Systemic inflammation and systemic oxidative stress in patients with acute exacerbations of COPD

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Summary

Background: In patients with chronic obstructive pulmonary disease (COPD), the inflammatory processes and oxidative stress are closely linked in the lung compartment. However, the relationships between systemic inflammation and parameters of oxidative stress in the systemic circulation during acute exacerbations of COPD remain to be explored.

Objective: To analyze relationships between erythrocytic glutathione peroxidase (GPx), a marker of systemic oxidative stress, and parameters reflecting systemic inflammation, such as circulating neutrophils, C-reactive protein (CRP), and interleukin (IL)-6, in patients with acute exacerbations of COPD.

Patients and methods: We measured erythrocytic GPx activity, circulating neutrophil count, and serum high-sensitivity (hs) CRP and IL-6 in patients with acute exacerbations of COPD.

Results: From GOLD Stage II to Stage III and IV, erythrocytic GPx activity significantly decreased [mean ± SEM: from 44.3 ± 1.7 U/g Hb to 40.8 ± 1.1 U/g Hb and to 38.4 ± 1.5 U/g Hb, p = 0.037], while serum hsCRP increased [median (25th, 75th percentile): from 9.6 (3.0, 23.0) mg/l to 23.3 (6.4, 46.8) mg/l, and to 26.7 (6.5, 117.2) mg/l, p = 0.004]. Erythrocytic GPx activity was significantly inversely related to both, log neutrophil count (r = −0.219, p = 0.003) and log hsCRP (r = −0.199, p = 0.008).

Conclusions: Our study suggests an association between systemic inflammation and systemic oxidative stress reflected by erythrocytic GPx in patients with acute exacerbations of COPD.

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Introduction

Many aspects of the airway and systemic oxidative stress and airway and systemic inflammation in chronic obstructive pulmonary disease (COPD) have been unraveled in recent years. Importantly, systemic inflammation may be involved in the pathogenesis of systemic complications in patients with COPD. In non-COPD patients, several recent studies reported associations between increased cardiovascular risk, elevated neutrophil count, and peripheral markers of oxidative stress. In addition, it has been suggested that oxidative stress might be a determinant of serum C-reactive protein (CRP) levels and promote pro-atherosclerotic inflammatory processes. Therefore, investigation of systemic oxidative stress in patients with COPD who are known to be at increased cardiovascular risk became particularly relevant.

Recently, we and others have demonstrated an inverse relationship between glutathione peroxidase (GPx) activity in erythrocytes and the degree of obstructive lung impairment in patients with COPD. On the other hand, chronic airflow limitation is associated with systemic inflammation as evidenced by significantly raised circulatory levels of CRP, fibrinogen, leukocytes, and tumor necrosis factor (TNF)-α. Importantly, high CRP levels were shown to be associated with clinically important outcomes such as lower performance in the 6-min walk distance test, impaired energy metabolism, respiratory distress, and increased mortality. Taken together, these data suggest a potential link between systemic oxidative stress and systemic inflammation in patients with COPD.

Several studies reported that, in the lung compartment, the inflammatory processes and oxidative stress are tightly linked. In patients with stable COPD, significant relationships were observed between sputum neutrophils and sputum concentrations of nitrosothiol- and oxidized glutathione, and between sputum neutrophils and levels of hydrogen peroxide and 8-isoprostanate in exhaled breath condensate. In addition, associations between parameters of oxidative stress markers and neutrophilia in the lungs, and inflammatory cytokines in the systemic circulation were reported. Nevertheless, the data on the potential relationships between systemic inflammation and markers of systemic oxidative stress in COPD are scarce. In a recent report, a relationship between GPx in blood and serum interleukin (IL)-8 in patients with stable COPD was observed. Similar relationships have not been yet studied during acute exacerbations of COPD. Therefore, the aim of the present study was to analyze relationships between erythrocytic GPx, a marker of systemic oxidative stress, and parameters reflecting systemic inflammation, such as circulating neutrophil count, high-sensitivity (hs) CRP, and IL-6, in patients with acute exacerbations of COPD.

