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АНАЛИЗ БИОМАРКЕРОВ ВОСПАЛЕНИЯ В КОНДЕНСАТЕ ВЫДЫХАЕМОГО ВОЗДУХА У ПАЦИЕНТОВ С ХОБЛ В СОЧЕТАНИИ С ОБЛИТЕРИРУЮЩИМ АТЕРОСКЛЕРОЗОМ АРТЕРИЙ НИЖНИХ КОНЕЧНОСТЕЙ

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Analysis of Inflammation Biomarkers in Exhaled Breath Condensate in Patients with COPD Combined with Peripheral Arterial Disease

Резюме

Актуальность. Хроническая обструктивная болезнь легких (ХОБЛ) является одной из наиболее значимых респираторных патологий, что связано с ее высокой распространенностью и влиянием на прогноз. Частота обострений и коморбидность — важные факторы, влияющие на течение ХОБЛ. Считается, что локальное и системное воспаление могут лежать в основе гетерогенного течения ХОБЛ. В этой связи оценка активности локального воспаления в дыхательных путях может быть полезна для оценки течения ХОБЛ. **Цель.** Изучить молекулярные механизмы ХОБЛ и оценить биомаркеры воспаления в конденсате выдыхаемого воздуха у пациентов с ХОБЛ с частыми обострениями в сочетании с периферическим атеросклерозом. **Материалы и методы.** Проведен биоинформационный анализ данных из Gene Expression Omnibus (GEO) с целью изучения генной онтологии дифференциально экспрессируемых генов при ХОБЛ. Далее проведено исследование провоспалительных цитокинов интерлейкина — 1 бета (interleukin (IL)-1β) и фактора некроза опухоли альфа (tumor necrosis factor alpha (TNFα)) в конденсате выдыхаемого воздуха (КВВ) у пациентов с ХОБЛ с частыми обострениями без сопутствующих атеросклеротических сердечно-сосудистых заболеваний (АССЗ) и у пациентов с ХОБЛ с частыми обострениями и облитерирующим атеросклерозом артерий нижних конечностей (ОААНК) в сравнении со здоровым контролем. **Результаты.** Дифференциально экспрессируемые гены вовлечены в биологические процессы и сигнальные пути по Киотской энциклопедии генов и геномов (Kyoto Encyclopedia of Genes and Genomes, KEGG пути), связанные с иммунным ответом, которые могут связывать развитие и прогрессирование ХОБЛ и атеросклероза. У пациентов с ХОБЛ в сочетании с атеросклерозом наблюдались более высокие значения IL-1β и TNFα в КВВ, по сравнению с контролем ($p < 0,001$). У пациентов с ХОБЛ с частыми обострениями и ОААНК были обнаружены наиболее высокие уровни IL-1β и TNFα в КВВ в сравнении с пациентами без АССЗ ($p=0,0038$ и $p=0,0005$ соответственно). **Вывод.** У пациентов с ХОБЛ с частыми обострениями и ОААНК повышены уровни TNFα и IL1β в КВВ, что может свидетельствовать о наличии локального воспаления в дыхательных путях, выраженность которого связана с клиническим течением ХОБЛ.

Ключевые слова: *ХОБЛ, воспаление, конденсат выдыхаемого воздуха, иммунная система, цитокины*

Конфликт интересов

Авторы заявляют, что данная работа, её тема, предмет и содержание не затрагивают конкурирующих интересов

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Abstract

Background. Chronic obstructive pulmonary disease (COPD) is one of the most significant diseases due to its high prevalence and impact on prognosis. The frequency of exacerbations and comorbidity are important factors influencing the course of COPD. It is believed that local and systemic inflammation may underlie this heterogeneous course of COPD. In this regard, assessment of local inflammation activity in the respiratory tract may be useful to assess the course of COPD. **Aim.** To study molecular mechanisms of COPD and assess inflammation biomarkers in the exhaled breath condensate (EBC) in patients with COPD with the phenotype of frequent exacerbations combined with peripheral atherosclerosis. **Materials and Methods.** Bioinformatic analysis of data from Gene Expression Omnibus (GEO) was performed to examine gene ontology of differentially expressed genes in COPD. Proinflammatory cytokines interleukin-1 beta (IL-1β) and tumor necrosis factor alpha (TNFα) in EBC in COPD patients without concomitant atherosclerotic cardiovascular disease (ASCVD) in the stable course phase, in patients with COPD with the phenotype of frequent exacerbations and peripheral artery disease (PAD) compared with healthy controls were examined. **Results.** Differentially expressed genes are involved in biological processes and signaling pathways according to the Kyoto Encyclopedia of Genes and Genomes (KEGG pathway) associated with the immune response that may link the development and progression of COPD and atherosclerosis. Patients with COPD combined with atherosclerosis had higher values of IL-1β and TNFα in EBC compared with controls (p <0.001). COPD patients with frequent exacerbations and PAD had the highest levels of IL-1β and TNFα in EBC compared with patients without ASCVD (p=0.0038 and p=0.0005, respectively). **Conclusion.** TNFα and IL1-β levels in EBC are elevated in COPD patients with frequent exacerbations and PAD, which may indicate the presence of local inflammation in the airways, the severity of which is associated with the clinical course of COPD.

