Role and significance of subpopulation spectrum changes of peripheral blood lymphocytes and their apoptosis in patients with COPD stage III in the exacerbation phase

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Abstract
A lot of papers are related to the study of the state of immune response in patients with chronic obstructive pulmonary disease (COPD), however, we are of the opinion that the development of immune alterations in this pathology is not fully disclosed, particularly at the level of functional activity of macrophages, subpopulations of peripheral blood lymphocytes and their synthesis of a range of inflammatory transmitters and thus globalization of systemic symptoms of the pathology at the level of the whole human body.

The paper presents data on pathogenetic peculiarities of immune response in the process of generation of COPD stage III in the exacerbation phase. The obtained results show significant role of lymphocytic subpopulations of lymphocytes in the formation of both, local manifestations and systemic effects of COPD.

Keywords: COPD, subpopulations of lymphocytes, apoptosis

1. Introduction
The definition of chronic obstructive pulmonary disease (COPD) itself bears unfavorable prognosis for the patient and is most often nosologically represented by chronic obstructive bronchitis and emphysema. Mortality in COPD in developed countries ranges from 90 to 400 deaths per 100 thousand cases among males and from 20 to 200 deaths per 100 thousand in females [21] at the age of 65-74 years. Over the last 40 years mortality rate associated with COPD in the USA has increased five times, death rate associated with emphysema has increased six times, while it remained stable in chronic bronchitis and bronchial asthma. People suffering from COPD start seeking medical advice at the age of 25 years and older while the peak of the disease can be attributed to the age range of 65-75 [14].

COPD is the most common disease, for it is considered that 11-13% of people are affected by this ailment, and the morbidity rate tends to double every five years [16]. At present there are nearly 600 million patients with COPD in the world [3]. According to the present day data the average number of patients suffering from this pathology in our country is 10-14% of adult population, which accounts for nearly 3 million people [4, 7].

COPD still remains one of the major causes of mortality, ranking sixth among all the causes of death of the world’s population [10]. Investigations, carried out in industrially advanced countries, show double increase of disability and mortality rates for COPD every ten years [3]. WHO predicts that if maintaining the increase rate of this pathology in 2020 COPD can become the third leading cause of death among population [6]. Ukraine is described in the “European Lung White Book” as one of the countries with the highest COPD death rate among male population [9]. In particular the mortality rate for the period of 2004-2005 made up 0.8% and is 38.4 per 100 thousand adult populations [4]. It’s worth mentioning that the level of intra-hospital lethality for COPD remains high and makes up 0.89%, while that for pneumonia is – 1.21% that indicates problems with both early diagnosis and inpatient treatment [4].

People who smoke are six times more likely to suffer from COPD than non-smokers and this dependence is more obvious in people who smoke a lot and for a long period of time [18]. Therefore, etiologically COPD is associated with inhalation of pollutants, while its risk factors include genetic predisposition, age, α1-antitrypsin deficiency, infection and others [11, 18].

Bronchial permeability is pathogenetically decreased as a result of bronchospasm, swelling of the bronchial mucosa, obliteration of the bronchial lumen with viscous sputum, collapse of bronchial tubes during expiration and sclerosis of the bronchial wall [10, 12, 15, 17, 20].
GOLD-2016 recommendations provide data showing that the risk of disease is associated with social-and-economic reasons and low quality of life [10]. Increase of the disease prevalence is predicted in the future as a result of the increased number of smokers, increase of population and environmental pollution [3]. Despite the large number of scientific studies concerning COPD problem, current understanding of the stages of disease development and advance of the pathology still remain under-investigated [5, 10]. Special attention should be given to the disorders of the immune response, activity of macrophage cells against oxidative stress and pathologic lipid metabolism of cell membranes that predetermine activation of functional and irreversible pathogenetic mechanisms of COPD development and advance [5, 8]. A lot of papers are related to the study of the state of immune response in patients with chronic obstructive pulmonary disease (COPD), however, we are of the opinion that the development of immune alterations in this pathology is not fully disclosed, particularly at the level of functional activity of macrophages, subpopulations of peripheral blood lymphocytes and their synthesis of a range of inflammatory transmitters and thus globalization of systemic symptoms of the pathology at the level of the whole human body [5, 8].

The goal of the research is to evaluate the state of subpopulation lymphocytes count of peripheral blood and their apoptosis in patients with COPD stage III in the exacerbation phase, groups’ C and D.

2. Materials and methods

The study involved 56 patients with COPD stage III, groups C and D. Verification of the diagnosis and its statement were carried out according to the order of the Ministry of Health Care of Ukraine №555 from June 27, 2013 “On approval and introduction of medical and technological documents for standardization of medical care in chronic obstructive pulmonary disease” [3]. The investigation was performed prior to initiating therapy with the use of common treatment patterns (according to the standards established under the order of the Ministry of Health Care of Ukraine №555 from June 27, 2013) [2]. Control group involved 18 apparently healthy individuals (AHI) without current symptoms of respiratory tract disorders or other pathologies of internal organs.