Patients and methods

Study population

Patients with diagnosis of COPD according to the American Thoracic Society/European Respiratory Society guidelines, admitted to the tertiary referral teaching hospital because of an exacerbation, participated in the study. The diagnosis of an acute exacerbation was established if the patient suffered from a sustained worsening of his/her conditions from the stable state, and beyond the normal day-to-day variations, that was acute in onset and warranted additional treatment. This definition is based on previously described symptomatic criteria. In agreement with Perera et al., exclusion criteria were respiratory disorders other than COPD, a new radiological infiltrate, malignancy, overt cardiac failure, recent surgery, chronic autoimmune disorders, and severe endocrine, hepatic or renal disease. Treatment of acute exacerbation consisted of inhaled bronchodilators, systemic corticosteroids, oxygen use in case of hypoxemia, and antibiotics in case of purulent sputum and/or evidence of microbial growth in sputum. The study had local ethics committee approval, and all subjects gave written consent to the study.

Pulmonary function tests

Pulmonary function tests were evaluated with the use of bodyplethysmography (Ganshorn, Germany). All pulmonary function testing was performed according to the European Respiratory Society standards with the patients in a sitting position by the same technician in order to ensure consistency of the technique. Three technically acceptable measurements were performed on each patient, and the highest value was included in the analyses.

Biochemical analyses

In all patients, peripheral venous blood samples from the antecubital vein were collected between 6.00 and 8.00 a.m. after 10 h fast, within first 36 h after admission. Arterial blood gases were determined in samples obtained by puncture of the radial artery with the patient seated. The samples for blood gases analyses were obtained while breathing room air in patients not treated with oxygen, and while breathing oxygen in those requiring oxygen therapy. Routine biochemical analyses were performed using standard techniques. GPx activity was determined in washed red blood cells obtained immediately after sampling from the whole blood anticoagulated with EDTA using commercially available kits (Ransel, Randox). GPx values are expressed as units per gram hemoglobin (U/g Hb).

Serum was separated from blood cells by centrifugation at 4000 cycles/min. All samples were stored at −70 °C until analyzed. hs serum CRP levels were assessed by chemiluminescent immunoassay (Tina-Quant, Roche Diagnostics). The analytical sensitivity of this CRP assay is 0.1 mg/l. Serum IL-6 levels were measured using commercially available enzyme-linked immunosorbent assay kits (Beckmann-Coulter Immunotech). The IL-6 assay sensitivity, defined as the lowest IL-6 concentration significantly different from the zero standard with a probability of 95%, was 3 pg/ml.
Statistical analyses

Data were analyzed using SPSS for Windows software (version 14.0). Power calculations were performed based on hsCRP. This variable was chosen based on its clinical importance in COPD.26–28 Published results of hsCRP patients with acute exacerbations of COPD reported an average standard deviation of 3.26 With this information, a power calculation indicated that we would need at least 44 patients per each GOLD Stage group to detect a difference of 2 mg/l in hsCRP with a power of 80% at a 0.05 significance level.

The Kolmogorov–Smirnov test of normality was applied. Exacerbation inflammatory indices not normally distributed were rendered so by log_{10} transformation. Continuous variables are shown as mean±SEM, non-normally distributed variables as median (25th, 75th percentile). Differences between groups in normally distributed variables were tested by one-way analysis of variance (ANOVA) with Tukey test for post hoc pairwise comparison procedures, and in non-normally distributed variables by ANOVA on ranks with Dunn’s test for post hoc pairwise comparison procedures. Chi-square test was used to compare the proportion of categoric variables between groups. To assess the relationships between selected variables, linear regression analysis was used. Because the distributions of peripheral neutrophil count, serum hsCRP, and IL-6 activity were all skewed, we used the log-transformed values of these variables in regression analyses.