Key words: *COPD, inflammation, exhaled breath condensate, immune system, cytokines*

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BODE — B — body mass index, O — obstruction, D — dyspnea (одышка), E — exercise tolerance, BP — Biological Processes, CC — Cellular Components, FDR — false discovery rate, GEO — The Gene Expression Omnibus, GO — GENE ONTOLOGY, GOLD — Global Initiative for Chronic Obstructive Lung Disease, IL-1β — Interleukin1β, KEGG — Kyoto Encyclopedia of Genes and Genomes, MCODE — Molecular Complex Detection, MF — Molecular Functions, mMRC –Modified Medical Research Council Dyspnea Scale, NCBI –The National Center for Biotechnology Information, PAD — peripheral artery disease, PPI — Protein-Protein Interaction, STRING — Search Tool for the Retrieval of Interacting Genes database, TLR– Toll-like receptor, TNFα — Tumor necrosis factor alpha, ACVD — atherosclerotic cardiovascular diseases, CI– confidence interval, CHD — coronary heart disease, EBC — expired breath condensate, LDLPs — low-density lipoproteins.; LLAOA — obliterating atherosclerosis of lower limb arteries, FEV_1 — forced expiratory volume per 1 second, COPD — chronic obstructive pulmonary disease

Introduction

The topicality of the problem of a combination of chronic obstructive pulmonary disease (COPD) and atherosclerotic cardiovascular diseases (ACVD) is caused by their high medical and social significance and economic burden both for patients and their families and for the healthcare in general. These diseases have high incidence and are among main causes of permanent disability and death [1]. Both conditions have several common risk factors, e.g., smoking and ageing, and are associated with a higher risk of unfavourable outcome. Therefore, it is essential to search for new diagnostic tools which will increase the efficiency of early diagnosis and management of such patients. Recently, the clinical significance of COPD and obliterating atherosclerosis of lower limb arteries (LLAOA) is of interest, since the patients with these diseases can have a higher mortality and morbidity risks. Studies show that patients with COPD and LLAOA are at a considerably higher risk of death compared to patients with any one of these conditions. It is assumed that a higher mortality risk is caused by general factors and the impact of these conditions on the cardiovascular system. Patients with COPD and LLAOA are also at a higher risk of hospitalisation and have lower quality of life. Understanding the clinical significance of both these conditions and their common risk factors is essential for development of an efficient management strategy and improved treatment [2]. It is worth mentioning that very

often GPs downplay the problem of COPD and LLAOA comorbidity and rather target other atherosclerosis locations, such as coronary heart disease.

The pathogenesis of atherosclerosis and COPD includes an array of various mechanisms with sophisticated regulation paths [3–5]. In the pathogenesis of both COPD and atherosclerosis, an important role is played by inflammation, the analysis of biomarkers of which is described in numerous papers [6]. COPD is known to affect smokers and is characterised by progressive disease caused by chronic airways inflammation. Various immune cells, such as macrophages and neutrophils, contribute to inflammation. They express an aray of chemokines and inflammatory cytokines that facilitate recruitment of new cells and make inflammation even worse [6]. In addition to a local bronchial inflammation, inflammation in COPD has also a system component underlying comorbid associations with other diseases, such as atherosclerosis [7]. The growing number of evidences contribute to the understanding that vascular wall inflammation is significant for atherogenesis. Endothelial cells, a monolayer of arterial walls, have a number of functions; they ensure a barrier and regulate vascular hemodynamics and other cells behaviour in vascular wall and blood flow [8,9]. Endothelial dysfunction is believed to be a key early event in atherogenesis [10]. Systemic inflammation commonly observed in COPD facilitates endothelial dysfunction, cell recruitment from the blood flow to the vascular wall, and atherosclerosis progression. Smoking-associated oxidative stress is another significant factor contributing to atherosclerosis in COPD [11]. Macrophages which differentiate in the vascular walls from recruited monocytes and macrophage progenitor cells in tissues participate in the intake of low-density lipoproteins (LDLPs), thus giving rise to in foam cells[12]. Macrophages in an atherosclerosis plaque have different polarisation, they are also of pro-inflammatory phenotype which is known to produce pro-inflammatory cytokines that facilitate inflammation [13].

Taking into account the role of airways inflammation in COPD, the information on the evaluation of inflammation markers in expired breath condensate (EBC) [14]can be of interest. Expired air is known to be saturated with evaporated water, which can condensate when cooled. Despite the fact that condensate consists primarily of evaporated water, it also contains aerosols of various particles from the lower respiratory tract. Thus, a local bronchial inflammation in COPD is a source of inflammatory agents in EBC. Inflammation biomarkers found in EBC can be clinically significant as they reflect local processes in bronchi and are a valuable source of diagnostic information [15].

