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Functional Activity of Blood Neutrophils in Patients with Stable Course and Exacerbation of Chronic Obstructive Pulmonary Disease

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We performed a comparative study of the parameters of chemiluminescence of blood neutrophils in patients with different severity of chronic obstructive pulmonary disease in its different periods. The maximum values of induced and spontaneous chemiluminescence were recorded at moderate severity of the disease during exacerbation. Low levels of chemiluminescence indicators were found in severe chronic obstructive pulmonary disease in the stable phase. The values of the induction period of the chemiluminescent response in patients with moderate chronic obstructive pulmonary disease were higher than in the control group. Correlations between the values of induced chemiluminescence of neutrophils and the respiration function parameter FEV_1 were established, which may indicate the influence of multidirectional changes in the functional activity of systemic neutrophils on the development and worsening of airway obstruction in chronic obstructive pulmonary disease patients.

Key Words: *COPD; chemiluminescence; neutrophils*

Chronic obstructive pulmonary disease (COPD) is characterized by chronic inflammatory reactions of the airways and progressive decline in lung function [1]. Among topical problems of COPD, an important direction is the search for additional diagnostic criteria of the severity of chronic inflammation, exacerbations, and a stable course of COPD based on the assessment of impaired immunomolecular indicators [2]. Inflammation of the respiratory tract in COPD is accompanied by a cascade of cellular interactions and infiltration of bronchopulmonary tissue by various cells of the immune system with a predominance of neutrophils. Neutrophils play the main role in both destruction and pathological remodeling of bronchopulmonary structures in patients with COPD, which is associated with accumulation of these cells and their mediators in both organ-specific (bronchial secretion) and systemic (peripheral blood) substrates [3-6]. It has been shown that the increase in the content of neutrophils in the peripheral blood is associated with the severity and frequency of COPD exacerbations [7], and their dysfunctional state is typical of patients with the COPD phenotype associated with frequent exacerbations [8].

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Despite ample data that neutrophils are the key effector cells of chronic inflammation in COPD, the functional activity of circulating neutrophils in patients with different severity and in different phases of the disease (exacerbation, stable course) remains the subject of discussion. A number of studies have demonstrated the relationship between the severity of COPD and some markers of neutrophils, in particular, neutrophil elastase and myeloperoxidase in the sputum and bronchoalveolar lavage fluid [7,9]. An important factor in the implementation of the effector potential of neutrophils is ROS generation; excessive release of ROS provokes the development of oxidative stress.

Currently, the pattern of ROS production by neutrophils in patients with different severity of COPD during exacerbation and stable course cannot be considered established. On the one hand, it has been shown that the spontaneous production of ROS by endobronchial neutrophils during exacerbation of COPD is higher than in the stable phase of the disease [10]. At the same time, reduced activity of blood neutrophils and reduced intracellular generation of ROS were observed in stable COPD and in frequent exacerbations of the disease [8,11]. These contradictions can be explained by the use of different methodological approaches in assessing ROS production by neutrophils, as well as different phases of the course of COPD in patients included in the study.

ROS generation by neutrophils is accompanied by energy release, which is realized by the emission of light quanta, which can be methodically recorded by the chemiluminescence (CL) method.

Our aim was to study the parameters of neutrophil CL in patients with moderate and severe COPD during exacerbation or stable course of the disease.

MATERIALS AND METHODS

The study was conducted in accordance with the Declaration of Helsinki (1964) and approved by the Ethics Committee of the Research Institute of Pulmonology (Protocol No. 05-19, October 10, 2019).

The study included 125 men: 104 patients with COPD aged 55.1±5.4 years (69.2% smokers and 30.8% ex-smokers) and 21 healthy non-smoker volunteers with normal lung function aged 54.3±7.6 years. COPD was diagnosed in accordance with the criteria of the Global COPD Initiative (GOLD) at $FEV_1/FVC<0.7$ (FEV_1 is forced expiratory volume in 1 sec, FVC is forced vital capacity of the lungs) after a test with a bronchodilator [1]. The patients with COPD were divided into four groups: moderate COPD during the exacerbation ($FEV_1>50\%$; group 1; $n=23$); moderate COPD in stable phase ($FEV_1>50\%$; group 2; $n=24$); severe COPD during the exacerbation ($FEV_1<50\%$; group 3; $n=29$); severe COPD in stable phase ($FEV_1<50\%$; group 4; $n=28$).