Results

Subjects characteristics

One hundred and seventy-seven patients (129 males and 48 females) with COPD were recruited to the study (Table 1). Forty-seven patients were classified as Stage II, 90 patients were Stage III, and 40 patients were Stage IV COPD.21 All patients had a previous history of the diagnosis of COPD, and were using short-acting bronchodilators (salbutamol or ipratropium bromide) to relieve their symptoms. In addition, 60% of patients were on long-term treatment with long-acting beta agonists, 58% with inhaled corticosteroids, 65% with theophyllines, 9% with oral corticosteroids, and 12% with long-term home oxygen therapy. Prior to the hospital admission, 15% were prescribed antibiotics for respiratory infection. The acute exacerbation of COPD required new or increased administration of both bronchodilators and systemic corticosteroids in all patients. Antibiotics were increased or introduced to the treatment in 86% patients, and oxygen therapy in 24% patients. At admission, patients were treated with hydrocortisone 200 mg intravenously per day, and the dose was reduced after 2–3 days. None of the patients smoked cigarettes during the admission. Medical records revealed an increased prevalence of ischemic heart disease from Stage II to Stage III and to Stage IV [from 21 (45%) to 60 (67%), and to 20 (50%) patients, p = 0.028]. No differences were observed between Stage II, III, and IV in the presence of ischemic heart disease.

Table 1 Demographic data and pulmonary function parameters in patients with COPD.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Entire group</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>177</td>
<td>47</td>
<td>90</td>
<td>40</td>
<td>0.002</td>
</tr>
<tr>
<td>Women (no., %)</td>
<td>48 (27)</td>
<td>22 (47)</td>
<td>16 (18)</td>
<td>10 (25)</td>
<td>0.002</td>
</tr>
<tr>
<td>Age (years)</td>
<td>65.8±0.8</td>
<td>62.8±1.8</td>
<td>67.0±1.1</td>
<td>67.2±1.4</td>
<td>0.072</td>
</tr>
<tr>
<td>Smoking (pack-years)</td>
<td>25.5±1.7</td>
<td>17.9±3.2</td>
<td>29.0±2.5*</td>
<td>26.4±2.9*</td>
<td>0.020</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.6±0.5</td>
<td>26.2±0.8</td>
<td>26.1±0.7</td>
<td>24.0±0.9</td>
<td>0.146</td>
</tr>
<tr>
<td>FEV₁ (% predicted)</td>
<td>41.9±1.0</td>
<td>60.8±1.2</td>
<td>39.1±0.6*</td>
<td>25.8±0.5*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FEV₁ (l)</td>
<td>1.1±0.03</td>
<td>1.5±0.1</td>
<td>1.1±0.1*</td>
<td>0.7±0.1*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FVC (%)</td>
<td>65.8±1.4</td>
<td>84.1±1.8</td>
<td>63.6±1.4*</td>
<td>49.4±2.3*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FVC (l)</td>
<td>2.2±0.1</td>
<td>2.7±0.1</td>
<td>2.2±0.1*</td>
<td>1.7±0.1*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FEV₁/FVC (%)</td>
<td>50.6±0.9</td>
<td>58.2±1.0</td>
<td>49.7±1.1*</td>
<td>43.7±1.9*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>RV (%)</td>
<td>178.7±4.2</td>
<td>156.0±5.0</td>
<td>177.3±5.7*</td>
<td>206.7±10.6*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>RV (l)</td>
<td>4.0±0.1</td>
<td>3.3±0.1</td>
<td>4.1±0.2*</td>
<td>4.6±0.3*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TLC (%)</td>
<td>108.4±1.7</td>
<td>107.9±2.1</td>
<td>107.7±2.5</td>
<td>110.7±4.6</td>
<td>0.768</td>
</tr>
<tr>
<td>TLC (l)</td>
<td>6.4±0.2</td>
<td>6.2±0.2</td>
<td>6.4±0.2</td>
<td>6.7±0.4</td>
<td>0.567</td>
</tr>
<tr>
<td>RV/TLC (%)</td>
<td>62.5±0.9</td>
<td>54.5±1.2</td>
<td>63.9±1.0*</td>
<td>69.5±2.1*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PaO₂ (kPa)</td>
<td>8.2±0.1</td>
<td>8.9±0.2</td>
<td>8.3±0.2</td>
<td>7.6±0.3</td>
<td>0.002</td>
</tr>
<tr>
<td>PaCO₂ (kPa)</td>
<td>5.6±0.1</td>
<td>4.8±0.1</td>
<td>5.6±0.2*</td>
<td>6.6±0.2*</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values given as mean±SEM.

