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Special features of systemic immunity in patients with acute exacerbation of chronic obstructive pulmonary disease depending of smoking status

Keywords: chronic obstructive pulmonary disease, smoking status, immune system, cytokines.

The pathogenesis of chronic obstructive pulmonary disease (COPD) traditionally associated with smoking. In recent years, is found that 25-45 % of patients with COPD have never smoked [19, 30, 31]. It is assumed that the occurrence of COPD in such cases may be due to the influence of many risk factors, including – biomass fuel, organic dust (animal, land), chronic asthma, the presence of atopy in farm workers and other factors [24, 31].

In addition, 18 % of mild-to-moderate COPD patients were atopic, and 15-45 % of patients with COPD may be associated with asthma – that makes some features of COPD [29]. Thus, the presence of COPD patients of an allergic phenotype is associated with the risk of increased respiratory symptoms and risk of COPD exacerbations [22] and with the risk of anaphylaxis among hospitalized patients [27]. Thus, the disease of some patients with COPD, who have never smoked may be associated with an allergic phenotype, the presence of which can approximately determine by the composition of the cytokine ratios in blood and evaluation of pro- and anti-inflammatory cytokines, which reflect the severity of responses of T-lymphocyte type 1 and 2.

In particular, it was found that compared with healthy individuals, who do not smoke, the percentage of cytotoxic T lymphocytes (CD8+) in the blood significantly higher in the same COPD patients, due to which they, in contrast to healthy is low ratio of CD4+/CD8+-T-lymphocytes compared to the same group of healthy individuals [28].

In this case, on the contrary, patients with a positive smoking status the percentage of CD8+-T-lymphocytes in the

blood is lower than in smokers without COPD, and the ratio of CD4+/CD8+-T-lymphocytes is increased in smokers with COPD compared to healthy smokers [26]. The total number of B-lymphocytes in the blood of patients with COPD negative smoking status lower compared in smokers COPD patients [28]. On the other hand, the number of T-lymphocytes (CD4+) in the blood of smokers COPD patients compared with smokers without airflow limitation may be both increased [26] and low [23]. In this case, the number of T-lymphocytes in bronchoalveolar lavage does not depend on the presence of COPD and smoking status.

So, remain unclear causes of COPD in individuals who have never smoked, and the features of COPD such individuals, – what makes the relevance of this study.

The aim of the present study was to investigate the immunological features of COPD, depending on the status of smoking patients by examining the T, B, phagocytic links of immunity and levels of pro- and anti-inflammatory cytokines and biologically active substances in the blood serum in COPD patients in the acute stage.

Materials and methods

There has been clinical, functional and immunological study of 65 COPD patients in the acute stage, held hospital treatment in pulmonary departments of the SO «National Institute of phthysiology and pulmonology named after F. G. Yanovsky NAMS of Ukraine». Clinical diagnosis of COPD was placed under the Ministry of Health of Ukraine Decree № 555 from 27.06.2013 h. [10].

Among surveyed were 42 men (64.6 %) and 24 women (35.4 %), average age ($61,5 \pm 1,6$) years control group consisted of 25 healthy individuals – donors available without signs of respiratory tract diseases, including there were 10 men and 15 women, average age ($45,9 \pm 2,2$) years.

Spirometry examination was conducted of patients using the device MicroLab (UK).

Depending on the availability of the smoking status examined COPD patients were divided into 2 groups: group 1 included 31 patients (47,7 %) (mean age $65,1 \pm 1,8$ years) who had smoked in the past or smoke in the present. Among surveyed were 26 men ($83,9 \pm 6,6$ %) and 5 women ($16,1 \pm 6,6$ %), average of indicators of lung function: forced expiratory volume in the first second (FEV1) – ($37,7 \pm 2,8$ %), and the FEV1 value/FVC – ($43,7 \pm 2,3$ %).

The group 2 included 34 patients (52.3 %) who had never smoked (mean age $58,1 \pm 2,5$) years, Among surveyed were 16 men ($47,1 \pm 8,6$ %), and 18 women ($52,9 \pm 8,6$ %), FEV1 – ($51,9 \pm 3,4$ %) and the FEV1/FVC – ($67,0 \pm 3,6$ %).