The respiratory function was studied on a SpiroLab III computer spirograph with assessment of FEV_1, FVC, and FEV_1/FVC ratio after a bronchodilator test (15 min after inhalation of 400 μg of salbutamol through a metered-dose aerosol inhaler). FEV_1 was assessed as a percentage of the required values.

ROS generation by neutrophils was assessed by spontaneous and induced CL on a Dynatech chemiluminometer. A leukocyte suspension consisting of neutrophils, eosinophils, monocytes, and lymphocytes was isolated from heparinized blood (0.4×10^6 cells in 200 μl of saline). It is believed that the development of the CL response is mainly determined by neutrophils. To measure spontaneous CL, 20 μl of 10^{-3} M luminol was added to the leukocyte mixture; induced CL was assessed after additional treatment with 20 μl of opsonized zymosan. Functional activity of blood neutrophils was assessed by measuring spontaneous CL of blood neutrophils (intrinsic cell luminescence, I_{spont}), CL induced by opsonized zymosan (I_{max}), cell activity coefficient (I_{max}-I_{spont}/I_{max}), and the time to maximum luminescence of cells after stimulation (induction period T, min). The results were expressed in relative luminescent units (RU).

Statistical analysis was performed using the Statgraphics Centurion XVI software package. Data are presented as *M*±*SD*. The Shapiro–Wilk test was used to check the normality of distribution. Further analysis was performed using Student's *t* test. The Pearson's test was used to calculate the correlation coefficient (*r*). The level of statistical significance was set at $p<0.05$.

RESULTS

FEV_1 values in all patients with COPD were significantly lower than in controls ($p=0.001$) (Table 1). In addition, significant differences in FEV_1 values between the groups were established, which indicates heterogeneity of the studied groups of COPD depending on activity of inflammation (exacerbation/stable course) and the severity of the disease (Table 1).

In comparison with the control group, the level of induced CL in group 1 was higher by 2.8 times ($p=0.01$), in group 2 by 1.4 times, and in group 3 by 1.5 times (Fig. 1). The maximum intensity of I_{max} was observed during exacerbation of COPD and $FEV_1>50\%$. I_{max} in group 1 was higher by 2.1 times than in groups 2 and 3 and by 2.5 times higher than in group 4. It should be noted that the disinhibition of the effector function of blood neutrophils was a characteristic feature for patients with exacerbation of severe COPD and in the stable phase of COPD of moderate severity. At the

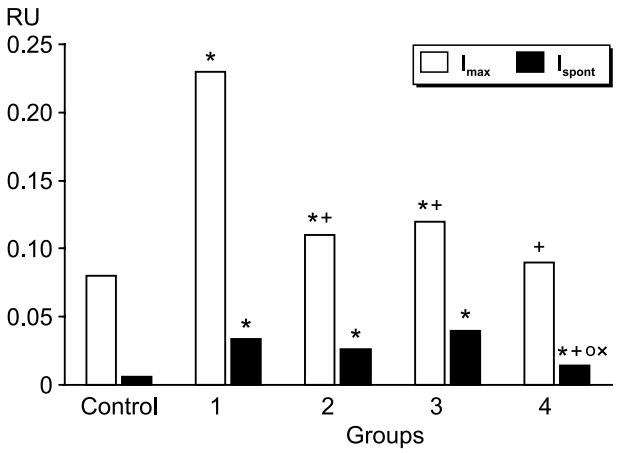

Fig. 1. Induced and spontaneous CL in COPD patients. $p<0.05$ in comparison with *control, +group 1, °group 2, ×group 3.

same time, in patients with severe COPD, I_{max} did not differ from the control.

The level of spontaneous CL in COPD also significantly exceeded the control values (0.006±0.003, $p=0.01$). The most marked increase in I_{spont} was observed in patients with exacerbation of COPD (groups 1 and 3). Excessive production of ROS by naive (not stimulated) phagocytes can serve as one of the reasons for the formation of chronic non-infectious inflammation of bronchopulmonary tissue, initiation of oxidative stress and damage to the alveoli with the development of emphysema [3,7]. Group 4 showed significantly lower I_{spont} values compared to groups 1-3 ($p<0.05$). The data obtained allow us to conclude that the severe course of COPD, especially in the stable phase, is associated with exhaustion of the oxidative-metabolic potential of neutrophils under conditions of progressive hypoxemia and in the stage of fibrotic changes.

