Role of neopterin among COPD patients

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Key words
Neopterin; COPD; Oxidative stress

Abstract Chronic Obstructive Pulmonary Disease (COPD) is a major public health problem. It is the fourth leading cause of chronic morbidity and mortality worldwide. COPD is characterized by a specific pattern of inflammation involving many cells and mediators. Neopterin serves as a marker of systemic immune activation, which is of importance in the pathogenesis and progression of various diseases.

Aim of the work: This study was conducted to elucidate the role of neopterin in the pathogenesis of COPD.

Methods: 55 COPD patients and 20 healthy control subjects of similar age and sex were included in the study. Neopterin levels in the serum and sputum samples were measured in all subjects.

Results: The mean values of neopterin in the serum (20.34 ± 4.70 nmol/l) and sputum (32.07 ± 8.14 nmol/l) samples of COPD patients were significantly higher than the mean values of neopterin in the serum and sputum samples of control subjects (6.27 ± 3.35 nmol/l and 4.13 ± 2.25 nmol/l respectively) (p < 0.001). Also, COPD patients with exacerbation had significantly higher (p < 0.001) serum and sputum neopterin levels (23.47 ± 2.56 nmol/l and 39.94 ± 5.47 nmol/l respectively) in comparison with stable COPD patients (18.69 ± 4.76 nmol/l and 27.91 ± 5.95 nmol/l respectively). There was statistically significant difference (p value < 0.05) between different COPD severity groups in the mean values of serum and sputum neopterin (nmol/l). There was statistically significant difference (p value < 0.05) in the mean values of serum and sputum neopterin between different COPD patients when they are classified according to the smoking state. Current smokers have higher mean values of serum (22.40 ± 3.04 nmol/l) and sputum (39.60 ± 7.56 nmol/l) neopterin than ex smokers (20.33 ± 4.67 nmol/l and 29.84 ± 6.33 nmol/l respectively) and nonsmokers (16.00 ± 5.3 nmol/l and 26.42 ± 6.60 nmol/l respectively).

Conclusions: This study found increased neopterin serum and sputum levels among COPD patients and suggests a role for neopterin in the pathogenesis of COPD. We recommend larger studies to support our results.

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Introduction

Chronic Obstructive Pulmonary Disease (COPD) is a major public health problem. It is the fourth leading cause of chronic morbidity and mortality in the United States, and is projected to rank fifth in 2020 in burden of disease caused worldwide, according to a study published by the World Bank/World Health Organization [1]. COPD is characterized by a specific pattern of inflammation involving neutrophils, macrophages, and lymphocytes. These cells release many inflammatory mediators and interact with structural cells in the airways and lung parenchyma [2]. A wide variety of inflammatory mediators that have been shown to be increased in COPD patients attract inflammatory cells from the circulation (chemotactic factors), amplify the inflammatory process (proinflammatory cytokines), and induce structural changes [3].

Neopterin is a catabolic product of guanosine triphosphate (GTP), a purine nucleotide. Neopterin belongs to the chemical group known as pteridines. It is synthesized by activated monocytes, macrophages, dendritic cells, and endothelial cells upon stimulation with the cytokine interferon-gamma and to a lesser extent by interferon alpha and beta with its release being enhanced by tumor necrosis factor [4,5]. Neopterin serves as a marker of systemic immune activation. Measurement of neopterin concentrations in body fluids like blood serum, cerebro-spinal fluid or urine provides information about cellular immune activation, which is of importance in the pathogenesis and progression of various diseases, e.g., in viral infections, in autoimmune or inflammatory diseases, rejection episodes following allograft transplantation, autoimmune-logical diseases and in several malignant diseases [6,7].

Aim of the work

This study was conducted to elucidate the role of neopterin in the pathogenesis of COPD.

Subjects and methods

This study included 55 patients with COPD (45 men, 10 women) with a mean age of 56.49 ± 8.52 years (ranging 42–78) that were selected from patients who visited our university hospitals and 20 healthy control subjects (15 men, 5 women, mean age 54.37 ± 7.33 years, range 39–74 years) (Table 1). Patients and control subjects were matched for age and gender. The COPD patient group included 36 stable COPD patients and 19 COPD patients with exacerbation.

The diagnosis of COPD was established by clinical symptoms, physical examination, chest radiography and pulmonary function tests according to guidelines of GOLD (2014) [1]. The COPD subjects were classified into (stages I–IV) on the basis of their post-bronchodilator forced expiratory volume in one second (FEV1)/forced vital capacity (FVC) and FEV1 % predicted. FEV1/FVC was < 70% and their FEV1 fell into set bands (stage 1: FEV1 ≥ 80%; stage 2: 50% ≥ FEV1 < 80%; stage 3: 30% ≥ FEV1 < 50%; stage 4: FEV1 < 30%).

