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Abstract

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LEVELS OF SYSTEMIC INFLAMMATORY RESPONSE MARKERS IN PATIENTS WITH PULMONARY HYPERTENSION AND COPD

Introduction. The prevalence of COPD worldwide is about 7.6 %, and it is one of the main causes of morbidity and mortality in today society. Today, much information has been obtained about the pathogenetic mechanisms of COPD development. However, more and more studies have recently shown that COPD patients have an increase in proinflammatory mediators that contribute to the development of systemic subclinical inflammation; this is due to the involvement of inflammatory cells from the bloodstream in the process, and the development of a systemic inflammatory response. An urgent medical and social problem of our time is the development of pulmonary hypertension in patients with COPD. The processes of inflammation and remodeling of the vascular wall are inseparable; they complement each other, leading to the formation of a clinical picture of pulmonary hypertension.

The aim of the study. To determine the levels of markers of systemic inflammatory response among patients with pulmonary hypertension on the background of COPD.

Materials and methods. The results of the study are based on data from a comprehensive survey of 170 patients aged 40 to 65 years with COPD, 123 of which had pulmonary hypertension and 47 ones had no PH.

Obtained results. The level of hs-CRP in the group of PH patients with COPD was 10.46 [6.24; 15.30] mg/l and was significantly higher, both against the value of 7.30 [6.22; 9.18] mg/l in the group of COPD patients without PH ($p < 0.05$), and in comparison with the group of healthy individuals, where this indicator was 1.08 [0.96; 1.41] mg/l, ($p < 0.05$). The increase in IL-6 levels was significantly higher by 57% in the group of PH patients with COPD compared to the value of 5.67 [4.44; 6.98] PG/ml, ($p < 0.05$) in the group of COPD without PH and amounted to 8.90 [7.76; 9.93] PG/ml, and a 7.4-fold increase in the value of 1.20 [0.95; 1.57] PG/ml in the group of healthy individuals, ($p < 0.05$). The median level of IL-10 was significantly higher in the group of healthy individuals by 11.2 % and 10 % compared to the groups of patients with PH on the background of COPD and COPD without PH, respectively, and was 5.35 [4.97; 6.86] PG/ml, ($p < 0.05$). In the groups of patients with PH on the background of COPD and

COPD without PH, there was a significant increase in this indicator by 8.8 and 6 times compared to the level of 0.21 [0.20; 0.25] in the group of healthy individuals, ($p < 0.05$). Comparing subgroups of patients depending on the type of COPD exacerbation, the level of hs-CRP, IL-6 and the ratio of IL-6/IL-10 levels were significantly higher in the subgroup with infectious type of exacerbation compared to the subgroup of non-infectious type of COPD exacerbation, ($p < 0.05$).

Key words: markers of systemic inflammatory response, pulmonary hypertension, chronic obstructive pulmonary disease, mean pulmonary artery pressure.

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Резюме

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РІВНІ МАРКЕРІВ СИСТЕМНОЇ ЗАПАЛЬНОЇ ВІДПОВІДІ У ПАЦІЄНТІВ З ЛЕГЕНЕВОЮ ГІПЕРТЕНЗІЄЮ НА ФОНІ ХОЗЛ

Вступ. Поширеність ХОЗЛ у всьому світі становить близько 7,6 %, і є однією з головних причин захворюваності та смертності в сучасному суспільстві. На теперішній день отримано багато відомостей про патогенетичні механізми розвитку ХОЗЛ. Однак останнім часом з'являється все більше робіт, в яких встановлено, що у хворих на ХОЗЛ спостерігається зростання прозапальних медіаторів. Актуальною медико-соціальною проблемою сучасності є розвиток легеневої гіпертензії у пацієнтів які мають ХОЗЛ. Процеси запалення і ремоделювання судинної стінки нероздільні, вони доповнюють один одного, приводячи до формування клінічної картини легеневої гіпертензії, що і визначило мету даного дослідження

Мета дослідження: визначити рівні маркерів системної запальної відповіді у пацієнтів з легеневою гіпертензією на фоні ХОЗЛ.