EBC is a biological material obtained with the use of non-invasive methods, which contains a lot of various biomolecules, including cytokines, that can give an idea of the pathophysiology of airways inflammation [16]. EBC biomarkers were proposed as sensitive and specific indicators of pulmonary inflammation and oxidative stress and they can provide important information on the pathogenesis and clinical course of COPD. Also, such pro-inflammatory cytokines as tumour necrosis factor-α (TNFα) and interleukin (IL)-1β, were found in EBC of patients with COPD and can be associated with the nature of COPD [14]. It is assumed that the local inflammation intensity can be associated with systemic inflammation and development of comorbidities [6]. COPD exacerbations are caused by inflammation intensification and are an important COPD-affecting factor.

Thus, molecular mechanisms underlying COPD development and progression, as well as mechanisms that bind COPD and LLAOA are of huge research and clinical interest.

The objective of this study is to evaluate inflammation biomarkers in EBC of patients with frequent COPD and LLAOA.

Materials and Methods

Identification of molecular mechanisms of COPD pathogenesis

We analysed the GSE5058 data set, which includes the data on the gene expression levels in bronchial epithelium samples of patients with COPD received from The Gene Expression Omnibus (GEO), The National Center for Biotechnology Information (NCBI), by fiberoptic bronchoscopy from 12 healthy non-smokers and 6 smokers with COPD [17]. The data were obtained using GPL570 [HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array. The data were normalised using Mas5 normalisation. GEO is an international open repository archiving and circulating data on microchips, next generation sequencing and other forms of high-performance functional genomics data provided by the research community [18].

Differentially expressed genes were evaluated with the help of a bioinformatic analysis in experimental groups using limma package in Bioconductor, R (v. 4.0.2) [19]. For analysis, data were normalised including log2 transformation and quantile normalisation. In order to adjust the level of statistical significance for multiple comparisons, the algorithm by Benjamini & Hochberg (false discovery rate, FDR) was used. The conditions for the screening of differentially expressed genes were the absolute value of logFC >1 and p value of FDR ≤ 5 %.

Protein-protein interactions (PPIs) of protein products of common differentially expressed genes were evaluated using an online tool, Search Tool for the Retrieval

of Interacting Genes database (STRING) [20]. Correlations bewteen differentially expressed genes were analysed using Cytoscape Network Analyzer plug-in module [21]. Also, gene clusters in the PPI network were searched for using Molecular Complex Detection, MCODE v. 2.0.2 [22].

The most important genes in the network were identified using cytoHubba app in Cytoscape (v. 3.9.1) [23]. Cytoscape cytoHubba plug-in was used for node ranking in the network by their network properties. Main proteins were analysed, predicted, and visualised in molecular PPI networks using Maximal Clique Centrality (MCC) algorithm. Biological processes Gene Ontology (GO) and KEGG paths for the key genes in teh network were identified with the help of GEO2Enrichr [24], ShinyGO v0.741 [25] and g:Profiler; [26] gene ontology (GO) was analysed in accordance with their biological processes obtained from THE GENE ONTOLOGY RESOURCE [27]; signal paths were identified according to KEGG (Kyoto Encyclopedia of Genes and Genomes) [28,29] and Reactome database [30]; and their functional enrichment and visualisation were performed in Weishengxin. The p value of < 0.05 adjusted using the algorithm by Benjamini & Hochberg was set as a threshold for identification of biological processes and paths.

Clinical characteristics of study subjects

The following groups wer formed in accordance with the study objective: 20 patients with frequent COPD without ACVD (males, mean age: 60.55 (95 % CI 57.21; 63.89) ears); 20 patients with frequent COPD and LLAOA (males, mean age: 63.9 (95 % CI 61.54; 66.26) years; and controls: 20 healthy subjects (males, mean age: 62.85 (95 % CI 61.6; 64.1) years).

All subjects provided their informed consent for participation and met inclusion criteria (no exclusion criteria).

Inclusion criteria for group 1 (patients with COPD without ACVD):

1. Inactive COPD
2. No clinical manifestations of ACVD

Inclusion criteria for group 2 (patients with COPD and LLAOA):

1. Inactive COPD
2. Clinical manifestations of LLAOA

Inclusion criteria for controls (healthy subjects):

1. Absence of broncho-obstructive diseases
2. No clinical manifestations of ACVD

Exclusion criteria:

1. Acute infectious diseases
2. Acute coronary heart disease (CHD)
3. Renal and hepatic insufficiency
4. Tumours
5. Mental disorders
6. Constant administration of anti-inflammatory agents, system glucocorticosteroids
7. Bronchial asthma

COPD was diagnosed on the basis of clinical data, medical history, and spirometery results using Global Initiative for Chronic Obstructive Lung Disease (GOLD) [31] criteria. Disease exacerbation was assessed using the criteria developed by N. R. Anthonisen.[32] In accordance with the clinical recommendations for the management of chronic obstructive pulmonary disease approved by the Scientific and Practical Board at the Ministry of Health of the Russian Federation, frequent exacerbations occur at least twice a year. Patients enrolled in this study had 3 and more exacerbation a year. LLAOA was stage IIB according to the classification developed by A. V. Pokrovskiy–Fontaine and was diagnosed on the basis of clinical and ultrasound results in accordance with the National Recommendations for the Diagnosis and Management of Lower Limb Arterial Diseases [33].