Immunological examination consisted of evaluation the total number of peripheral blood leukocytes (PB), calculation of lymphocyte content (Lf), monocytes (Mc) and neutrophils (Ng), (ABX Micros 60, France).

The T-lymphocytes (T-Lf) populations in peripheral blood were analyzed with two color flow cytometry (flow cytometer FACScan) with the monoclonal antibody (Caltag laboratories, USA). Thus, the relative and absolute content of T lymphocytes (CD3+19-), subpopulations of T helpers/inducers (CD4+8-) and T suppressor/killer (CD4-8+) were determined [9].

To assess their activity used as indicator of the intensity of fluorescence (IF), which is due to the amount of labeled monoclonal antibodies that bound to the ligand on the cell surface [5]. Luminescence intensity or brightness, after cell attachment luminescent monoclonal antibodies depends on the number of specific receptors on the cell surface and their affinity characterized by density and surface receptors. The proliferative activity of T lymphocytes characterized by the reaction of blast transformation of lymphocytes with PHA (RBTL with PHA).

Condition B – immune system was evaluated by determining the concentration of immunoglobulin (Ig) serum classes (IgA, M, G) and the level of circulating immune complexes (CIC). The functional activity of phagocytic cells was determined by their ability to absorbing latex particles, hoping index of phagocytosis (PF) – the percentage of cells with latex particles and the phagocytes number (Fn) – number of shares latex in a cell; the level oxygen-dependent metabolism NG and peripheral blood monocytes in the test with nitro-blue tetrazolium (NBT-test) and by the cytochemical index (CChI) (13).

The cytokines and biologically active substances in the serum (E-selectin, soluble intercellular adhesion molecule ICAM-1, high-sensitivity C-reactive hsS-RP protein) was determined by enzyme-linked immunosorbent assay (ELISA) using a commercial test kits for the determination of tumor necrosis factor (TNF-a), interleukin (IL) IL-4 (Protein contour, Russia), of IL-8 (Cytokines, St. Petersburg, Russia), E-selectin (Biomedica Gruppe,

Austria), sICAM-1 (Bender MedSystems, Vienna, Austria), high-sensitivity C-reactive protein (hsS-RP) (DA, USA).

Storage of research results and their mathematical processing was carried out using licensed software products that are included in the package Microsoft Office Professional 2000 (license Russian Academic OPEN No Level № 17016297).

The arithmetic mean value (M), mean deviation (σ) and error of the arithmetic mean (m) were determined. Comparison of the average values of the group and assessment of the significance of differences was performed parametric and non-parametric methods of variation and rank statistics using t-test probability Students or two selective Wilcoxon test [8]. Calculation of criterion values and confidence intervals (CI) was held at a given confidence level $p \leq 0,05$. This work was funded by the state budget of Ukraine.

Results and Discussion

The clinical features of COPD patients with the negative status of smoking are the younger age – ($58,1 \pm 2,5$) years against ($65,1 \pm 1,8$) years in group 1, the predominance of females (52,9 % and 16,1 %, $p < 0,05$ respectively) and significantly higher rates of respiratory function ($51,9 \pm 3,4$ %) and ($37,7 \pm 2,8$ %), $p < 0,05$, FEV1/FVC – ($67,0 \pm 3,6$ %) against ($43,7 \pm 2,3$ %), $p < 0,05$ respectively). Shows the aggravation of COPD in both groups of patients was accompanied by a significant increase in blood leukocytes absolute content due to the growth in the absolute number of neutrophils and monocytes. This data indicate the high activity of the inflammatory process in both groups (Table 1). The terms haemograms of patients group 1 was observed a more pronounced leukocytosis on the base of a relative lymphopenia probably due to more pronounced inflammation.

It has been shown that exacerbation of COPD patients accompanied by an increase in the absolute number of lymphocytes CD3+19- while reducing the intensity of fluorescence (Table 2). Increasing the number of T cells was due, in particular, by the increase in the absolute number of T-helper lymphocytes (CD4+8-). The increase in the absolute content of T-cytotoxic lymphocytes (CD4-8+) of smokers COPD patients (group 1), was observed.

The distinguishing feature of COPD patients who had never smoked (group 2), were more distinct decrease in the density of surface receptors on lymphocytes to CD4+ differentiation antigen (by immunofluorescence intensity), – indicating that the functional activity of T- helper lymphocytes (CD4+8) significantly decreased.

