



Correlation analysis between inflammatory biomarkers and significant clinical phenotypes of chronic obstructive pulmonary disease

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Abstract

Introduction: Chronic obstructive pulmonary disease (COPD) is the leading cause of mortality. Using the evidence obtained about various clinical phenotypes of patients with the same disease allowed us to expand our understanding of the treatment of COPD. Nowadays the only option for solving the problem will be the definition of the clinical phenotype of COPD, and the receipt of expanded data on its correlation with respiratory and other significant biomarkers.

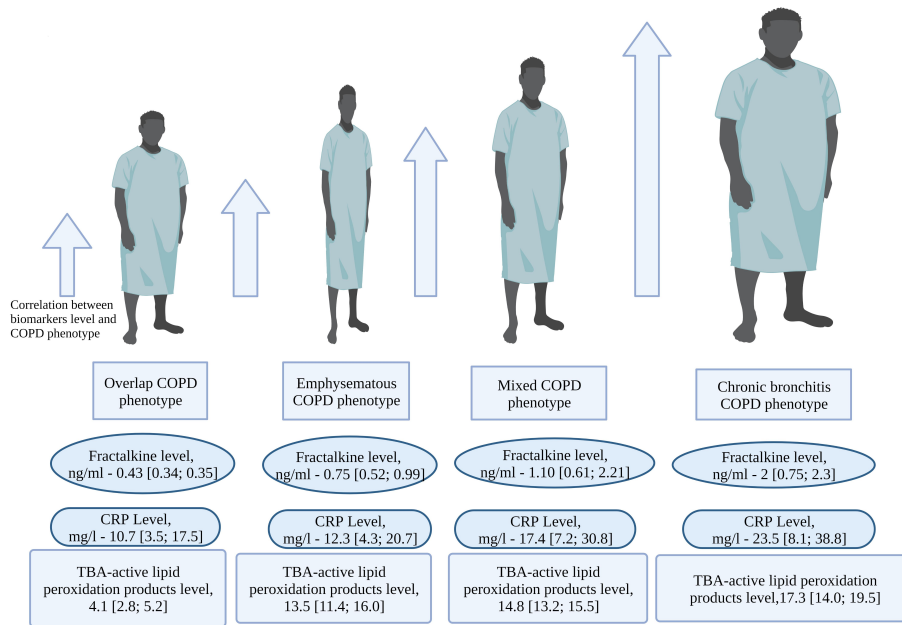
Materials and methods: We analyzed the correlations between FKN, CRP and TBA-active lipid peroxidation products in 373 patients with various COPD phenotypes and 60 healthy volunteers. Enzyme immunoassay was used to study the levels of inflammatory biomarkers.

Results: We have identified a statistically significant increase in the levels of inflammatory biomarkers in patients with COPD compared with the control. The FKN level in the group of patients with COPD was 1.3 ng/ml, which was higher ($p < 0.001$) than in the control (FKN level of 0.3 ng/ml, $p < 0.001$). The CRP level in patients with COPD was 27.8 mg/L, whereas in control the CRP level was 1.2 mg/L ($p < 0.001$). The TBA-active lipid peroxidation products level in patients with COPD was 14.5 mmol/L, which was higher when compared to the control ($p < 0.001$).

Discussion: The correlation analysis revealed very strong relationships between the levels of all the biomarkers studied. The highest values of the Kendall rank correlation coefficient (τ) were determined between the levels of all the inflammatory biomarkers in subgroups of patients with chronic bronchitis and mixed COPD phenotypes.

Conclusion: Detection of the COPD phenotype will help actively monitor the therapy of COPD exacerbations.

Graphical Abstract



Keywords

COPD, phenotyping, inflammation.

Introduction

Nowadays chronic obstructive pulmonary disease (COPD) is a worldwide medical and social problem and holds a special place among respiratory diseases (Agustí et al. 2020). COPD is a common disease and develops in 4–6% of men and 1–3% of women (Agrawal et al. 2019).