Dyspnea was evaluated using mMRC scale (modified Medical Research Council Dyspnea Scale); comorbidities were evaluated with Charlson comorbidity index [34]. Also, the BODE index was calculated which is a multiple index comprising the following parameters: B — body mass index; O — obstruction; D — dyspnea; E — exercise tolerance [35].

Clinical characteristics of patients enrolled in the study are presented in Table 1.

EBC sampling

EBC was sampled using a portable R-Tube device (Respiratory Research, USA) in accordance with the manufacturer's method. Examination was perform before noon after a thorough mouth rinse with distilled water. EBC was sampled for 10 minutes using an RTube colled to -20˚C. Collected samples were placed into polypropylene tubes, frozen, and stored in a freezer at -80ºC until analysis.

Enzyme-linked immunosorbent assay

TNFα and IL-1β levels in EBC were measured using appropriate ELISA kits. Cytokine concentrations were used with Cloud-Clone Corp. kits (USA). High Sensitive ELISA Kit for TNFa and ELISA Kit for IL-1β had sensitivity of 0.52 pg/mL and 0.49 pg/mL, respectively.

Statistical data processing

Statistical data processing was performed using MedCalc (v. 20.1.4) and R packages (v. 4.2.2). Categorical data were compared between subgroups using chi square and continuous variables with the help of Student t-test or Mann–Whitney–Wilcoxon test, ANOVA or ANOVA

Table 1. *Characteristics of comparison groups*

Parameter	Control group (n=20)	Patients with COPD without ASCVD (n=20)	Patients with COPD and PAD (n=20)	p
Age, years	62,85 (95 % ДИ 61,6; 64,1)	60,55 (95 % ДИ 57,21; 63,89)	63,9 (95 % ДИ 61,54; 66,26)	$p^{1,2}$= 0,1877 $p^{1,3}$= 0,4344 $p^{2,3}$ =0,1033
Smokers	0	100 %	100 %	-
Pack-years index	0	35,8 (95 % ДИ 31,09; 40,51)	39,2 (95 % ДИ 35,4; 43)	p= 0,0861
FEV_1, % predicted	98,58 (95 % ДИ 97,82; 99,34)	44,96 (95 % ДИ 36,97; 52,96)	44,21 (95 % ДИ 38,28; 50,15)	$p^{1,2}$ <0,001 $p^{1,3}$ <0,001 $p^{2,3}$ =0,4516
Стадия ХОБЛ/ COPD stage	-	2,85 (95 % ДИ 2,54; 3,16)	3,0 (95 % ДИ 2,66; 3,34)	p= 0,4528
Dyspnea, MRC	0,65 (95 % ДИ 0,27; 1,03)	2,85 (95 % ДИ 2,47; 3,23)	3,15 (95 % ДИ 2,77; 3,53)	$p^{1,2}$ <0,001 $p^{1,3}$ <0,001 $p^{2,3}$ = 0,1339
Body mass index, kg/m^2	26,04 (95 % ДИ 25,04; 27,05)	28,15 (95 % ДИ 26,32; 29,98)	26,93 (95 % ДИ 25,44; 28,42)	$p^{1,2}$ =0,0231 $p^{1,3}$= 0,2706 $p^{2,3}$ =0,2706
Charlson Comorbidity Index	1,45 (95 % ДИ 0,8; 2,1)	4,6 (95 % ДИ 3,87; 5,33)	7,4 (95 % ДИ 6,37; 8,43)	$p^{1,2}$ <0,001 $p^{1,3}$ <0,001 $p^{2,3}$ <0,001
Arterial hypertension	9 (45 %)	11 (55 %)	19 (95 %)	$p^{1,2}$ =0,593 $p^{1,3}$ <0,001 $p^{2,3}$ =0,0027
Coronary artery disease	0	0	20 (100 %)	-
Chronic heart failure	0	3 (15 %)	10 (50 %)	$p^{2,3}$ <0,001
Total cholesterol, mmol/l	4,68 (95 % ДИ 4,47; 4,89)	4,83 (95 % ДИ 4,27; 5,39)	6,27 (95 % ДИ 5,81; 6,72)	$p^{1,2}$= 0,6142 $p^{1,3}$ <0,001 $p^{2,3}$ <0,001
LDL, mmol/l	2,7 (95 % ДИ 2,47; 2,93)	2,85 (95 % ДИ 2,49; 3,21)	3,96 (95 % ДИ 3,61; 4,31)	$p^{1,2}$ =0,5054 $p^{1,3}$ <0,001 $p^{2,3}$ <0,001
Blood glucose, mmol/l	4,43 (95 % ДИ 4,16; 4,7)	5,02 (95 % ДИ 4,59; 5,45)	6,14 (95 % ДИ 5,38; 6,9)	$p^{1,2}$= 0,0427 $p^{1,3}$ <0,001 $p^{2,3}$= 0,0093

Kruskal-Wallis test after evaluation of criteria for teh use of parametric tests. Differences were statistically significant at $p < 0.05$. Data were presented with 95 % confidence interval (CI) of the mean.