Stable Chronic Obstructive Pulmonary Disease

Stable COPD is defined by the absence of any exacerbation for 3 months preceding the study [8].

Acute exacerbation of COPD (AECOPD)

The American Thoracic Society (ATS) and European Respiratory Society (ERS) define an exacerbation as an acute change in a patient’s baseline dyspnea, cough, or sputum that is beyond normal variability, and that is sufficient to warrant a change in therapy [9].

Methods

All persons included in this study were subjected to the following:

1. Full history taking.
2. Thorough medical examination.
3. Plain chest radiography (postero-anterior and lateral).
4. Pulmonary function testing.
5. Complete blood picture (CBC) and differential.
6. CRP.
7. Measurement of neopterin levels in the serum and sputum samples of COPD patients and control subjects.

Blood samples for laboratory assessments were obtained from all subjects included in this study. Sera were separated by centrifugation at 1500 g for 10 min and were frozen at −70 °C and protected from light. Sputum samples were also collected from all included subjects. All COPD patients gave their sputum samples spontaneously, but control subjects gave their samples after the induced sputum technique [10]. Sputum samples were kept in a cold place (temp. 4 °C) and processed within two hours. They were solubilized with DTT (dithiothreitol) followed by Dulbecco’s phosphate buffer saline (D-PBS). Then the mixture was filtered and centrifuged. The supernatant is aspirated and frozen at −70 °C for neopterin analysis.

Neopterin concentrations were determined in the serum and sputum samples by the ELISA method using commercially available kit manufactured by IBL, Hamburg (Germany) [11].

Results

The mean values of neopterin in the serum (20.34 ± 4.70 nmol/l) and sputum (32.07 ± 8.14 nmol/l) samples of COPD patients were significantly higher than the mean values of neopterin in the serum and sputum samples of control subjects (6.27 ± 3.35 nmol/l and 4.13 ± 2.25 nmol/l respectively) (p < 0.001) (Table 2 and Fig. 1). Also, COPD patients with exacerbation had significantly higher (p < 0.001) serum and sputum neopterin levels (23.47 ± 2.56 nmol/l and 39.94 ± 5.47 nmol/l respectively) in comparison with stable COPD patients (18.69 ± 4.76 nmol/l and 27.91 ± 5.95 nmol/l respectively) (Table 3 and Fig. 2).
There was a statistically significant difference (p value < 0.05) between different COPD severity groups in the mean values of serum and sputum neopterin (nmol/l) (Table 4). The serum and sputum neopterin levels increased with increased severity of COPD. There was a significant positive correlation (p value < 0.001) between COPD severity and serum (r = 0.482) and sputum (r = 0.611) neopterin levels (Figs. 3 and 4). Also, there is positive correlation between the serum and sputum neopterin levels (r = 0.679).

There was statistically significant difference (p value < 0.05) in the mean values of serum and sputum neopterin between different COPD patients when they are classified according to the smoking state. Current smokers have higher mean values of serum (22.40 ± 3.04 nmol/l) and sputum (39.60 ± 7.56 nmol/l) neopterin than ex smokers (20.33 ± 4.67 nmol/l and 29.84 ± 6.33 nmol/l respectively) and non smokers (20.34 ± 4.70 nmol/l and 32.07 ± 8.14 nmol/l).

Table 1 Characteristics of COPD patients and control groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Patients</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>55</td>
<td>20</td>
</tr>
<tr>
<td>Male/female</td>
<td>45/10</td>
<td>15/5</td>
</tr>
<tr>
<td>Age yrs</td>
<td>56.49 ± 8.52</td>
<td>54.37 ± 7.33</td>
</tr>
<tr>
<td>FEV1 % pred</td>
<td>51.88 ± 17.52</td>
<td>93.66 ± 5.42</td>
</tr>
<tr>
<td>FEV1/FVC %</td>
<td>60.7 ± 5.33</td>
<td>90.75 ± 4.1</td>
</tr>
<tr>
<td>PO2</td>
<td>62.54 ± 15.25</td>
<td>95.88 ± 2.70</td>
</tr>
<tr>
<td>PCO2</td>
<td>41.10 ± 7.55</td>
<td>39.40 ± 2.62</td>
</tr>
</tbody>
</table>

Table 2 Mean values of serum and sputum neopterin among COPD patients and control groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Serum neopterin mean values ± SD (nmol/l)</th>
<th>Sputum neopterin mean values ± SD (nmol/l)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>COPD patients</td>
<td>20.34 ± 4.70</td>
<td>32.07 ± 8.14</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>No. = 55</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control subjects</td>
<td>6.27 ± 3.35</td>
<td>4.13 ± 2.25</td>
<td></td>
</tr>
</tbody>
</table>

Table 3 Mean values of serum and sputum neopterin among stable COPD patients and COPD patients with exacerbation.