On the other hand, all COPD patients showed an increase in the absolute number of B-lymphocytes, the functional activity of which (by IF) unlike IF of T-cells was increased. This increase was significantly higher in smoking COPD patients and accompanied by expressive dysfunction (probably depletion) of these cells, which shows a significant decrease in the levels of serum IgM and IgA.

The functional activity of neutrophils and monocytes in blood of COPD patients with exacerbation irrespective of smoking status was reduced due to inhibition of absorption capacity of latex particles (PF, Fn), oxygen-dependent metabolism (NBT-test) and production of superoxide anion (CChI) (Table 3).

Indexes	Groups of patients		
	Healthy persons (n = 25)	1 (n = 31)	2 (n = 34)
Leucocytes (x109)	5,9 ± 0,2	10,4 ± 0,9 **	8,5 ± 0,6 #
Lymphocytes (x109)	2,1 ± 0,1	3,3 ± 0,3 #	3,0 ± 0,2 #
Lymphocytes (%)	34,5 ± 1,0	29,6 ± 2,0 **	35,9 ± 2,1
Neutrophils (x109)	3,6 ± 0,2	6,5 ± 0,6 #	5,3 ± 0,5 #
Neutrophils (%)	59,0 ± 1,2	60,2 ± 2,6	55,6 ± 2,7
Monocytes (x109)	0,20 ± 0,03	0,53 ± 0,07 #	0,41 ± 0,06 #
Monocytes (%)	3,8 ± 0,4	4,8 ± 0,4	4,7 ± 0,5
Eosinophils (%)	2,0 ± 0,4	1,5 ± 0,3	2,3 ± 0,6

Notes: # the difference with an indicator of healthy statistically significant (p < 0,05); * the difference corresponding figure in 1 and 2 groups statistically significant (p < 0,05).

In the serum of all 65 people registered increases in levels of proinflammatory cytokines TNF- α , IL-8, E-selectin, C-reactive protein as well as anti-IL-4 (Table 4), that reflect the activity of inflammatory process in patients at the time of the survey.

In the analysis of data depending on the smoking status is established, this factor does not depend only levels of E-selectin and C-reactive protein, which were elevated in both groups. These indicators are used as markers of active inflammation and often associated with active bacterial infection [7, 18, 21].

Patients of the second group defined the statistical evidence of increase in serum level of anti-inflammatory IL-4. Thus the ratio of the levels of TNF- α /IL-4 in serum of patients of the second group was significantly reduced with respect to this indicator of the 1st group and the group of donors – indicating a greater severity of inflammatory reactions in patients of the 2nd group associated with more pronounced activation of T-lymphocytes cells of the 2nd type.

These references for a system of production of pro- and anti-inflammatory cytokines in patients with COPD are somewhat contradictory. On the one hand, it is found that at an exacerbation of COPD T-helper (CD4+) cells synthesize predominantly blood cytokines that characterize the response of T-helper 1 type. In particular, the elevated levels of TNF- α in the blood (due to its hyperproduction of mast and lymphoid blood cells) and in the sputum [15, 20], IL-8 in serum and in bronchoalveolar lavage [25], especially in smokers [16] correlates with the severity of the process and a decrease in lung function [33]. Thus, the concentration of circulating IL-8 in serum did not depend of smoking cessation [25]. In other studies, a large part of the patients examined had a low level of TNF- α in the blood serum that, according to the authors, may indicate a loss of the ability of cells to synthesis of this cytokine [12]. Several studies have shown that low rates of basal and stimulated