Ethical approval

The clinical part of the study was conducted in accordance with the ethical principles of the Good Clinical Practice, Declaration of Helsinki, medical regulations of the Russian Federation and was approved by the Local Ethics Committee at the Federal State Budgetary Educational Institution of Higher Education Ryazan State Medical University of the Ministry of Health of Russia. All patients received detailed information on participation in the study and consented to take part in the study.

Results

Identification of differentially expressed genes and analysis of their gene ontology

The biological information analysis made it possible to identify 1355 differentially expressed genes with higher expression and 370 differentially expressed genes with lower expression (Fig. 1).

The obtained differentially expressed genes with up-regulation were used to plot a protein-protein interactions network where MCODE plug-in was used to identify 35 clusters. Further analysis was performed using the most significant cluster from the common subnetwork with MCODE value of 16.857 and 36 nodes (**Fig. 2**). cytoHubba plug-in was used to identify the following gene concentrators (**рис. 3**) in the selected cluster.

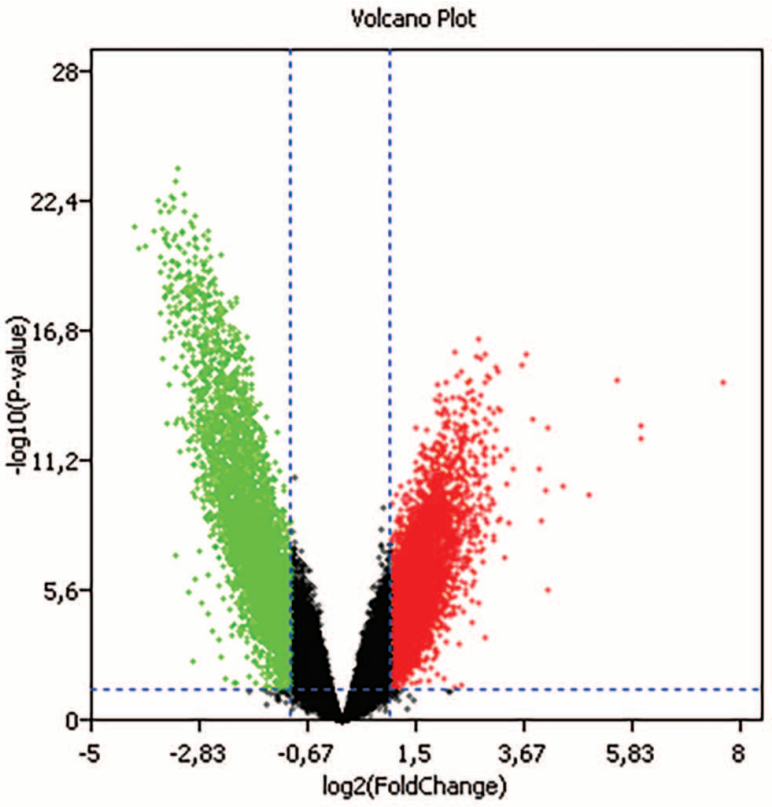

Figure 1. *Volcano plot characterizing differentially expressed genes*

Note: The colored dots in the graph indicate genes that show differential expression with | log2 FC |> 1 and p <0.05, while the black dots do not meet these criteria. Red dots indicate differentially expressed genes with up-regulation and are displayed on the right side of the graph, and green dots indicate differentially expressed genes with down-regulation (they are displayed on the opposite side)

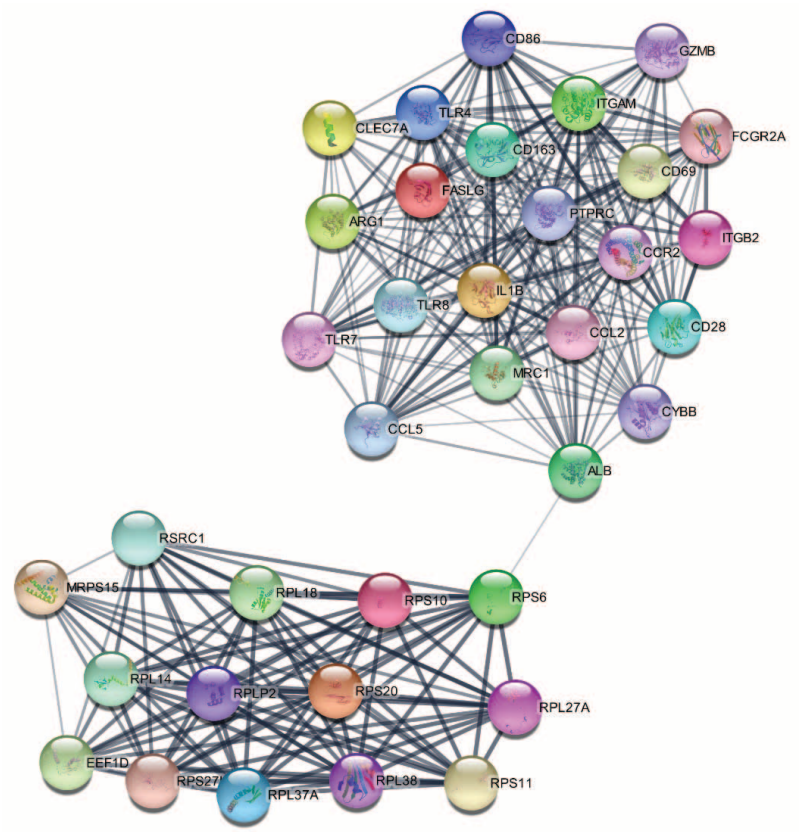

Figure 2. *Cluster 1 identified in a network of protein-protein interactions derived from differentially expressed genes in COPD*

