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# SYSTEMIC INFLAMMATORY PROFILE IN PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE

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**Abstract**

Chronic obstructive pulmonary disease (COPD) is heterogeneous condition with various phenotypes that have their own pathogenetic mechanisms and certain inflammatory mediators, as C-reactive protein, interleukins, circulating leukocytes. Uncovering the inflammatory profile may identify disease biomarkers. We aimed to compare the values of systemic inflammatory parameters in patients with different clinical phenotypes and determine their correlation with clinical parameters.

In 30 COPD patients we analyzed demographic and clinical data, history of allergies, cigarette smoking and history of exacerbations. We phenotyped them into non-exacerbator, exacerbator and COPD with asthma phenotype. COPD assessment test, modified dyspnea scale and the BODE (Body mass index, Obstruction, Dyspnea, Exercise capacity) index were calculated. Spirometry and lung X-ray were performed. Peripheral blood was taken for analysis of inflammatory parameters.

There were 16 patients (53.33%) with phenotype of non-exacerbator, and 7 (23.33%) with exacerbator and COPD with asthma phenotype each. COPD assessment test had significantly lowest value in non-exacerbator and modified dyspnea scale significantly highest value in exacerbator phenotype. There were no mild grade patients in exacerbator, and no very severe grade in nonexacerbator phenotype. C-reactive protein and interleukin 8 had significantly lowest value in non-exacerbator; leucocytes significantly highest value in exacerbator; eosinophyls and interleukin 4 significantly highest value in COPD with asthma phenotype. There was no significant difference among the three phenotypes in neutrophyls and interleukin 18.

The three clinical phenotypes: non-exacerbator, exacerbator and COPD with asthma have their own specific clinical and inflammatory features that have clinical, prognostic and therapeutic implications.

***Keywords:*** COPD, inflammatory parameters, phenotypes.

## Introduction

Chronic obstructive pulmonary disease (COPD) is a common disease characterized by persistent respiratory symptoms and airflow limitation due to abnormalities of the airways and alveoli, caused by exposure to harmful particles or gases and influenced by host factors, which can be prevented and treated [1].

Inhaling cigarette smoke or other harmful particles causes lung inflammation [2].

In the airways, lung parenchyma and pulmonary blood vessels, the number of macrophages, activated neutrophils and lymphocytes (Tc1, Th1, Th17, ILC3) increases. There may also be an increase in eosinophils, Th2 or ILC2. Inflammatory cells, along with epithelial cells and other structural cells, release multiple inflammatory mediators that attract inflammatory cells from the circulation (chemotactic factors), amplify the inflammatory process (proinflammatory cytokines) and induce structural changes (growth factors) [3].

The inflammatory process is transferred to the systemic circulation, which is seen in increased values of acute phase proteins, circulating cytokines, chemokines or as abnormalities in circulating cells (eg.leukocytes). Systemic inflammation is thought to be associated with disease severity, with

accelerated decline in function, higher mortality and more frequent exacerbations, and with increased risk of cardiovascular disease, diabetes, lung cancer, and pneumonia [4].

Several inflammatory parameters stand out from the inflammatory profile of COPD. C reactive protein (CRP) is an acute-phase protein that is elevated in patients with COPD, particularly during acute infectious exacerbations. In stable COPD, the concentration of CRP in the plasma is associated with total mortality, also with a progressive decline in lung function. CRP is produced in the liver in response to circulating interleukin 6, its concentration increases in plasma, and therefore may be a biomarker of systemic inflammation [5, 6].

Interleukin 4 (Il-4) is a pleotropic cytokine. It mainly promotes the proliferation of T cells and stimulates the production of antibodies by B cells. It can also stimulate the proliferation, differentiation and activation of fibroblasts, endothelial and epithelial cells and increases the recruitment of inflammatory cells [7].

Interleukin 6 (Il-6) is increased in patients with COPD, especially during exacerbations. It is a significant pro-inflammatory cytokine and a potent stimulator of acute phase proteins such as CRP. It is involved in the systemic characteristics of COPD and the worsening of comorbid conditions [8].