Indexes	Groups of patients		
	Healthy persons (n = 25)	1 (n = 31)	2 (n = 34)
Absolute number TL (CD3+19-) (x109)	1,4 ± 0,1	2,1 ± 0,2 #	2,0 ± 0,2 #
Relative content TL (CD3+19-) (%)	69,7 ± 1,5	65,3 ± 2,1	66,5 ± 1,9
FI TL (CD3+19-) (s. u.)	624,4 ± 4,5	586,4 ± 15,5 #	550,4 ± 16,7 #
Abs content T-h (CD4+8-) (x109)	0,8 ± 0,06	1,2 ± 0,1 #	1,2 ± 0,1 #
Relative content T-h (CD4+8-) (%)	42,2 ± 2,3	38,3 ± 1,8	38,6 ± 2,1
FI T-h (CD4+8-) (s. u.)	574,2 ± 4,3	577,9 ± 13,4	533,9 ± 14,6 **
Abs content T-ctl (CD4-8+) (x109)	0,6 ± 0,04	1,2 ± 0,2 #	0,8 ± 0,1
Relative content T-ctl (CD4-8+) (%)	28,6 ± 1,7	32,1 ± 1,7	29,7 ± 1,7
FI T-ctl (CD4-8+) (s. u.)	736,2 ± 4,6	734,8 ± 16,2	715,8 ± 15,3
CD4/CD8 (s. u.)	1,6 ± 0,2	1,4 ± 0,1	1,5 ± 0,1
RBTL with PHA (%)	55,8 ± 1,6	49,2 ± 2,0	54,5 ± 2,3
Abs content B-L (CD3-19+) (x109)	0,21 ± 0,03	0,39 ± 0,05 **	0,32 ± 0,05 #
Relative content B-L (CD3-19+) (%)	9,3 ± 0,8	11,7 ± 1,3	9,9 ± 1,0
FI B-L (CD3-19+) (s. u.)	456,7 ± 28,2	642,24 ± 25,9 **	554,71 ± 32,4 #
IgM (g/L)	1,44 ± 0,12	1,12 ± 0,07 **	1,41 ± 0,10
IgG (g/L)	13,9 ± 0,5	12,4 ± 0,5	13,0 ± 0,7
IgA (g/L)	2,8 ± 0,1	2,3 ± 0,2 #	2,7 ± 0,2
CIC (s. u.)	92,67 ± 4,03	109,0 ± 28,6	78,8 ± 7,3

Notes: # the difference with an indicator of healthy statistically significant (p < 0,05); * the difference corresponding figure in 1 and 2 groups statistically significant (p < 0,05).

Table 3
Functional activity of peripheral blood phagocytic cells in COPD patients according to smoking status (M ± m)

Indicators of cell activity		Groups of patients		
		Healthy persons (n = 25)	1 (n = 31)	2 (n = 34)
Neutrophils	Index of phagocytosis (%)	66,6 ± 2,4	49,5 ± 2,6 #	52,0 ± 2,1 #
	Phagocytes number (s. u.)	6,8 ± 0,3	5,6 ± 0,1 #	5,6 ± 0,1 #
	NBT-test (%)	64,2 ± 3,1	56,1 ± 2,6 #	52,7 ± 2,8 #
	Cytochemical index (CChI) (s. u.)	0,90 ± 0,10	0,71 ± 0,05	0,64 ± 0,05 #
Monocytes	Index of phagocytosis (%)	31,2 ± 1,7	12,4 ± 0,5 #	13,0 ± 0,7 #
	Phagocytes number (s. u.)	5,1 ± 0,1	2,3 ± 0,2 #	2,7 ± 0,2 #
	NBT-test (%)	32,6 ± 2,0	109,0 ± 28,6 #	78,8 ± 7,3 #
	Cytochemical index (CChI) (s. u.)	0,40 ± 0,01	0,31 ± 0,03 #	0,26 ± 0,02 #

Notes: # the difference with an indicator of healthy statistically significant (p < 0,05).

levels of IL-8, which are obtained during examination of patients with COPD contribute to chronic carriage of intracellular parasites and a constant slow infection process in the bronchopulmonary system (Aldonyte R. et al., 2003; Karoli N. A. and Rebrov A. P., 2004) [6, 17].

Some studies [1, 14] registered reduced level of IL-4 in serum of COPD patients during exacerbation compared to healthy donors. According to other authors, the level of IL-4 in serum exceeds the normal of 1.76 [2] to 9 times [3] which may be directed to limit the «hyperinflammatory» reactions and is a natural consequence of the persistence of microorganisms in bronchopulmonary system and activation of effector cells (macrophages and T-lymphocytes) to destroy microbes. In this case, IL-4 can be a key inhibitor of the inflammatory response with the direct participation in the activation of reparative processes, confirmation of which may be retention of the predominance of levels IL-4 over TNF- α in COPD patients after treatment. On the other hand, it is believed that one of the causes of hyperproduction of IL-4 may be a violation of the process of differentiation of T-helper cells: by increasing the level of T-helpers of the type-2, there is excessive secretion of IL-4 [4, 9]. It is also possible that the increased activity of T-helper type-2 in COPD patients, who had never smoked, may be due to allergic reactions.