BMI, body mass index; FEV₁, forced expiratory volume in 1 s; FVC, forced vital capacity; RV, residual volume; TLC, total lung capacity; PaCO₂, arterial carbon dioxide partial pressure; PaO₂, arterial oxygen partial pressure.

P value refers to trend from GOLD Stage II, to Stage III and to Stage IV (ANOVA).

* p<0.05 versus GOLD Stage II.

† p<0.05 versus GOLD Stage III.
of arterial hypertension [29 (61%) versus 52 (58%), and versus 18 (45%), respectively, \( p = 0.259 \)].

All patients experienced worsened dyspnea as a manifestation of acute exacerbation of COPD, and reported the average modified MRC dyspnea score of 3.0 ± 0.1 ("stops for shortness of breath after walking about 100 meters or after a few minutes on the level"). The majority also experienced worsening cough (79%), an increase in sputum production (77%) or a change in sputum color (51%). Wheezing was reported by 48%, sore throat or coryza by 2% of patients. Body temperature was increased (\( > 37 \)°C) in 34% patients. The reported duration of symptoms averaged 2.6 ± 0.4 days before hospital admission. Sputum cultures were obtained from 159 of 177 patients. Of these, potentially pathogenic microorganisms were revealed in 49 (31%): Haemophilus influenzae in 15, Escherichia coli in 10, Pseudomonas aeruginosa in 6, Acinetobacter in 6, Enterobacteriaceae in 4, Klebsiella pneumoniae in 3, Streptococcus pneumoniae in 3, and Staphylococcus aureus in 2 patients.

Peripheral neutrophil count ranged from 1.68 to \( 19.7 \times 10^9 \text{l}^{-1} \) with a median of \( 5.56 \times 10^9 \text{l}^{-1} \), while IL-6 value ranged from 1.2 to 350.0 pg/ml with a median of 14.7 pg/ml, and hsCRP ranged from 0.1 to 282.8 mg/l with a median of 17.9 mg/l. The activity GPx ranged from 20.9 to 74.1 U/g Hb with a median of 39.5 U/g Hb.

Table 1 displays demographic data, pulmonary function tests, \( P_{O_2} \) and \( P_{CO_2} \) in the GOLD Stage II–IV groups of patients. The proportion of women was significantly higher in Stage II compared to Stage III and Stage IV groups \( (p = 0.003) \). In addition, there was a trend towards lower age in Stage II compared to Stage III and IV groups \( (p = 0.072) \). \( P_{O_2} \) decreased, whereas \( P_{CO_2} \) increased from Stage II to Stage III and Stage IV group \( (p = 0.002 \) and \( p < 0.001 \), respectively).

Relationships between GOLD Stage, circulating neutrophil count, serum hsCRP, IL-6, and erythrocyte GPx activity

From GOLD Stage II to Stage III and IV, erythrocytic GPx activity significantly decreased \( [\text{mean} \pm \text{SEM}: \text{from } 44.3 \pm 1.7 \text{U/g Hb to } 40.8 \pm 1.1 \text{U/g Hb and to } 38.4 \pm 1.5 \text{U/g Hb, } p = 0.037] \) (Fig. 1), while serum hsCRP increased \( [\text{median (25th, 75th percentile): from } 9.6 (3.0, 23.0) \text{mg/l and to } 26.7 (6.5, 117.2) \text{mg/l, } p = 0.004] \) (Fig. 2). No differences were observed in either circulating neutrophil count or serum IL-6 between the three GOLD Stages [circulating neutrophil count: \( 5.5 (1.1, 7.9) \) versus \( 5.7 (4.2, 8.0) \) versus \( 5.6 (4.2, 8.7) \times 10^9 \text{l}^{-1}, \) \( p = 0.487 \); IL-6: \( 16.8 (8.4, 33.2) \text{pg/ml versus } 15.5 (10.6, 37.1) \text{pg/ml, and versus } 12.8 (8.0, 25.0) \text{pg/ml, } p = 0.550 \)].