This rate increases significantly after the age of 40, reaching 11.8% in men and 8.5% in women (Casas Herrera et al. 2016). COPD is characterized by a progressive course with the inevitable addition of systemic effects that aggravate the patient's quality of life and reduce the expected effectiveness of the treatment (Fermont et al. 2019). Most scientific publications note the hypodiagnosics of COPD as the most common cause of the delay in the timely implementation of treatment and rehabilitation measures (Hassan et al. 2016; Khalil et al. 2019; Vogelmeier et al. 2020).

Over the past 10–15 years, data on the significant heterogeneity of the clinical picture of COPD have appeared in literature. It should be noted that using the evidence obtained about various clinical phenotypes of patients with the same disease allowed us to expand our understanding of the progression and treatment pathways of COPD. Unfortunately, in modern conditions, the above-mentioned clinical descriptions have not been able to fully reflect the heterogeneity of patients who are diagnosed with COPD. One of the solutions to this problem may be the maximum possible

definition of the clinical phenotype of COPD, and with it the receipt of expanded data on its correlation with informative respiratory and other significant biomarkers (Manian 2019; Corlateanu et al. 2020; Brat et al. 2021). A potential condition for such studies may also be a comprehensive study of some inflammatory biomarkers, such as: fractalkine (FKN), C-reactive protein (CRP) and thiobarbituric acid (TBA-active lipid peroxidation products), which are involved in the justification of the clinical phenotype of the patient and the prediction of the course of COPD (Hoffman et al. 2016; Karayama et al. 2019; Manian 2019).

Our aim was to study and analyze the relationships between the levels of FKN, CRP, and TBA-active lipid peroxidation products in patients with significant clinical COPD phenotypes.

Materials and methods

Study design

One-stage study

This study was conducted in the conditions of the therapeutic hospital of the Clinical City and lasted 3 years (April/12/2016–May/16/2018).

Patient Characteristics

73 patients with COPD exacerbation were selected into the main group, divided according to the clinical

phenotype of the disease into 4 subgroups: 98 patients (26.3%) had an emphysematous phenotype, 90 patients (24.1%) had a chronic bronchitis phenotype, 93 patients (24.9%) had a combination of COPD with bronchial asthma (BA) and 92 patients (24.7%) had a mixed phenotype. The definition of a mixed phenotype corresponded to “emphysematous+chronic bronchitis”. The combination of “COPD+BA” is presented by us as an overlap-phenotype. The control group was represented by 60 somatically healthy individuals comparable in age and sex to the main group. The median age of patients with COPD was 59 [45; 65] years, and in the somatically healthy group – 58 [44; 63] years ($p=0.079$). In the main group, a comparison by sex was found between men and women ($p=0.879$).

Criteria for inclusion: previously verified COPD of II-III stages, the age of patients under 65 and the presence of their informed voluntary consent form to participate in this study. The exclusion criteria included COPD of I and IV stages, the age of patients over 65 years, oncological and concomitant diseases in the acute phase. To formulate the diagnosis and to determine the severity of COPD, the Global Initiative for Chronic Obstructive Lung Disease (GOLD) program was used (Halpin et al. 2021).

The clinical symptoms of exacerbation of COPD were cough of varying degrees of dissipation, sputum production and shortness of breath; besides, the appearance and intensification of distance wheezing were also observed.

Enzyme immunoassay was used to study the levels of inflammatory biomarkers: FKN, CRP and TBA-active lipid peroxidation products on the first day of the COPD exacerbation. The content of FKN in the blood plasma was determined using a commercial test of the rayBio® Human Fractalkine system, from RayBiotech, Inc., USA. To determine the level of CRP, diagnostic kits from CRP (HS) Wide Range Multi-Calibrator Set HTI and High Technology Inc., USA, were used. The total determination of the content of TBA-active lipid peroxidation products was carried out using the diagnostic kits “TBK-AGAT” by Biokont Company, Moscow, Russia.