Interleukin 8 (Il-8) plays an important role in the activation of neutrophils and monocytes in patients with COPD. Its concentration is significantly elevated in the sputum of patients and correlates with the severity of the disease. It has also been found elevated in serum of exacerbation prone COPD patients [9].

Interleukin 18 (Il-18) is a proinflammatory cytokine with key host defense and antitumor activities. It induces a wide variety of inflammatory and remodeling responses in the lung and also mixed type 1, type 2 and type 17 cytokine responses [10].

COPD is a heterogeneous condition with various phenotypes representing subgroups of patients with common characteristics that have clinically significant outcomes. Widely accepted COPD phenotypes are non-exacerbator (NE), exacerbator (AE), and COPD with asthma phenotype (C/A).

Each of these subgroups has its own pathogenetic mechanisms, and therefore certain systemic inflammatory mediators. Uncovering the systemic inflammatory profile in individual phenotypes may help identify biomarkers of the disease, which will aid in early diagnosis, phenotyping, and a personalized approach to therapy [11,12].

The aim of this study was to determine the values of systemic inflammatory parameters: CRP, total leukocytes, neutrophils and eosinophils, interleukin 4, interleukin 6, interleukin 8 and interleukin 18 in patients with COPD, to compare their values in patients with different clinical phenotypes and to determine their correlation with clinical parameters.

## Material and methods

This cross sectional study was conducted at the University Clinic for Pulmonology and Allergology in Skopje in the period from June 1, 2020 to May 31, 2021. The research sample consisted of 30 COPD patients diagnosed according to the current version of the 2021 Global Initiative for Chronic Obstructive Lung Disease (GOLD), in a stable phase of the disease (> 4 weeks without exacerbation), over 18 years of age [1].

We did not include patients with an acute exacerbation, pneumonia or other clinically manifest infection four weeks before the inclusion, patients with other clinically significant lung diseases: cystic fibrosis, tuberculosis, pulmonary fibrosis, lung cancer, as well as neuromuscular diseases, neoplastic diseases, immunological disorders and systemic diseases.

In all patients, we made medical interview and review of the medical documentation for demographic and clinical data, history of allergies, and cigarette smoking (smoker was considered any patient who had smoked more than 100 cigarettes in his life) [13] and a history of exacerbations.

We included only the moderate and severe exacerbations, which represent deteriorations in the condition that require additional treatment in outpatient or hospital setting [1].

The presence of chronic bronchitis (CB) was considered the presence of cough with sputum for a period of at least 3 months per year for two consecutive years [14].

We assessed the symptoms using COPD Assessment Test (CAT) composed of 8 questions, with a total score of up to 40, with a higher score indicating more severe symptoms [15]; and the dyspnea with the modified British Medical Research Council dyspnea scale (mMRC -modified Medical Research

Council Dyspnea scale) on a scale of 0 to 4 [16]. As a predictor of mortality risk of COPD, we used the BODE (Body mass index, Obstruction, Dyspnea, Exercise capacity) index, which is a complex measure of body mass index (BMI), degree of obstruction, degree of dyspnea and physical capacity expressed through the 6-minute walking test [17].

Spirometry was performed using standard methodology, we expressed the forced expiratory volume in the first second (FEV1) after inhalation of a bronchodilator (200 μg salbutamol) as a % of the predicted value [18].

Presence of emphysema was determined by lung radiography in posteroanterior direction and left profile [19].

Patients were divided into 3 clinical phenotypes. A non-exacerbator (NE) phenotype was defined by the presence of at most one moderate and no severe exacerbation in the last 12 months [20]. Exacerbator phenotype (AE) was defined by the presence of two or more moderate or at least one severe exacerbation with hospital treatment in the last year [21].

A COPD with asthma phenotype was defined in patients meeting criteria for COPD and with a previous diagnosis of asthma or a positive methacholine test, positive allergy history, or positive bronchodilator reversibility; regardless of the number of exacerbations [22].