Table 2 displays linear relationships between FEV\(_1\), GPx, and log-transformed values of inflammatory markers. Importantly, erythrocytic GPx activity was significantly inversely related to both, log hsCRP \( (p = 0.008) \) and log neutrophil count \( (p = 0.003) \) (Fig. 3). Also, a relationship between log neutrophil count and log hsCRP was observed \( (r = 0.247, p < 0.001) \). Furthermore, GPx activity was related to FEV\(_1\) \( (r = 0.233, p = 0.002) \).

Discussion

The present study provides a novel observation of the relationships between markers of systemic inflammation and GPx, a parameter of systemic oxidative stress, during acute exacerbations of COPD: erythrocytic GPx activity was inversely related to log-transformed serum hsCRP. In addition, circulating neutrophils were inversely related to GPx activity, and directly to hsCRP levels. A close link between oxidative stress and inflammatory processes was recognized in the lung compartment of patients with COPD before.16–18 Our data extend these previous findings by demonstrating that oxidative stress is related to inflammatory markers also in the systemic compartment.

In the lung compartment, the links between inflammatory processes and oxidative stress were studied repeatedly by analyzing various inflammatory markers and parameters of
oxidative stress in induced sputum or exhaled breath condensate. In healthy individuals, sputum induction with hypertonic saline causes an inflammatory response that is paralleled by increases in exhaled nitric oxide concentrations. In patients with stable COPD, Beeh et al. reported a significant relationship between sputum neutrophilia, and sputum concentrations of nitrosothiols and oxidized glutathione; Kostikas et al. observed analogical associations between sputum neutrophils and levels of hydrogen peroxide and 8-isoprostane in the exhaled breath condensate. Prominent neutrophilia has been described in bronchoalveolar lavage fluid and induced sputum from patients with COPD, and neutrophilic infiltration within the smooth muscle of such patients may have a pathogenetic role of smoking-induced airflow limitation. Importantly, the enhanced oxidative stress within the airways is paralleled by activation of neutrophils and their increased ability to synthesize chemotactic factors such as leukotriene B4. Therefore, it has been suggested that oxidative stress in vivo enhances local inflammation, and may ultimately contribute to the infiltration/activation of neutrophils into the airways of subjects with COPD.

Associations between parameters of oxidative stress markers in the lungs and inflammatory cytokines in the systemic circulation were also reported. In exacerbated COPD patients, raised levels of hydrogen peroxide in the exhaled air, in association with increased serum IL-8 and cell adhesion molecules at admission were observed. During subsequent treatment, all the markers significantly declined. Nevertheless, the data on the potential relationships between systemic inflammation and markers of systemic oxidative stress in COPD are scarce. In a recent report, Sadowska et al. observed a significant relationship between GPx in blood and serum IL-8 in patients with stable COPD. In our study, we have demonstrated an inverse relationship between the circulating neutrophil count and erythrocytic GPx activity, and a direct relationship between neutrophil count and log-transformed serum hsCRP. Importantly, GPx activity was inversely related to log-transformed hsCRP levels. Therefore, our study provides a novel evidence of significant relationships between oxidative stress and systemic inflammation markers during acute exacerbations of COPD, complementary to those observed in patients with stable disease before.