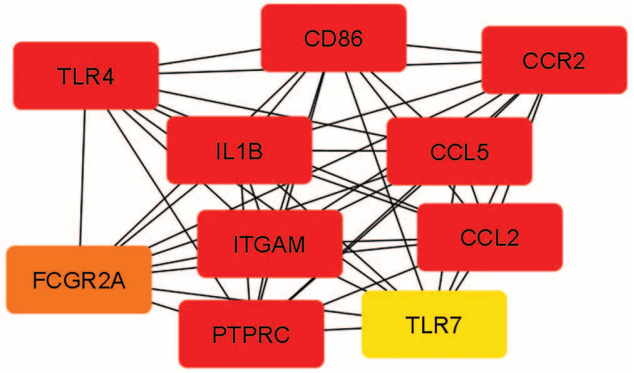

Figure 3. *The most important genes identified in the PPI network using the MCC algorithm in CytoHubba*
Note: The hub genes are ranked as follows: the most important genes are highlighted in red, the least important genes are highlighted in orange, and the least important genes are highlighted in yellow

The analysis of the functional enrichment in biological processes of identified gene concentrators in the modules was related primarily with the immune system (**Fig. 4**).

The analysis of the functional enrichment of these gene concentrators in KEGG paths was related primarily to the following: TLR receptor signal paths (hsa04620); TNF (hsa04668) signal path; interaction between cytokines and receptors (hsa04060) and between lipid signal path and atherosclerosis (hsa05417), characterising the presence of common molecular links of COPD with comorbidities (**Fig. 4**). Molecular functions of gene concentrators were related to cytokine activity, whereas functional gene enrichment in cell components was associated with cell plasma membrane.

Thus, the obtained data allowed identifying objectives for further experimental studies. It was found out that airway inflammation in COPD is characterised by innate immune system involvement with the help of cytokines, e.g., IL-1β and TNFα.

Results of EBC cytokine analysis

Study results demonstrated that COPD patients had higher IL-1β and TNFα levels in EBC vs. controls ($p < 0.001$). At the same time, the group of COPD patients with frequent exacerbations and LLAOA demonstrated higher TNFα levels vs. both controls and teh group of COPD patients without ACVD (**Fig. 5**).

EBC IL-1β levels demonstrated moderate correlation with the rate of exacerbations (r=0.612 (95 % CI 0.453; 0.733), $p < 0.0001$) and FEV1 values vs. expected values (r=-0.650 (95 % CI -0.761; -0.503), $p <0.0001$) and high correlation with BODE index (r=0.711 (95 % CI 0.582; 0.805), $p < 0.0001$). EBC TNFα levels had moderate correlation with the rate of exacerbations (r=0.557 (95 % CI 0.384; 0.692, $p < 0.0001$), FEV1 values vs. expected values (r=-0.646 (95 % CI -0.758; -0.497), $p < 0.0001$) and high correlation with BODE index (r=0.757 (95 % CI 0.645; 0.838), $p <0.0001$).

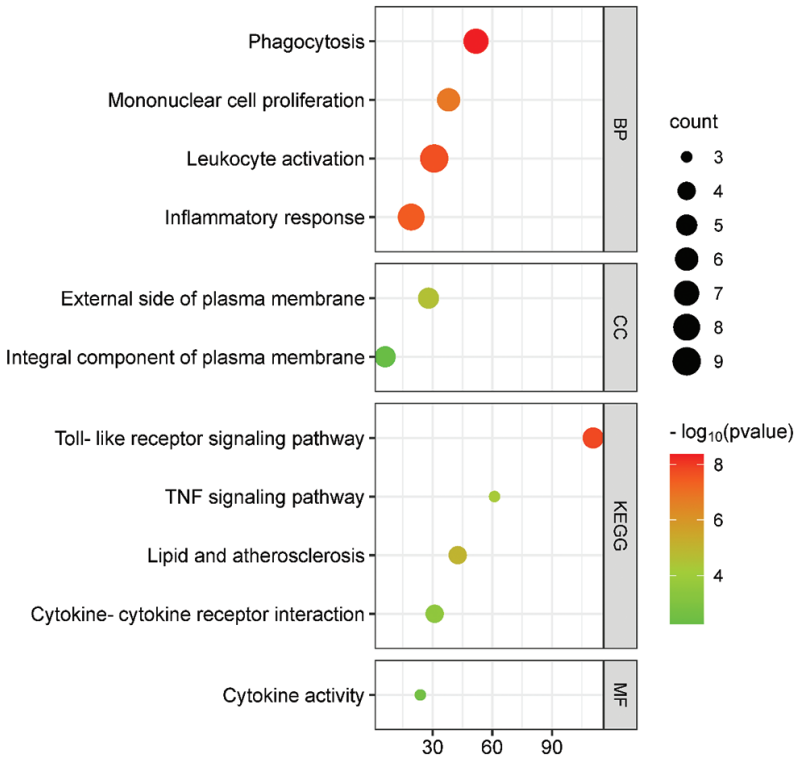

Figure 4. *Biological Processes (BP), KEGG Pathways (KEGG), Cellular Components (CC), and Molecular Functions (MF) in which Hub genes are involved. The data are ranked by Fold Enrichment values*