Матеріали і методи. Результати дослідження ґрунтуються на даних комплексного обстеження 170 хворих у віці від 40 до 65 років з ХОЗЛ, з яких 123 мали легеневу гіпертензію та 47 осіб були без неї.

Отримані результати. Рівень ВЧ С-РБ в групі хворих ЛГ на фоні ХОЗЛ був достовірно вище, як проти значення 7,30 [6,22; 9,18] мг/л в групі хворих ХОЗЛ без ЛГ ($p < 0,05$), так і в порівнянні з групою здорових осіб, де цей показник був 1,08 [0,96; 1,41] мг/л, ($p < 0,05$). Підвищення рівня ІЛ-6 було в групі хворих ЛГ на фоні ХОЗЛ достовірно вище на 57 % у порівнянні зі значенням 5,67 [4,44; 6,98] пг/мл, ($p < 0,05$) у групі ХОЗЛ без ЛГ і склав 8,90 [7,76; 9,93] пг/мл, та перевищувало в 7,4 рази значення 1,20 [0,95; 1,57] пг/мл у групі здорових осіб, ($p < 0,05$). Медіана рівня ІЛ-10 була достовірно вищою в групі здорових осіб на 11,2 % та 10 % у порівнянні з групами хворих ЛГ на фоні ХОЗЛ та ХОЗЛ без ЛГ відповідно і склала 5,35 [4,97; 6,86] пг/мл, ($p < 0,05$). У групах хворих з ЛГ на фоні ХОЗЛ та ХОЗЛ без ЛГ відзначалося достовірне збільшення співвідношення ІЛ-6/ІЛ-10 в 8,8 та 6 рази проти рівня 0,21 [0,20; 0,25] у групі здорових осіб, ($p < 0,05$). Порівнюючи підгрупи хворих в залежності від варіанту загострення ХОЗЛ рівень ВЧ С-РБ, ІЛ-6 та співвідношення рівнів ІЛ-6/ІЛ-10 були

достовірно вищим в підгрупі з інфекційним типом загострення порівняно з підгрупою неінфекційного типу загострення ХОЗЛ, (p < 0,05).

Ключові слова: маркери системної запальної відповіді, легеневa гіпертензія, хронічне обструктивне захворювання легенів, середній тиск в легеневій артерії.

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Introduction

Chronic obstructive pulmonary disease (COPD) significantly affects the quality of life, greatly limiting the physical capabilities of sufferers. The prevalence of COPD worldwide is about 7.6 %, and it is one of the main causes of morbidity and mortality in today society. According to experts of GOLD, the mortality rate due to COPD by 2030 will move to the third place among all causes of death [1, 2].

Today, much information has been obtained about the pathogenetic mechanisms of COPD development. However, more and more studies have recently shown that COPD patients have an increase in proinflammatory mediators that contribute to the development of systemic subclinical inflammation, this is due to the involvement of inflammatory cells from the bloodstream in the process, and the development of a systemic inflammatory response. An imbalance in the cytokine profile system is of great importance in the development of pathological changes in the respiratory system [3].

An urgent medical and social problem of our time is the development of pulmonary hypertension (PH) in patients with COPD. In Ukraine, there are no data on the prevalence and mortality from PH and its various forms, which is due to the lack of a single training and consultation center and registry of these patients [4].

The processes of inflammation and remodeling of the vascular wall are inseparable, they complement each other, leading to the formation of a clinical picture of PH. Researchers are asking what is the role of inflammatory mechanisms in the development of PH, and if is it possible to use laboratory markers of inflammation as diagnostic criteria for determining PH in COPD? Determination of systemic inflammatory response biomarkers can be a useful way to identify patients with PH, which determined the purpose of this study [5, 6].

The aim of the study. To determine the levels of markers of systemic inflammatory response among patients with pulmonary hypertension on the background of COPD.

Materials and methods. The results of the study are based on data from a comprehensive survey of 170 patients aged 40 to 65 years with COPD, 123 of which had pulmonary hypertension and 47 ones had no PH. In the period of 2015-2018, we conducted a survey of patients who were on inpatient treatment in the pulmonology Department of the municipal institution "Zaporizhzhia Regional Clinical Hospital" of the Zaporizhzhia Regional Council. Almost healthy 31 people were examined on an outpatient basis.