It is known that T-helper lymphocytes (CD4+) are the main subpopulation of T-cells that regulate a protective cellular immune response, including in patients with COPD. Dynamic balance functions of T-helper type 1 and type 2 provides greater flexibility and plasticity of the immune response, and their imbalance contributes to the development of diseases of the bronchopulmonary system. [11, 32].

Thus, in our study was found that imbalance of T helper type 1 and type 2 (according to reduced levels of TNF- α /IL-4 ratio in the serum) in the direction of the prevalence of humoral immune response in COPD patients with a negative smoking status evidenced about relatively more severity of anti-inflammatory reactions in these patients.

Conclusions

1) Among COPD patients almost half of them (52 %) had never smoked.

2) The clinical features of COPD in patients with a negative smoking status include: younger age (58,1 ± 2,5 years), female predominance (52.9 %), better lung function (FEV1 – (51, 9 ± 3,4) %, FEV1/FVC – (67,0 ± 3,6) %).

Table 4
Content of cytokines and biologically active substances in the serum of patients with exacerbation of COPD, depending on the smoking status (M ± m)

Indicators	Groups of patients			
	Healthy persons (n = 25)	Total (n = 65)	1 (n = 31)	2 (n = 34)
TNF- α (pg/ml)	7,9 ± 0,8	15,4 ± 3,9 #	14,3 ± 5,8	16,5 ± 5,5
IL-8 (ng/ml)	45,0 ± 3,4	61,6 ± 6,1 #	58,8 ± 9,8	63,3 ± 7,6 #
IL-4 (pg/ml)	7,6 ± 0,8	68,3 ± 21,7 #	42,7 ± 21,6	92,8 ± 37,2 #
E-селектін (ng/ml)	54,3 ± 7,1	119,8 ± 8,2 #	114,6 ± 9,9 #	124,7 ± 13,1 #
sICAM-1 (ng/ml)	292,8 ± 24,2	336,7 ± 22,7	316,5 ± 24,3	358,3 ± 39,2
hsC-RP (mg/l)	1,8 ± 0,4	7,5 ± 1,1 #	7,4 ± 1,6 #	7,5 ± 1,6 #
TNF α /IL-4 (s. u.)	1,28 ± 0,23	1,42 ± 0,38	2,61 ± 0,81	0,66 ± 0,15 **

Notes: # the difference with an indicator of healthy statistically significant p < 0,05); * the difference corresponding figure in 1 and 2 groups statistically significant (p < 0,05).

3) Do not depend on smoking status and are observed in all COPD patients with exacerbation: elevated absolute number of leukocytes in blood by increasing the absolute number of neutrophils and monocytes; increase in the absolute number of T lymphocytes (CD3+) due in particular subpopulation of T-helper (CD4+); decrease in functional activity of T-lymphocytes (CD3+) (by immunofluorescence intensity – IF); increase in the absolute number of B-lymphocytes (CD19+) with an increase in their functional activity (IF); increase in serum levels of E-selectin and C-reactive protein – that demonstrates the high activity of the inflammatory process in patients with acute exacerbation of COPD, regardless of smoking status.

4) In COPD patients with positive smoking status in the acute phase there is a more pronounced leukocytosis on the background of a relative lymphopenia in blood, with the

growth of the absolute number of cytotoxic T lymphocytes (CD8+), a more pronounced increase in the absolute number of B-lymphocytes with their pronounced dysfunction / exhaustion which shows a significant decrease in the level of serum IgM and A, – probably due to more pronounced inflammation

5) For COPD patients with a negative smoking status characterized by a more pronounced decrease in functional activity of T-helper lymphocytes (CD4+) (fluorescence intensity), increased serum levels of anti-inflammatory IL-4, the decrease ratio of the levels of TNF- α /IL-4, – which indicates a greater expressiveness inflammatory reactions associated with a more pronounced activation of T-helpers of type 2.

6) Different pathogenesis of COPD in patients depending on the smoking status causes various immunological features of the course of disease exacerbation.

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