<table>
<thead>
<tr>
<th>Group</th>
<th>Serum neopterin mean values ± SD (nmol/l)</th>
<th>Sputum neopterin mean values ± SD (nmol/l)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stable COPD No.</td>
<td>18.69 ± 4.76</td>
<td>27.91 ± 5.95</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>COPD No. = 36</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exacerbated COPD No.</td>
<td>23.47 ± 2.56</td>
<td>39.94 ± 5.47</td>
<td></td>
</tr>
<tr>
<td>COPD No. = 19</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4 Mean values of serum and sputum neopterin (nmol/l) among different COPD severity groups.

<table>
<thead>
<tr>
<th>Severity</th>
<th>Serum neopterin (nmol/l) (mean ± SD)</th>
<th>Sputum neopterin (nmol/l) (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild (No. = 6)</td>
<td>14.50 ± 2.58</td>
<td>24.16 ± 3.06</td>
</tr>
<tr>
<td>Moderate (No. = 19)</td>
<td>20.36 ± 5.34</td>
<td>29.42 ± 7.12</td>
</tr>
<tr>
<td>Severe (No. = 25)</td>
<td>20.92 ± 3.60</td>
<td>34.48 ± 8.36</td>
</tr>
<tr>
<td>Very severe (No. = 5)</td>
<td>24.40 ± 3.20</td>
<td>39.60 ± 0.89</td>
</tr>
<tr>
<td>ANOVA F</td>
<td>5.57</td>
<td>6.02</td>
</tr>
<tr>
<td>p value</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Figure 1 Mean values of serum and sputum neopterin among COPD patients and control groups.

Figure 2 Mean values of serum and sputum neopterin among stable COPD patients and COPD patients with exacerbation.

Figure 3 Mean values of serum and sputum neopterin among stable COPD patients and COPD patients with exacerbation.
In this study, the mean values of neopterin in the serum (20.34 ± 4.70 nmol/l) and sputum (32.07 ± 8.14 nmol/l) samples of COPD patients were significantly higher than the mean values of neopterin in the serum and sputum samples of control subjects (6.27 ± 3.35 nmol/l and 4.13 ± 2.25 nmol/l respectively) (p < 0.001). Also, COPD patients with exacerbation had significantly higher (p < 0.001) serum and sputum neopterin levels (23.47 ± 2.56 nmol/l and 39.94 ± 5.47 nmol/l respectively) in comparison with stable COPD patients (18.69 ± 4.76 nmol/l and 27.91 ± 5.95 nmol/l respectively). Other workers found similar results. Takabatake and his colleagues [12] evaluated circulating levels of interferon-gamma (IFN-gamma), soluble interleukin-2 receptor (sIL-2R), neopterin, and soluble intercellular adhesion molecule-1 (sICAM-1) in 35 clinically stable patients with COPD and in 22 age-matched healthy controls. They found that circulating levels of sIL-2R (1.52 ± 1.25 vs. 0.97 ± 0.48 ng/ml; p < 0.05), neopterin (7.23 ± 4.24 vs. 4.95 ± 1.52 nmol/l; p < 0.05), and sICAM-1 (665 ± 302 vs. 328 ± 164 ng/ml; p < 0.001), but not IFN-gamma (7.55 ± 4.72 vs. 6.65 ± 1.13 pg/ml; p = NS) were significantly higher in patients with COPD than those in the controls. Warwick et al. [13] detected that, among COPD patients, levels of the novel markers neopterin and IP-10 were significantly increased in induced sputum supernatant (pooled groups pre and post exacerbation: IP-10: 188.6 ± 102.1 vs. 5.40 ± 1.28 pg/ml, p = 0.006; neopterin: 15.81 ± 2.50 vs. 5.38 ± 0.45 nmol/l, p < 0.001), as was TNF-α (137.8 ± 49.64 vs. 71.56 ± 45.03 pg/ml, p = 0.018).

Our study revealed a statistically significant difference (p value < 0.05) between different COPD severity groups in the mean values of serum and sputum neopterin (nmol/l). The serum and sputum neopterin levels increased with increased severity of COPD. There was a significant positive correlation (p value < 0.001) between COPD severity and serum (r = 0.482) and sputum (r = 0.611) neopterin levels. Also, there is a positive correlation between the serum and sputum neopterin levels (r = 0.679). Our findings are in agreement with that of others. Garrod et al. [14] found that measures of systemic inflammation (neopterin, TNF-α, IL6, and in particular CRP) are correlated with COPD severity in primary care.