After signing the "Voluntary informed consent of the patient to participate in the study", the subjects underwent General clinical, instrumental and laboratory examinations in order to verify the diagnosis and determine concomitant pathology. Research methods were chosen according to orders the Ministry of health of Ukraine No. 555 dated 27 June, 2013 and No. 614 dated 21 June, 2016, taking into account the recommendations of GOLD (2016).

The criteria for inclusion in the study were: male and female patients aged 40-65; known duration of COPD more than 1 year; informed consent of the patient to participate in the study.

Exclusion criteria from the study were: clinically significant comorbid pathology; the presence of decompensated diabetes, the presence of myocardial infarction in anamnesis, chronic heart failure of IIB–III stage; cancer; presence of contraindications to the administration of drugs and their components; drug addiction, alcohol addiction, mental illness; refusal of a patient to participate in the study.

All patients underwent General clinical, instrumental and laboratory examinations according to the algorithm of this study. The diagnosis of COPD was verified in accordance with order No. 555 of the Ministry of health of Ukraine dated 27 July, 2013, taking into account the

recommendations of GOLD (Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease, updated 2016). Pulmonary hypertension was determined on the basis of order No. 614 of the Ministry of health of Ukraine dated 21 June, 2016. Patients were divided into groups after determining their compliance with the criteria for inclusion/exclusion of the study, depending on the presence of pulmonary hypertension:

- the first group included 123 patients with PH and COPD (median age was 59.0 [51.0; 65.0] years);
- the second group consists of 47 patients with COPD without PH (median age was 58.0 [50.0; 65.0] years);
- the third group consisted of 31 practically healthy individuals (the median age was 56.0 [54.0; 58.0] years).

Characteristics of patients who are under the study. In the group of patients with PH on the background of COPD, 24 (19 %) patients had stage II of the disease and 99 (80.5 %) had stage III; in the group of patients with COPD without PH, there were 11 (23.4 %) people with stage II and 36 (76.6 %) had stage III. Groups of patients were comparable in COPD stage ($p > 0.05$).

In the PH group with COPD, the median mean pressure in the artery pulmonary (MPAP) was 31.00 [29.00; 42.00] mmHg. There were 84 (68.3 %) patients with the first degree of PH and 39 (31.7 %) ones with the II degree of PH. Infectious exacerbation was detected in 70 (56.9 %) of 123 patients.

Determination of high sensitivity C-reactive protein (hs-CRP), interleukin-6 (IL-6), interleukin-10 (IL-10) was performed in blood plasma by the ELISA method using standard kits: "hs-CRP-ELISA-best" "IL-6-ELISA-best" "IL-10-ELISA-best" according to the attached instructions. The optical density was estimated using spectrophotometry at a wavelength of $\lambda = 450$ nm. The extent value was determined using a semi-automatic tablet analyzer "SUNRISE TS" (Austria). The content of hs-CRP in blood plasma was expressed in mg/l, interleukin-6 (interleukin-10) was determined in PG/ml.

Statistical processing of the results obtained. We determined the distribution of data using the Shapiro – Wilk criterion, then used the method of descriptive statistics with the calculation of the median and interquartile range Me [25; 75], indicated the volume of the analyzed group (n). Comparison of two groups with a parametric distribution was performed using the Student's test (t-test). When comparing more than two independent variables, ANOVA analysis was used, followed by post-hoc analysis. When the distribution was different from the normal one, we analyzed it using nonparametric tests: when comparing two independent samples, we used the Mann - Whitney method (U-test), and in the case of more than two, we used the Kruskal-Wallis method. For the level of statistical significance (p), it is recommended for biomedical research below 0.05.

The relationships between quantitative indicators were determined using correlation analysis with the calculation of the correlation coefficient, strength and direction of the links. The Pearson method (r) was used for parametric data distribution, and the Spearman method (R) was used for nonparametric data distribution.

Obtained results. We evaluated the levels of markers of systemic inflammatory response in the examined individuals. The results are presented in Table 1.