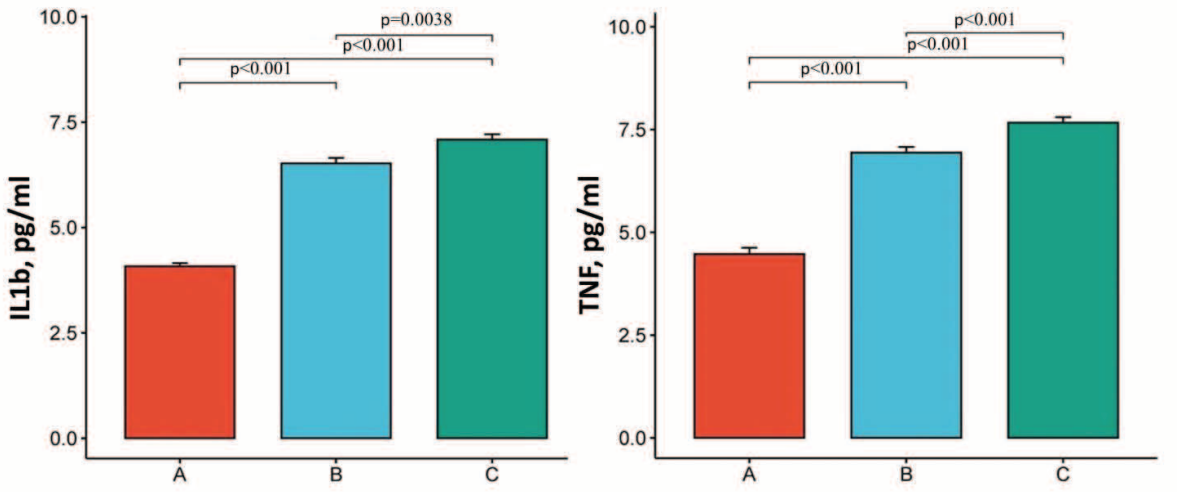

Figure 5. *Plot of changes in TNFα and IL-1β levels in the EBC in the comparison groups.*
Note: A — cytokine levels in the control group; B — cytokine levels in the group of COPD patients with frequent exacerbations without ASCVD; C -cytokine levels in the group of COPD patients with frequent exacerbations and PAD

Therefore, analysis of inflammation biomarkers in EBC showed that COPD is characterised with a marked local inflammation; COPD patients with LLAOA have higher inflammation biomarker levels, which can be related to the intensity of a system inflammation and more severe disease.

Discussion

In this study, we performed a biological information analysis of the data obtained from GEO, which allowed identifying signal paths involving differentially expressed genes with higher expression to airway epithelium in COPD patients vs. controls (non-smokers). Taking into account these data, we evaluated proinflammatory biomarkers in EBC of patients with heterogeneous COPD. The study enrolled patients with COPD without any clinically manifested ACVDs. Controls were subjects without COPD and LLAOA. The data for both groups were compared to the data on healthy volunteers without COPD and clinically manifested ACVDs, who did not smoke and were of a similar age to that of the study patients. COPD patients and controls had an expired breath condensate sample taken using RTube in accordance with the manufacturer's protocol for proinflammatory cytokine evaluation using ELISA.

It was found out that differentially expressed genes from bronchi endothelium in COPD were related to the innate immune system, namely to TLR, TNF signal paths, interaction with cytokines, such as IL-1β. COPD patients demonstrated higher TNFα and IL-1β levels in EBC vs. controls, potentially showing a local inflammation in COPD. At the same time, COPD patients with LLAOA had higher TNFα and IL-1β levels vs. both controls and COPD patients without ACVD. These data demonstrate that a local bronchial inflammation is involved in COPD pathogenesis and its clinically heterogeneous progression.

The data in this study improve the understanding of the role of inflammation in comorbid COPD progression. Previous studies demonstrated that COPD is associated with a higher risk of peripheral arterial diseases and mortality [36,37]. At the same time, immune biological processes and signal paths can connect the development and progression of COPD and atherosclerosis [38].