We detected a statistically significant difference (p value < 0.05) in the mean values of serum and sputum neopterin between different COPD patients when they are classified according to the smoking state. Current smokers have higher mean values of serum (22.40 ± 3.04 nmol/l) and sputum (39.60 ± 7.56 nmol/l) neopterin than ex smokers (20.33 ± 4.67 nmol/l and 29.84 ± 6.33 nmol/l respectively) and nonsmokers (16.00 ± 5.3 nmol/l and 26.42 ± 6.60 nmol/l respectively) (Table 5).

### Table 5 Mean values of serum and sputum neopterin (nmol/l) among COPD patients regarding smoking status.

<table>
<thead>
<tr>
<th>Group</th>
<th>Serum neopterin (mean ± SD)</th>
<th>Sputum neopterin (mean ± SD)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smokers (No. = 15)</td>
<td>22.40 ± 3.04</td>
<td>39.60 ± 7.56</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>Ex smokers (No. = 33)</td>
<td>20.33 ± 4.67</td>
<td>29.84 ± 6.33</td>
<td></td>
</tr>
<tr>
<td>Non smokers (No. = 7)</td>
<td>16.00 ± 5.35</td>
<td>26.42 ± 6.60</td>
<td></td>
</tr>
</tbody>
</table>

Discussion

In this study, the mean values of neopterin in the serum (20.34 ± 4.70 nmol/l) and sputum (32.07 ± 8.14 nmol/l) samples of COPD patients were significantly higher than the mean values of neopterin in the serum and sputum samples of control subjects (6.27 ± 3.35 nmol/l and 4.13 ± 2.25 nmol/l respectively) (p < 0.001). Also, COPD patients with exacerbation had significantly higher (p < 0.001) serum and sputum neopterin levels (23.47 ± 2.56 nmol/l and 39.94 ± 5.47 nmol/l respectively) in comparison with stable COPD patients (18.69 ± 4.76 nmol/l and 27.91 ± 5.95 nmol/l respectively). Other workers found similar results. Takabatake and his colleagues [12] evaluated circulating levels of interferon-gamma (IFN-gamma), soluble interleukin-2 receptor (sIL-2R), neopterin, and soluble intercellular adhesion molecule-1 (sICAM-1) in 35 clinically stable patients with COPD and in 22 age-matched healthy controls. They found that circulating levels of sIL-2R (1.52 ± 1.25 vs. 0.97 ± 0.48 ng/ml; p < 0.05), neopterin (7.23 ± 4.24 vs. 4.95 ± 1.52 nmol/l; p < 0.05), and sICAM-1 (665 ± 302 vs. 328 ± 164 ng/ml; p < 0.001), but not IFN-gamma (7.55 ± 4.72 vs. 6.65 ± 1.13 pg/ml; p = NS) were significantly higher in patients with COPD than those in the controls. Warwick et al. [13] detected that, among COPD patients, levels of the novel markers neopterin and IP-10 were significantly increased in induced sputum supernatant (pooled groups pre and post exacerbation: IP-10: 188.6 ± 102.1 vs. 5.40 ± 1.28 pg/ml, p = 0.006; neopterin: 15.81 ± 2.50 vs. 5.38 ± 0.45 nmol/l, p < 0.001), as was TNF-α (137.8 ± 49.64 vs. 71.56 ± 45.03 pg/ml, p = 0.018).

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Oxidative stress induced by reactive oxygen and nitrogen species (ROS and RNS) plays a central role in the pathophysiology of COPD [16]. Previous data indicate a possible participation of neopterin derivates within the cytotoxic mechanism of white blood cells [17]. On the one hand, a strong correlation between detection of neopterin and ability of monocytes/macrophages/neutrophils to set free reactive oxygen species (ROS and RNS) plays a central role in the pathophysiology of COPD [16]. Previous data indicate a possible participation of neopterin derivates within the cytotoxic mechanism of white blood cells [17]. On the one hand, a strong correlation between detection of neopterin and ability of monocytes/macrophages/neutrophils to set free reactive oxygen species (ROS and RNS) plays a central role in the pathophysiology of COPD [16]. Previous data indicate a possible participation of neopterin derivates within the cytotoxic mechanism of white blood cells [17].
Role of neopterin among COPD patients

iNOS-gene [22]. Similar to this, progranulin cell death, apoptosis, is induced or intensified by neopterin derives [23–27]. These findings suggest a new role for neopterin in the pathogenesis of COPD. Previous reports show that neopterin and 7,8-dihydroneopterin upregulate expression of proto-oncogene c-fos, so these substances may be even involved in malignant transformation of cells [28,29].

Conclusions

This study found increased neopterin serum and sputum levels among COPD patients and suggests a role for neopterin in the pathogenesis of COPD. We recommend larger studies to support our results.

Conflict of interest

There is no conflict of interest.

References