Table 1 – The levels of markers of inflammation in the examined individuals (Me [25; 75], n = 201)

Variable	Patients with PH and COPD (n = 123)	Patients with COPD without PH (n = 47)	Healthy individuals (n = 31)
hs-CRP, mg/l	10,46 [6,24; 15,30]	7,30 [6,22; 9,18]	1,08 [0,96; 1,41]
P-value	$p_{1-2} = 0,02$	$p_{2-3} < 0,001$	$p_{1-3} = < 0,001$
IL-6, PG/ml	8,90 [7,76; 9,93]	5,67 [4,44; 6,98]	1,20 [0,95; 1,57]
P-value	$p_{1-2} = < 0,001$	$p_{1-2} = < 0,001$	$p_{1-3} = < 0,001$
IL-10, PG/ml	4,81 [4,29; 5,21]	4,87 [4,43; 5,28]	5,35 [4,97; 6,86]
P-value	$p_{1-2} = 1,0$	$p_{1-2} = < 0,01$	$p_{1-3} = < 0,001$
IL-6/IL-10	1,84 [1,62; 2,07]	1,26 [0,95; 1,41]	0,21 [0,20; 0,25]
P-value	$p_{1-2} = < 0,001$	$p_{2-3} < 0,001$	$p_{1-3} = < 0,001$

The level of hs-CRP in the group of PH patients with COPD was 10.46 [6.24; 15.30] mg/l and was significantly higher, both against the value of 7.30 [6.22; 9.18] mg/l in the group of COPD patients without PH ($p < 0.05$), and in comparison with the group of healthy individuals, where this indicator was 1.08 [0.96; 1.41] mg/l, ($p < 0.05$). Significantly, the level of hs-CRP in the PH group with COPD was higher by 43.3 % compared to the group of patients with COPD without PH and 9.7 times higher than the median of this indicator in the group of healthy individuals ($p < 0.05$).

The increase in IL-6 levels was significantly higher by 57% in the group of PH patients with COPD compared to the value of 5.67 [4.44; 6.98] PG/ml, ($p < 0.05$) in the group of COPD without PH and amounted to 8.90 [7.76; 9.93] PG/ml, and a 7.4-fold increase in the value of 1.20 [0.95; 1.57] PG/ml in the group of healthy individuals, ($p < 0.05$). The level of IL-6 was significantly higher in the COPD group without PH of 5.67 [4.44; 6.98] PG/ml versus a value of 1.20 [0.95; 1.57] PG/ml in healthy individuals, ($p < 0.05$).

The median level of IL-10 was significantly higher in the group of healthy individuals by 11.2 % and 10 % compared to the groups of patients with PH on the background of COPD and COPD without PH, respectively, and was 5.35 [4.97; 6.86] PG/ml, ($p < 0.05$). There were no significant differences between the groups of patients with PH on the background of COPD and COPD without PH ($p > 0.05$).

The ratio of IL-6/IL-10 among PH patients with COPD was 1.84 [1.62; 2.07] and significantly exceeded by 46 % the median of this indicator in the group of COPD without PH, where this indicator was 1.26 [0.95; 1.41], ($p < 0.05$). In the groups of patients with PH on the background of COPD and COPD without PH, there was a significant increase in this indicator by 8.8 and 6 times compared to the level of 0.21 [0.20; 0.25] in the group of healthy individuals, ($p < 0.05$).

The levels of markers of inflammation in patients with PH were determined depending on the exacerbation of COPD. The results are presented in Table 2.

Table 2 – The levels of markers of inflammation in patients with PH depending on the type of COPD exacerbation (Me [25; 75], n = 123)

Variable	The type of COPD exacerbation	
	Non-infectious type (n = 53)	Infectious type (n = 70)
hs-CRP, mg/l	6,14 [4,94; 11,19]	13,24 [9,47; 17,13]
P-value	p < 0,001	
IL-6, PG/ml	7,78 [6,48; 9,05]	9,28 [8,56; 10,27]
P-value	p < 0,001	
IL-10, PG/ml	4,90 [4,38; 5,66]	4,77 [4,26; 5,07]
P-value	p = 0,02	
IL-6/IL-10	1,61 [1,46; 1,74]	1,97 [1,84; 2,18]
P-value	p < 0,001	