The induction period of induced neutrophil CL (T, min) in group 1 exceeded the control values by 1.6 times ($p=0.001$) and in group 2 by 1.4 times ($p=0.019$) (Table 2), which can be explained by inhibition functional activity of the receptor apparatus of systemic neutrophils. At the same time, there were no significant differences in the indicators in these groups. In severe COPD (groups 3 and 4), the induction period did not differ from the control. Normal values of the induction period in patients of group 3 should not be regarded as a favorable sign. In our opinion, this phenomenon in severe COPD during the exacerbation can be associated with an increase in the permeability of the cell membrane of circulating neutrophils. Cell activity coefficient in patients of group 1 did not differ from the control. In other groups, this parameter was significantly below the control ($p<0.05$). The most significant inhibition of cellular activity was observed in group 3, which can indicate progression of cytotoxic failure of circulating neutrophils against the background of severe respiratory problems and intensification of inflammation.

In patients with moderate COPD, negative correlations were found between I_{max} and FEV_1; in severe COPD, on the contrary, positive associations of induced CL of blood neutrophils with FEV_1 were recorded (Table 3). These results demonstrate a relationship of the decrease in the functional activity of blood neutrophils and significant deterioration in bronchial patency in COPD patients. According to

TABLE 1. Parameters of Pulmonary Function in Patients with COPD and Healthy Non-Smoking Men (*M*±*SEM*)

Parameters	Control	FEV_1 >50%		FEV_1 <50%	
		group 1 (exacerbation)	group 2 (stable phase)	group 3 (exacerbation)	group 4 (stable phase)
FEV_1, %	101.5±4.5	56.9±9.3*	62.1±8.1*+	39.3±5.4*++oo	43.7±7.4*++ooxx
FEV_1/FVC	102.5±3.9	55.7±8.3*	65.4±8.9*+	46.5±7.1*++oo	51.2±9.3*++oox

Note. *$p=0.001$ in comparison with the control; +$p=0.01$, ++$p=0.001$ in comparison with group 1; oo$p=0.001$ in comparison with group 2; x$p=0.04$, xx$p=0.02$ in comparison with group 3.

TABLE 2. Parameters of the Induction Period of Stimulated CL of Neutrophils and Cell Activity in COPD Patients

Parameter	Control	FEV_1 >50%		FEV_1 <50%	
		group 1 (exacerbation)	group 2 (stable phase)	group 3 (exacerbation)	group 4 (stable phase)
T, min	15.1±1.3	24.7±0.9**	21.5±2.1*	16.2±1.5+++o	17.2±1.3++
Cell activity coefficient	0.92±0.03	0.85±0.04	0.76±0.02**+	0.67±0.02***+++ooo	0.84±0.02*oox

Note. *$p=0.02$, **$p=0.001$, ***$p=0.0001$ in comparison with the control; +$p=0.04$, ++$p=0.001$, +++$p=0.0001$ in comparison with group 1; o$p=0.05$, oo$p=0.01$, ooo$p=0.001$ in comparison with group 2; x$p=0.001$ in comparison with group 3.

TABLE 3. Correlations (r) between Blood Neutrophil CL Intensity and FEV_1 in COPD Patients

Parameters of neutrophil CL	Moderate COPD (FEV_1>50%)	Severe COPD (FEV_1<50%)
I_{max}	r=-0.42; p=0.041	r=0.51; p=0.024
I_{spont}	r=-0.19; p=0.42	r=-0.25; p=0.23

the results of the correlation analysis, the effect of reduced activity of oxidative stress on progression of airway obstruction and clinical manifestations of the disease is clearly seen.

The study of CL parameters of systemic neutrophils in patients with COPD in comparison with healthy non-smokers revealed their significant differences, which is associated with activity of the inflammatory process and the stage of COPD. The decrease in the intensity of stimulated CL of blood neutrophils and cell activity coefficient in patients with severe COPD can indicate exhaustion of the reserve capacity of neutrophils and be considered as a diagnostic criterion for an unfavorable course of the disease.

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Conflict of interest. The authors declare no conflict of interest.

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