The study was carried out in accordance with the standards of Good Clinical Practice and the principles of the Helsinki Declaration.

This study was approved by the regional independent ethics committee (Minutes № 3, 18.10.2016). Written informed consent form was obtained from all persons examined to participate in the study.

Statistical analysis

For statistical processing of the data obtained, the program IBM SPSS Statistics 28.0.1.1 was used. In the study groups of patients, a different distribution of data from the normal one was revealed, which was the basis for the use of non-parametric criteria. For each

indicator, the median (Me) and percentiles [5-95] were calculated. For intergroup comparisons in three or more groups, the Kruskal-Wallis Test was used. In similar comparisons for two groups, we used the Mann-Whitney U Test. For correlation analyses, we used 2 methods: Spearman rank correlation coefficient (r) and Kendall rank correlation coefficient value (τ). The level of critical p -value was <0.05 .

Results

In 63.7% of patients older than 55 years in the exacerbation clinic, a more pronounced deterioration in the general condition and significant respiratory discomfort were detected. In the same age-related cohort, 66.1% of men with clinical and radiographic signs of emphysema of the lungs had shortness of breath, resulting in a noticeable restriction of daily activity and a decrease in exercise tolerance. In accordance with the visual analog scale MRC (Medical Research Council Scale), shortness of breath in these patients corresponded mainly to moderate severity. In the main group of patients, the indicators of FEV1, Tiffeneau-Pinelli index and body weight were reduced, which were 41 [30; 59], 55 [43; 67] and 24 [21; 27], respectively. With a duration of COPD of at least 17 years and the frequency of its exacerbations twice a year, there were tendencies to moderate leukocytosis and an increase in ESR, and the calculated smoking index, which reached 35.7 [20; 50] “pack/year”, confirmed the presence of an intensive smoking factor.

As Table 1 shows, all the studied biomarkers in patients with COPD were significantly increased and statistically significantly differed from those in the control group.

Table 1. Levels of studied biomarkers in main group and control group

Studied biomarker	Patients with COPD (main group), n=373	Control group, n=60
FKN, ng/ml	1.3 [0.38; 2.5] $p<0.001$	0.3 [0.28; 0.33]
CRP, mg/L	27.8 [3.5; 38.8] $p<0.001$	1.2 [0.5; 2]
TBA-active lipid peroxidation products, mcmol/L	14.5 [2.8; 20.6] $p<0.001$	2.3 [1.5; 3.5]

Note: FKN – fractalkine; CRP – C-reactive protein; COPD – chronic obstructive pulmonary disease.

The highest values of FKN were detected in the patients with the chronic bronchitis COPD phenotype. The biomarker level reached 2 ng/ml and was statistically significantly higher than in the patients with other COPD phenotypes ($p<0.001$) (Table 2). A similar

trend was again observed in the change of the CRP level in patients with chronic bronchitis COPD phenotype. The increase in CRP levels in this subgroup was statistically significant (23.5 mg/L ($p < 0.001$)). With exacerbation of COPD in this category of patients, there was also a significant increase of TBA-active lipid

peroxidation products (17.3 mmol/L). At the same time, the quantitative value of the studied indicator was statistically significantly higher than that in the patients with emphysematous, mixed and overlap phenotype ($p < 0.001$).

