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The Biomarkers of Inflammation and Endothelial Dysfunction in Detection Patients with Pulmonary Hypertension on the Background of COPD

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Abstract

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

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.

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 ($p < 0.05$). 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, ($p < 0.05$). The highest sensitivity of Se = 86.18% among the analyzed parameters was IL-6 (AUC = 0.872) with cut off >6.98 pg/ml.

Keywords: endothelial dysfunction, pulmonary hypertension, chronic obstructive pulmonary disease

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].

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

Material 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.

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 comorbide 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.

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 ones (80.5 %) - stage III, in the group of patients with COPD without PH, there were 11 (23.4 %) people had stage II and 36 ones (76.6 %) - 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 I 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 endothelin-1. The quantitative content of endothelin-1 was determined by an enzyme immunoassay using a set of reagents "Endothelin-1 ELISA kit" (Biomedica, Austria) in accordance with the attached instructions. The optical density was estimated using a spectrophotometric method at a wavelength of λ = 540 nm. The extent value is determined using a semi-automatic tablet analyzer "SUNRISE TS" (Austria). The content of endothelin-1 in blood plasma was expressed in fmol/ml.

Determination of final stable metabolites of nitric oxide. The method based on the reduction of nitrates to nitrites with the determination of the latter by a reaction with a Gris reagent was conducted. The optical density was measured using a SF-46 spectrophotometer (FEK) at a wavelength of λ = 540 nm. The calculation of the amount of nitrites was carried out according to the calibration schedule that was built for nitrogen nitrite. The study obtained three results: the content of nitrite ions (NO_2) (mmol/l), the content of nitrate ions (NO_3) (mmol/l) and the total content of nitrite and nitrate ions (NO_2+ NO_3) (mmol/l).

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 λ = 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.

We used ROC analysis (Receiver Operating Characteristic curve analysis), and calculated the area under the ROC curve (AUC-Area under the ROC curve), and its 95% confidence interval (95% CI). We considered the AUC value greater than 0.5 to be statistically significant. The cut-off point was found using the J-Youden index and sensitivity (Se) and specificity (SP) were calculated for it.

Results and Discussion

The levels of biomarkers among the examined persons are presented in Table 1.

Table 1. The levels of biomarkers among patients examined (Me [25-75], n = 201)

Variable	Patients with PH and COPD (n = 123)	Patients with COPD without PH (n = 47)	Healthy individuals (n = 31)
	1	2	3
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	$p_{1-2} < 0,001$	$p_{2-3} < 0,001$	$p_{1-3} = < 0,001$
Endothelin-1, fmol/l	3,17 [2,19 ; 4,14]	1,78 [1,25 ; 2,18]	0,31 [0,19 ; 0,36]
P-value	$p_{1-2} < 0,001$	$p_{2-3} < 0,001$	$p_{1-3} < 0,001$
NO_2, μmol/l	7,00 [5,00 ; 8,00]	8,00 [8,00 ; 9,00]	10,00 [9,00 ; 11,00]
P-value	$p_{1-2} = < 0,001$	$p_{2-3} < 0,001$	$p_{1-3} = < 0,001$
NO_3, μmol/l	12,00 [11,00 ; 14,00]	14,00 [12,00 ; 14,00]	16,00 [14,00 ; 17,00]
P-value	$p_{1-2} = 0,007$	$p_{2-3} < 0,001$	$p_{1-3} < 0,001$
NO_3+NO_2, μmol/l	19,00 [17,00 ; 21,00]	22,00 [21,00 ; 23,00]	26,00 [25,00 ; 28,00]
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 Endothelin-1 among PH patients with COPD was 3.17 [2.19; 4.14] fmol/ml and was considerably higher both against 1.78 [1.25; 2.18] fmol/ml in the group of COPD patients without PH ($p < 0.05$), and 10.2 times higher than the level of 0.31 [0.19; 0.36] fmol/ml in the group of healthy individuals ($p < 0.05$). The level of 1.78 [1.25; 2.18] fmol/ml in the group of COPD patients without PH was 5.7 times higher than in the group of healthy individuals ($p < 0.05$).