COPD is known to be a disease underlied by a chronic inflammation in airways associated with long-term exposure to tobacco smoke components. This inflammation involves numerous cells which express various cytokines. Inflammation in COPD has local and systemic components characterised by an increase in cytokine levels, including TNFα and IL-1β, which are associated with the disease severity [39, 40]. Inflammation intensity and cytokine production are higher in COPD exacerbations that are a significant clinical characteristic of the disease. It was demonstrated that COPD exacerbations caused a significant rise in IL-1β and TNFα levels in EBC vs. stable COPD [41]. A systemic inflammation and circulating cytokines are an important link between COPD and atherosclerosis, including LLAOA. At the same time, high cytokine levels can be potential biomarkers for forecasting atherosclerosis progression [42]. Recent studies demonstrated an association between COPD exacerbations and a higher risk of cardiovascular events. Prevention of exacerbations can reduce the risk of cardiovascular catastrophes at a later stage [43, 44]. Of note, even one moderate exacerbation in a COPD patient with a number of symptoms increases the riks of exacerbations and death within next three years [45].

IL-1β is an important cytokine of the innate immune system and is an important inflammation biomarker.

A growing number of proofs improves the understanding of the importance of this cytokine in COPD [46]. Besides, IL-1β is involved in atherosclerosis pathogenesis, as demonstrated in clinical studies of Canakinumab CANTOS (Canakinumab Antiinflammatory Thrombosis Outcome Study) [47]. IL-1β is produced by various cell types, including monocytes, macrophages and endothelial cells, in response to various stimuli, such as bacterial and viral infections, oxidative lipoproteins, and local hemodynamics disorders. It was demonstrated that IL-1β facilitates atherogenesis via a number of mechanisms, including pro-inflammatory gene induction, stimulation of reactive forms of oxygen and endothelial cell activation. IL-1β can activate and recruit immune cells in the inflammation site, including monocytes and T-cells, which contribute to atherosclerosis plaques [48]. There are evidences that IL-1β stimulates production of other pro-inflammatory cytokines, such as TNF-α and interleukin-6 (IL-6), which can boost the inflammatory response. In addition to its pro-inflammatory effect, IL-1β can directly facilitate atherosclerosis plaque development. Studies demonstrated that IL-1β can stimulate expression of adhesion molecules on endothelial cells, facilitating adhesion and migration of monocytes to the subendothelial space [49, 50].

TNFα is another pro-inflammatory cytokine with an array of functions and it demonstrates involvement in pathogenesis of various diseases. TNFα levels can be associated with muscle weakness caused by sarcopenia and cachexia in COPD patients [51]. TNFα is a cytokine which plays an important role in atherogenesis [52].

Therefore, a systemic inflammation is a significant factor contributing to atherosclerosis in COPD patients. At the same time, a high rate of exacerbations can be associated with an increase in the local and systemic inflammation in COPD, a mechanism of disease progression and a cardiovascular comorbidity [6].

A clinical assessment of inflammation biomarkers, such as IL-1β and TNFα, in COPD is possible with the use of available methods, including measurements of serum, induced sputum and EBC levels. Recently EBC has been of interest as a non-invasive tool for measuring biomarkers reflecting the course of chronic respiratory diseases [14]. Literature data show that EBC, which is mostly water from the expired air, contains a large number of dissolved substances, including numerous biologically active organic substances [16]. It was demonstrated that EBC can be used to diagnose and monitor the course of bronchial asthma and COPD [53, 54]. Our data demonstrated that EBC tests can be used to monitor COPD progression. A high frequency of COPD exacerbations is associated with a more severe local inflammation, biomarkers of which can be found in EBC. Overall, these studies show that EBC cytokine levels can be a useful biomarker of COPD severity; they can be used to monitor therapeutic interventions and manage patients with severe disease.

It is worth mentioning that this study has a number of limitations associated with a small sample size and participation of male patients only. Besides, this study did not include an analysis of other factors facilitating production of pro-inflammatory cytokines. On the other hand, this study emphasises the significance of inflammation in COPD and LLAOA comorbidity. For better understanding of comorbid relations, further studies are required which will include a more detailed multivariate analysis of clinical data making it possible to take into account the multifactor nature of pro-inflammatory cytokines. Perspective areas of future studies are analysis of inflammation biomarkers taking into account clinical heterogeneity of COPD, identification of comorbid relations between various inflammation endotypes in COPD.

Therefore, our results add to the data obtained during previous studies which demonstrated the role of a COPD-associated system inflammation in comorbid atherosclerosis. Our study provides an insight into the diagnostic value of biomarkers in expired breath condensate in order to assess COPD comorbidity. These results have high clinical value since they allow suggesting that system inflammation diagnosis and correction can be an important strategy for prevention and management of LLAOA progression in COPD patients. A comprehensive clinical and immunological assessment of COPD with an early identification of inflammation biomarkers and the use of adequate therapeutic strategies can be a perspective objective for disease improvement and forecast.

Conclusions

Therefore, IL-1β and TNFα levels were elevated in EBC of COPD patients; COPD with LLAOA was associated with a higher level of pro-inflammatory cytokines, thus evidencing the intensity of a local bronchial inflammation in COPD and comorbidity. The obtained data demonstrate the significant role of an inflammation in COPD and LLAOA comorbidity. It necessitates an increase in the quality of COPD exacerbation diagnostics and analysis of their clinical and immunological characteristics in COPD monitoring. Identification of immune biomarkers in expired breath condensate is an efficient non-invasive clinical tool for COPD monitoring.

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