Findings of the present study might be meaningful especially in the light of three recently published sets of observations. First, population-based studies suggest that elevated white blood cell count is directly associated with increased incidence of coronary heart disease, ischemic stroke, and death from cardiovascular disease, with greater predictive ability provided by high neutrophil count compared to total white blood cell count. Second, considerable amount of data indicates the clinical importance and predictive value of CRP in patients with COPD: high CRP levels correlate with poorer performance in the 6-min walk distance test, impaired energy metabolism, respiratory distress, and, in addition, they relate to increased mortality. Third, peripheral markers of oxidative stress were shown repeatedly to be related to increased cardiovascular risk in non-COPD patients. Elevated levels of antioxidative enzyme myeloperoxidase in the blood, in association with increased leukocyte count, were associated with increased risk of coronary artery disease, whereas depletion of the GPx activity in erythrocytes was independently associated with an increased risk of cardiovascular

<table>
<thead>
<tr>
<th>Variable</th>
<th>FEV1 (% predicted)</th>
<th>GPx (U/g Hb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutrophils ((\log \text{cells} \times 10^8 \text{ml}^{-1}))</td>
<td>(-0.120 (0.114))</td>
<td>(-0.219 (0.003))</td>
</tr>
<tr>
<td>hsCRP ((\log \text{mg/l}))</td>
<td>(-0.312 (0.001))</td>
<td>(-0.199 (0.008))</td>
</tr>
<tr>
<td>Interleukin-6 ((\log \text{pg/ml}))</td>
<td>0.073 (0.414)</td>
<td>0.087 (0.333)</td>
</tr>
</tbody>
</table>

FEV1, forced expiratory volume in 1 s; GPx, glutathione peroxidase; hsCRP, high-sensitivity C-reactive protein.

### Figure 3
Relationship between erythrocytic glutathione peroxidase activity and log-transformed high-sensitivity C-reactive protein \((r = -0.199, p = 0.008)\), and between erythrocytic glutathione peroxidase activity and log-transformed neutrophil count \((r = -0.219, p = 0.003)\) in the entire group of 177 patients with an acute exacerbation of COPD.
events.\textsuperscript{13} Indeed, it was suggested that oxidative stress may be a determinant of CRP levels and promote pro-atherosclerotic inflammatory processes.\textsuperscript{14} To date, no data are available on the potential relationship between reduced erythrocytic GPx as a marker of systemic oxidative stress, and increased cardiovascular risk in patients with COPD. Importantly, given the current knowledge on the association between cardiovascular risk and CRP or GPx, several observations of the present study might stimulate future research to address this issue: first, we demonstrated a link between increased hsCRP and reduced GPx activity; second, an inverse relationship between FEV\textsubscript{1} and hsCRP, and a direct relationship between FEV\textsubscript{1} and erythrocytic GPx activity were observed; third, a significant trend towards higher prevalence of ischemic heart disease with increased COPD severity was seen, in agreement with previous reports.\textsuperscript{32}

There are several limitations to this study. The study was based on observational cross-sectional data, and therefore it remains uncertain whether clinical improvement during acute COPD exacerbations results in reductions in systemic inflammation in association with the restoration of systemic antioxidative mechanisms. Furthermore, treatment with systemic corticosteroids at the admission could have affected neutrophil counts in our study. Nevertheless, since all patients were treated by steroids using the same treatment regimen, the differences in the neutrophil counts, GPx, and CRP observed between the different GOLD Stages are relevant. It has to be emphasized that our study was not designed and powered to analyze a series of serum biomarkers and their correlation with clinical parameters. In a recent report, Pinto-Plata et al.\textsuperscript{33} demonstrated that a panel of 25 serum biomarkers was significantly related to FEV\textsubscript{1}, diffusion capacity, physical performance, and exacerbation frequency. Our data raise a question whether parameters of systemic oxidative stress such as erythrocytic GPx could potentially represent additional markers of important clinical outcomes in patients with COPD. Such a hypothesis requires to be tested in future studies.

In conclusion, the present study in patients with acute exacerbations of COPD demonstrates a link between erythrocytic GPx activity, a parameter of systemic oxidative stress, and serum CRP—a clinically important marker of systemic inflammation. This observation may have several potential implications, and raises some intriguing questions. First, these observations suggest that inflammation and oxidative stress are co-dependent and strongly interrelated processes not only in the lung\textsuperscript{16–18} but also in the systemic compartment. Second, a question regarding the origin of systemic oxidative stress, and its relationship to the origin of systemic inflammation remains to be explored. Further studies are needed to address these questions in more details.

Acknowledgments

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