The NO_2 level in the group of PH patients with COPD was 7.00 [5.00; 8.00] mmol/l and was much more lower both against 8.00 [8.00; 9.00] mmol/l in the group of COPD patients without PH ($p < 0.05$), and in comparison with healthy individuals, where the NO_2 level was 10.00 [9.00; 11.00] mmol/l, ($p < 0.05$). Significantly, the NO_2 level was 14.3 % lower in the PH group with COPD compared to the value of 8.00 [8.00 ; 9.00] mmol/l in the COPD group without PH.

Such indicator as NO_3 in the groups of COPD patients with and without PH was 12.00 [11.00; 14.00] mmol/l and 14.00 [12.00 ; 14.00] mmol/l, respectively, and was significantly lower compared to the level of 16.00 [14.00; 17.00] mmol/l in healthy individuals, ($p < 0.05$). The great difference in NO_3 level in PH patients with COPD was lower by 16.7 % compared to the value in patients with COPD without PH ($p < 0.05$).

The amount of nitric oxide metabolites (NO_2+ NO_3) was considerably lower by 15.8% in patients with PH with COPD - 19.00 [17.00; 21.00] mmol/l versus 22.00 [21.00; 23.00] mmol/l in the group of patients with COPD without PH ($p < 0.05$). The level of this indicator was 37 % lower in the PH subgroup with COPD compared to the healthy group, where the level was 26.00 [25.00; 28.00] mmol/l, ($p < 0.05$). In the group of COPD patients without PH, NO2+ NO3 was 22.00 [21.00; 23.00] mmol/l and was significantly lower by 18.2 % against the group of healthy individuals ($p < 0.05$).

In order to determine the diagnostic value of biomarkers, ROC analysis was used for the calculation. We used two groups of patients with PH on the background of COPD (n = 123) and COPD without PH (n = 47). The data obtained is presented in table 2.

Table 2. Predictive value of markers in relation to the detection of pulmonary hypertension (n = 170)

Variable	Cut off	AUC	95 % CI AUC	Se, %	Sp, %
hs-CRP, mg/l	>11,28	0,655	0,578 to 0,726	46,34 %	95,74 %
IL-6, pg/ml	>6,98	0,872	0,812 to 0,918	86,18 %	76,60 %

Table 2 (continued). Predictive value of markers in relation to the detection of pulmonary hypertension (n = 170)

Endothelin-1, fmol/l	>2,36	0,823	0,757 to 0,877	70,73 %	97,87 %
NO_2, μmol/l	≤7	0,792	0,723 to 0,850	65,85 %	82,98 %
NO_3, μmol/l	≤12	0,669	0,593 to 0,739	54,47 %	70,21 %
NO_3+NO_2, μmol/l	≤20	0,808	0,741 to 0,865	71,54 %	76,60 %

The results of the ROC analysis showed more higher (AUC = 0.823) predictor value of endothelin-1, with cut off > 2.2 fmol/l sensitivity was 70.73% and specificity 97.87 %. The highest sensitivity of Se = 86.18% among the analyzed parameters was IL-6 (AUC = 0.872) with cut off >6.98 pg/ml. For the NO_2 level at cut off ≤7 μmol/l, the sensitivity was 65.85 % and the specificity was 82.98 %. The least quality models had levels NO_3 (AUC = 0.669) and hs-CRP (AUC = 0.655).

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 [5].

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 [6], [7].

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 [8]. According to a number of researchers dealing with this problem, the increased content of such proinflammatory cytokines contributes to the development of endothelial dysfunction [9], [10].

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 in blood plasma.

2. In patients with COPD, endothelial dysfunction occurs, characterized by an increase in the concentration of endothelin-1, and a decrease in nitric oxide metabolites in the blood plasma.

3. When pulmonary hypertension occurs in COPD patients, there is a further increase in the level of interleukin-6, whose level >6.98 pg/ml with a sen-

sitivity of 86.18 % is capable of the detection of pulmonary hypertension.

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Ethical Declaration. The study was carried out in conformity with the Declaration of Helsinki.The study was approved by the local ethics committee of State Institute «Zaporizhzhia Medical Academy of Postgraduate Education of Ministry of Health of Ukraine».

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