Table 2. Biomarkers level in patients with various COPD phenotypes

Studied biomarker	Overlap phenotype, n=93	Emphysematous phenotype, n=98	Mixed phenotype, n=92	Chronic bronchitis phenotype, n=90	Kruskal-Wallis Test
FKN, ng/ml	0.43 [0.34; 0.55]	0.75 [0.52; 0.99] $p_1 < 0.001$	1.10 [0.61; 2.21] $p_1 < 0.001$ $p_2 < 0.001$	2 [0.75; 2.3] $p_1 < 0.001$ $p_2 < 0.001$ $p_3 < 0.001$	$\chi^2 = 229.48$; df=3; $p < 0.001$
CRP, mg/L	10.7 [3.5; 17.5]	12.3 [4.3; 20.7] $p_1 = 0.003$	17.4 [7.2; 30.8] $p_1 < 0.001$ $p_2 < 0.001$	23.5 [8.1; 38.8] $p_1 < 0.001$ $p_2 < 0.001$ $p_3 < 0.001$	$\chi^2 = 98.14$; df=3; $p < 0.001$
TBA-active lipid peroxidation products	4.1 [2.8; 5.2]	13.5 [11.4; 16.0] $p_1 < 0.001$	14.8 [13.2; 15.5] $p_1 < 0.001$ $p_2 < 0.001$	17.3 [14.0; 19.5] $p_1 < 0.001$ $p_2 < 0.001$ $p_3 < 0.001$	$\chi^2 = 209.43$; df=3; $p < 0.001$

Note: FKN – fractalkine; CRP – C-reactive protein; COPD – chronic obstructive pulmonary disease.

The next challenge was to study the relationships between the levels of FKN, CRP and TBA-active lipid peroxidation products in the COPD patients. As can be seen from Table 3, the results of the correlation analysis indicate that there is a very strong positive relationship between the levels of FKN and CRP. We found a strong positive correlation between the levels of FKN and TBA-active lipid peroxidation products and a strong positive correlation between the levels of CRP and TBA-active lipid peroxidation products (Table 3).

Table 3. Correlations between biomarkers level in COPD patients

Biomarkers	Main group, n=373
FKN and CRP	$r = 0.88$, $p < 0.001$
FKN and TBA-active lipid peroxidation products	$r = 0.93$, $p < 0.001$
CRP and TBA-active lipid peroxidation products	$r = 0.82$, $p < 0.001$

Note: FKN – fractalkine; CRP – C-reactive protein; COPD – chronic obstructive pulmonary disease.

Correlation analysis showed a strong positive relationship between the levels of FKN and CRP, and it follows that a quantitative change in the level of FKN causes an indirect change in the CRP level. In this case, a common reason for increasing biomarkers level was an exacerbation of COPD.

In the final phase of the study, it seemed important to assess clinically significant changes in the levels of inflammatory biomarkers corresponding to a particular COPD phenotype. According to the results of the correlation analysis, the presence of statistically significant relationships between the levels of FKN,

CRP and TBA-active lipid peroxidation products, and all COPD phenotypes were revealed (Table 4).

We found a strong positive relationship between the FKN level in patients with chronic bronchitis and mixed COPD phenotypes. In patients with emphysematous COPD phenotype with the level of this marker, an average positive correlation was revealed. The presence of a weak positive relationship with the level of FKN was identified in the patients with overlap phenotype. In a subgroup of patients with chronic bronchitis and mixed COPD phenotypes with a quantitative value of CRP, an average positive relationship was established. At the same time, a statistically significant relationship of weak force was revealed between the CRP level, emphysematous and overlap phenotypes.

Discussion

The study presents the results of studying the correlations between the levels of inflammatory biomarkers in patients with clinical significant COPD phenotypes. To improve diagnostic capabilities of determining the phenotype of the disease, we selected COPD patients exclusively in the acute phase. The necessity of such randomization of patients in the real-time hospital phase stems from the poorly studied genetic basis of COPD in Russia and abroad. It should be noted that the relevance of this problem has been mentioned in different years in the world experience of scientific and clinical practice (Casas Herrera et al. 2016; Fermont et al. 2019; Agustí et al. 2020). Various studies of recent years have also noted low informational value of inflammatory biomarkers in

Table 4. Kendall rank correlation coefficient value (τ) between levels of inflammatory biomarkers and clinical COPD phenotypes