The immuno-phenotyping of subpopulations of lymphocytes and their apoptosis [CD8+, CD4+, CD95+] and (Fas/APO-I) were performed using monoclonal antibodies. Lymphocytes were isolated from the peripheral blood according to the method of L.B. Heifets, V.F. Abalkin (1973). The specimens were studied under luminescence microscope, complete with phase-contrast device (“Liuman-13”). Under the phase contrast we initially counted the total number of cells in the field of view and then counted cells which glow in the luminescence excitation spectrum (order of filters: C3, C24, CSF, flint glass B85). Cells with circular and spot luminance were also taken into account. Percentage of lymphocytes carrying certain antigen on their surface was determined after counting 100 cells in the specimen.

3. Results and their discussion

The conducted investigation showed that the CD4+ cell count in COPD stage III patients was significantly reduced to 1.28 times (p<0.05) as compared with the control group (Table 1). State of subpopulations of peripheral blood lymphocytes in patients with COPD stage III, groups C and D in the exacerbation phase (M±m)

<table>
<thead>
<tr>
<th>Index, %</th>
<th>AHI, (n=18)</th>
<th>COPD stage III (n=56)</th>
</tr>
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<tbody>
<tr>
<td>CD4+</td>
<td>47.26±2.74</td>
<td>37.14±2.41*</td>
</tr>
<tr>
<td>CD8+</td>
<td>32.12±2.15</td>
<td>39.85±2.37*</td>
</tr>
<tr>
<td>CD95+</td>
<td>7.62±0.72</td>
<td>12.16±0.58*</td>
</tr>
</tbody>
</table>

Table 1

Note: index of probable difference between study groups and control: * p<0.05.

CD-4 receptors are carried by cells described as T-lymphocytes with helper (inductor) properties, the function of which is to recognize antigens, produce lymphokines and thus trigger and regulate all the T-cell-dependent immune reactions [11]. That is why, their deficiency in COPD stage III is a clear evidence of immune system malfunctioning in this category of patients.

At the time of initial examination the CD8+ index of peripheral blood lymphocytes was increased to 1.24 times and made up (39.85±2.37)% in patients with COPD stage III as compared with CD8+ indices in apparently healthy individuals — (32.12±2.15)% (p<0.05). Increase in the subpopulation of CD8+ peripheral blood lymphocytes in patients with COPD stage III in the exacerbation phase correlates (r=−0.89; p<0.05) with the decrease of CD4+ cell count. Our experimental data are supported by other scientists who identify the increase of CD8+ cell count also in the biotopes of bronchi of COPD patients [8, 10]. We believe that the discovered alterations of CD4+ and CD8+ peripheral blood lymphocytes in patients with COPD stage III in the exacerbation phase is a prominent evidence of abnormalities in the process of lymphocytic cooperation in immune response in terms of imbalance between cellular and humoral immune responses. We quite agree with the statements made by professor Drannik H.M. (2006), and corresponding member of National Academy of Medical Sciences of Ukraine Chernushenko K.F. (2007), that generation, course and advance of many diseases, with varied etiology and pathogenesis, are largely dependent on the state of immune system [11, 12]. Pathology of the respiratory system is not an exception. It is known that the character and magnitude of immune responses depend on the degree of antigenic action, as well as functional state of various components of immune system [11]. Immune response is triggered by a number of cell populations, including antigen-presenting cells (macrophages, dendritic cells of lymph nodes, etc.) which pass information about the antigen to T-cells, that have T-cell receptor (TCR), and B-cells with Ig-receptors [11-13]. The resulting signal causes activation of a combination of enzyme systems that is followed by cytokine synthesis – growth factors in certain specific cell clone [12, 13]. T-lymphocytes comprise two regulatory subpopulations: T-helpers-effectors (CD4) and T-killers-suppressors (CD8), which determine the course of immune response to different substances [9, 11]. The quantitative composition alterations of which in peripheral blood were determined in patients with COPD stage III in the exacerbation phase.

Over recent years major role in the regulation of immune response is assigned to two major types of T-helper (CD4) cells– Th-1 and Th-2. Antigens activate CD4-cells and production of IL-10, which promote transformation of Th-0 into Th-1 [12]. Th-1 regulates intensity of cellular response synthesizing IL-2, IL-12, γ-INF, TNF-α [11-13]. The second type of T-helper cells (Th-2) synthesizes cytokines– IL-4-, 6, 10, 13 and stimulates humoral reactions which promote production of immunoglobulins by B-cells [11-13]. Various vectors of influence of Th-1 and Th-2 types of cells are very important regulation factors, but since these cytokines may be produced by other cells, one cannot say that regulation of
immune response is triggered by these types of helper populations only [12, 13]. We found out that the expression of CD95+(Fas/APO-I) on the lymphocytes of peripheral blood of patients with COPD stage III in the exacerbation phase had increased by 1.60 times ($p<0.05$) as compared to control group, where this index was – (7.62±0.72)% (table 1). Taking into consideration the fact that the Fas/Fas-L system is not familiar with other functions except for activation of apoptosis [11, 13], the obtained findings point to the increase of apoptosis of peripheral blood lymphocytes in the examined patients and decrease of subpopulation content of T-helper cells count in contrast to the increase of Tsuppressors.

4. Conclusions
Development of the exacerbation phase of COPD stage III shows itself in the decrease of subpopulation content of T-helpers (CD4+) by 1.28 times ($p<0.05$) in peripheral blood of patients with simultaneous increase of T-suppressors (CD8+) by 1.24 times ($p<0.05$) on the background of increase of lymphocyte apoptosis (CD95+ marker) by 1.60 times ($p<0.05$) as compared to apparently healthy individuals.

5. References