined in patients with COPD (median [interquartile range], 1340 [601, 6227] vs. 101 [77, 110] vs. 68 [50, 89], respectively; p<0.001) (4).

In another study, it was observed that OP level increased in BAL cells in smoking-related pulmonary diseases (11). This data indicates that OP level increases in COPD due to inflammatory response.

In our study, when cases with COPD exacerbation and pneumonia were compared, serum osteopontin levels were found to be statistically significantly higher in the pneumonia group. Mean serum osteopontin level was determined to be 53.15 ng/mL in COPD exacerbation group and 93.47 ng/mL in the pneumonia group. In ROC analysis, AUC value for osteopontin in the diagnosis of pneumonia was found to be 0.847 (95% CI: 0.733 – 0.962) (p<0.01).
At Osteopontin values > 84 ng/mL, sensitivity was determined to be 80% and specificity was determined to be 92% for diagnosing pneumonia. It was observed that osteopontin level >84 ng/mL is an independent marker that increases the risk for pneumonia more than 8-fold.

In the literature, there is no study comparing serum osteopontin levels between COPD exacerbation and pneumonia groups.

Only one study has compared osteopontin levels among COPD exacerbation, stable COPD, and healthy control groups. In the study conducted by Lee et al., 64 individuals were included in the COPD exacerbation group, 68 individuals were included in the stable COPD group and 30 individuals were included in the healthy control group. Serum osteopontin level was measured as 32.6±29.6 ng/mL in COPD exacerbation, as 17.6±11.1 in stable COPD and as 8.4±6.1 ng/mL in the healthy control group (p<0.001). Osteopontin levels were found to be significantly higher in individuals who experienced exacerbations more frequently (p=0.008) (12). In our study, no statistically significant difference was observed between COPD exacerbation and stable COPD in terms of serum osteopontin values. Furthermore, in the COPD group, serum osteopontin levels did not differ between infectious exacerbations and non-infectious exacerbations (56.7±22.1 and 47.0±22.0; p=0.071). It was observed that serum OP levels of patients who experienced infection-induced COPD exacerbation did not differ from those in the stable period. On this aspect, we can say that serum OP level is not a determinant of the need for antibiotherapy in COPD exacerbation.

In addition to the fact that serum osteopontin level is a marker related to inflammation, serum Osteopontin levels to be higher in the stable period in COPD cases supports that inflammation continues also in the stable period and systemic immune response is active. In addition to this, in our study, serum OP level to be correlated with other inflammatory markers (CRP, PCT) supports this assertion. Thus, the importance and necessity of anti-inflammatory treatment in COPD have been underscored once again.

In this study, an increase in osteopontin levels was determined as frequency of exacerbation increased; however, this was not found to be statistically significant. Whereas the mean osteopontin value was determined to be 55.2±22.4 ng/mL in ones who experienced exacerbations frequently (≥2 exacerbations), the mean osteopontin value was determined to be 50.6±22.4 ng/mL in ones who experienced less than two exacerbations (p>0.05). It was speculated that the relatively small sample size in our study may have influenced the obtained results.

Serum osteopontin levels were found to be increased as stage of COPD increased. Whereas mean osteopontin level was 42.30±25.90 ng/mL in Group A, it was measured as 57.38±20.80 ng/mL in Group D (p=0.270). Similarly, also in the study conducted by Lee et al., stages of COPD exacerbations were grouped as from A to D. Osteopontin level increased as stage of the disease increased, but this was not determined to be statistically significant (12). Under light of this data, it can be suggested that OP level reflects a local inflammation.

In our study we observed that the NGAL level is increased as the severity of COPD increased (p=0.032) (fig- 4). This result is shown that NGAL may be a biomarker to predict the severity of COPD. In the literatures; there is no study about NGAL and COPD stage. In the study by A. Paul et al. investigated NGAL in sepsis and found that a screening tool with a score >7 can be used in the emergency department (ED) to identify bacterial sepsis (13).

**Study Limitations:** This study has some limitations. This study, despite its strengths such as prospective data collection and estimation of biomarkers with low measurement error, had limitations, such as a small sample size and being restricted to a single center. Additionally, all patients with COPD exacerbations in our study required hospitalization. Consequently, the osteopontin levels in patients who do not require hospitalization remain unknown. Furthermore, serial NGAL and OP levels were not measured to evaluate the response to antibiotherapy in patients with pneumonia, and there was a lack of a healthy control group.

**CONCLUSION**

Our findings suggest that serum osteopontin level can serve as an independent predictor in distinguishing between COPD exacerbation and pneumonia. The consistent serum osteopontin levels observed in both exacerbation and stable periods in COPD patients may reflect underlying chronic inflammation. Furthermore, as COPD severity increases, NGAL levels also rise, indicating its potential as a biomarker for predicting COPD severity. However, further comprehensive multicenter studies are warranted to validate these findings and provide additional insights into the utility of these biomarkers.

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**Author Contributions:** SG, TÖ, SSÖ, AÖ: Designed and supervised the study, conducted literature search, collected data, drafted the article, and made final revisions. All authors reviewed the results and approved the final version of the manuscript.

**Ethical approval:** The present study was conducted in strict accordance with the principles outlined in the Declaration of Helsinki. Informed consent was obtained from the participant of this study.

**REFERENCES**