Biomarker/COPD phenotype	Overlap phenotype, n=93	Emphysematous phenotype, n=98	Mixed phenotype, n=92	Chronic bronchitis phenotype, n=90
FKN, ng/ml	$\tau=0.33$; $p<0.001$	$\tau=0.51$; $p<0.001$	$\tau=0.75$; $p<0.001$	$\tau=0.85$; $p<0.001$
CRP, mg/L	$\tau=0.30$; $p<0.001$	$\tau=0.48$; $p<0.001$	$\tau=0.55$; $p<0.001$	$\tau=0.67$; $p<0.001$
TBA-active lipid peroxidation products	$\tau=0.35$; $p<0.001$	$\tau=0.55$; $p<0.001$	$\tau=0.63$; $p<0.001$	$\tau=0.76$; $p<0.001$

Note: FKN –fractalkine; CRP – C-reactive protein; COPD – chronic obstructive pulmonary disease.

stable COPD course (Agrawal et al. 2019; Vogelmeier et al. 2020). Given the multidimensionality of the state of the problem, the logistics of our study initially provided for preferential treatment of such a key condition as achievement of maximum possibility to verify the disease phenotype. To optimize the results obtained, at the first stage of the study we created subgroups of COPD patients formed with regard to clinically determined phenotypes. Subsequent phenotype identification and supplementation of biochemical characteristics of the COPD exacerbation that had occurred were carried out by a combined study of selected inflammatory biomarkers. In the present study, the unidirectional character of an increase in quantitative indices of FKN, CRP, and TBA-active lipid peroxidation products at the beginning of COPD exacerbation was accentuated. The studied inflammatory markers clarify the characteristic of the acute phase of the inflammatory process. At the same time, different responses of FKN, CRP and TBA-active lipid peroxidation products levels in patients of the main group contribute to verification not only of causal relationship, but also of COPD exacerbation severity.

The discussion of the obtained data allows the authors to make a correct statement of some of their opinions. Thus, the increased level of FKN reflects the involvement of this chemokine in the development of pulmonary inflammation, and the increased quantitative value of CRP during exacerbation of COPD indicates the presence of significant prevalence of the inflammatory process. A high level of TBA-active lipid peroxidation products in the bloodstream of a patient is caused by initiation of oxidative damage cascade of the body. The obtained result coincides with the opinion of other researchers about the initiation of lipid peroxidation as the leading link in the pathogenesis of COPD (Manian 2019; Brat et al. 2021). It is necessary to note the clinical significance of increased levels of inflammatory biomarkers increased in blood serum in the majority of patients with chronic bronchitis COPD

phenotype. This judgment creates a good clinical perspective, as it is guaranteed by the reliability of the results of complex study and clear differentiation of quantitative indices.

Conclusion

As a result of the study, in all patients with COPD, compared with the control group, a statistically significant increase in the reference levels of FKN, CRP and TBA-active lipid peroxidation products were revealed, reflecting their representation as markers of the inflammatory process. The highest quantitative values of FKN, CRP and TBA-active lipid peroxidation products were revealed in patients with chronic bronchitis COPD phenotype. At the same time, all the above biomarkers significantly exceeded those with emphysematous, mixed and overlap phenotypes. Correlation analysis also confirmed the existence of a very strong positive relationship between the levels of FKN, CRP and TBA-active lipid peroxidation products. A strong positive relationship was established with the FKN level in subgroups of patients with chronic bronchitis and mixed phenotypes, and also a strong relationship with the biomarker of TBA-active lipid peroxidation products is fixed only with the chronic bronchitis COPD phenotype. In subgroups of patients with chronic bronchitis, mixed and emphysematous COPD phenotypes with a quantitative value of FKN and CRP, an average positive relationship was established. However, a weak positive relationship with the levels of FKN, CRP and TBA-active lipid peroxidation products was found in patients with emphysematous and overlap phenotypes.

Conflict of interests

The authors declare no conflict of interest.

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