Comparing subgroups of patients depending on the type of COPD exacerbation, the level of hs-CRP was significantly higher in the subgroup with infectious type of exacerbation by 2 times compared to the subgroup of non-infectious type of COPD exacerbation and amounted to 13.24 [9.47; 17.13] mg/l versus 6.14 [4.94; 11.19] mg/l, respectively,($p < 0.05$). The level of IL-6 was 9.28 [8.56; 10.27] PG/ml in the subgroup with infectious type of COPD exacerbation and was significantly higher by 19.3 % compared to the subgroup of non-infectious type of COPD exacerbation, where this indicator was 7.78 [6.48; 9.05] PG/ml, ($p < 0.05$).

The median IL-10 index of the subgroup of non-infectious type of COPD exacerbation was 4.90 [4.38; 5.66] PG/ml and greatly exceeded this indicator in the subgroup with infectious type of COPD exacerbation, where the level was 4.77 [4.26; 5.07] PG/ml, ($p < 0.05$). The ratio of IL-6/IL-10 levels was significantly higher in the subgroup with infectious type of COPD exacerbation - 1.97 [1.84; 2.18] compared with the subgroup of non - infectious type of COPD exacerbation-1.61 [1.46; 1.74] by 22.4 %, ($p < 0.05$). Then the correlation analysis is performed. Reliable relationships were determined between the following indicators: MPAP and hs-CRP (R =

+0.64, p = 0.001); MPAP and IL-6 (R = +0.67, p = 0.001); MPAP and IL-6/IL-10 (R = +0.42, p = 0.001).

Discussion Systemic inflammatory response syndrome is a typical pathological process characterized by total inflammatory reactivity of endotheliocytes, plasma and cellular factors of blood, connective tissue, and in the final stages – microcirculatory disorders in vital organs and tissues. In addition, it is manifested by an increase in the concentration of circulating cytokines and the activation of numerous inflammatory cells that synthesize their own mediators [7].

Our data correlate with the results of other studies, which indicate that the average concentration of CRP in COPD patients is increased, especially in smokers. In their study, P. Joppa et al. it was determined that the level of CRP in patients with COPD was significantly higher when combined with pulmonary hypertension [8, 9].

Systemic inflammation is now recognized as a component of COPD. The results of this study indicate the role of inflammation, or rather IL-6, in the pathogenesis of PH in COPD patients. In patients with COPD, IL-6 levels were correlated with MPAP. Our data are consistent with the data obtained by A. Chaouat et al., which determined that circulating levels of the proinflammatory cytokine IL-6 were higher in COPD patients than in the control group [10].

The formation of subclinical persistent inflammation occurs not only locally, in the bronchopulmonary system, but also leads to the development of systemic effects, due to a violation of the balance of cytokines in the blood. A number of studies have found that interleukin-6 negatively affects the number and function of endothelial cells that are mobilized from the bone marrow and participate in vasculogenesis. According to a number of researchers dealing with this problem, the increased content of such proinflammatory cytokines contributes to the development of endothelial dysfunction [11, 12].

Thus, the immune system reacts to the exacerbation of COPD and participates in the development of comorbid pathology. Changes in the cytokine profile make a very large contribution to the development of pathological changes in the bronchopulmonary system. However, the mechanisms of developing an imbalance of these reactions, as well as their significance in the process of remodeling the cardiovascular system, remain poorly understood.

Conclusions

1. In patients with COPD, the level of markers of systemic inflammatory response increases, which is characterized by an increase in the concentration of hs-CRP, IL-6 and a decrease in the level of anti-inflammatory interleukin-10 in blood plasma.

2. When pulmonary hypertension occurs in patients with COPD, there is a further increase in the level of interleukin-6 and hs-CRP in the blood plasma.

Prospects for future research

Systemic inflammatory response is strongly prevalent among patients with COPD, vascular dysfunction impairs gas exchange, and when combined with PH, may be a major factor in the survival of these patients. Already in the early stages of COPD, the systemic inflammatory response contributes to the pathogenesis of PH, and this opens up a potentially new approach to the treatment of these patients, which requires further research.

Conflict of interest

The authors declare no conflict of interest.

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