Leukocytes (Le) and leukocyte formula with eosinophils (Eo) and neutrophils (Ne) were measured in venous blood on an automated hematology analyzer. The value of CRP was determined on a biochemical analyzer ROCHE Hitachi C501, by turbidimetric method. Luminex technology was used to determine the concentration of interleukin 4, 6, 8 and 18 [23].

Participation in the study was voluntary with a signed informed consent from the subjects preceded by a detailed description of the research and a statement on the anonymity of the data and their use exclusively for scientific purposes.

The data was statistically processed using the SPSS software package, version 20.0 for Windows (SPSS, Chicago, IL, USA). Qualitative series were analyzed by determining relationship coefficients, proportions and rates. Quantitative series were analyzed with measures of central tendency (mean, median, 25% and 75% percentiles, minimum and maximum values), as well as measures of dispersion (standard deviation).

Shapiro-Wilk W test was used to determine the normality of the frequency distribution of the studied variables. Fisher Feeman Halton test was used to determine the association between certain attributive features.

Pearson Correlation was used to determine the association between inflammatory and clinical variables.

One Way ANOVA and Kruskal-Wallis H test were used to test the significance of the difference between certain numerical parameters with correct or irregular frequency distribution.

Difference test was used to compare the proportions. A two-sided analysis with a significance level of $p<0.05$ was used to determine statistical significance.

**Results**

The sample consisted of a total of 30 patients with COPD of which 8 (26.67%) were women and 22 (73.33%) were men, with a gender ratio of 0.36:1, and a significantly higher percentage of men compared to female gender - Difference 46.7% ((21.51 – 64.32) 95% CI); p=0.0003.

The mean age in the sample was 64.13±7.23, with min/max 50/78 years and 50% patients older than 64 years. The average age for women was 62.62±6.25, with min/max 56/74 years, and for men 64.68±7.62, with min/max 50/78 years.

Over 62 years of age were 50% of women for Median IQR=62.5 (57-66), and over 64.5 years of age were 50% of men for Median IQR=64.5 (60-69).

There was no significant difference between the two sexes in terms of age (Mann-Whitney U Test: Z=0.750); p=0.4530.

**Table 1a.** Analysis according to phenotypes and selected clinical parameters

| Clinical parameters | Mean±SD | Min/Max | Median [IQR] | p |
|---|---|---|---|---|
| **FEV 1 [%]** | | | | |
| **NE** | 52,37±17,04 | 30/ 83 | 48,5 [36,5-63] | F=0,498; df=2; p=0,6151 |
| **AE** | 44,43±18,53 | 23/ 73 | 43 [23-62] | |
| **C/A** | 48,28±19,93 | 22/ 75 | 46 [31-70] | |
| **Total** | 49,57±17,73 | 22/ 83 | 46,5 [36-63] | |
| **CAT** | | | | |
| **NE** | 12,62±5,78 | 3/ 21 | 13,5 [8-17] | F=3,158; df=2; p=0,0486* |
| **AE** | 19,01±5,67 | 9/ 25 | 19 [15-24] | |
| **C/A** | 16,86±6,82 | 9/ 26 | 19 [9-22] | |
| **Total** | 15,11±6,41 | 3/ 26 | 16 [9-20] | |
| **mMRC** | | | | |
| **NE** | 1,56±0,73 | 0/ 3 | 2 [1-2] | $X^2$ [2]=5,618; p=0,0463* |
| **AE** | 2,57±0,97 | 1/ 4 | 3 [2-3] | |
| **C/A** | 1,57±0,97 | 0/ 3 | 2 [1-2] | |
| **Total** | 1,80±0,92 | 0/ 4 | 2 [1-2] | |
| **BODE** | | | | |
| **NE** | 2,69±2,06 | 0/ 7 | 2,5 [1-4,5] | $X^2$ [2]=2,514; p=0,2845 |
| **AE** | 4,29±2,29 | 1/ 7 | 4 [2-7] | |
| **C/A** | 2,71±2,43 | 0/ 6 | 3 [0-5] | |
| **Total** | 3,07±2,23 | 0/ 7 | 3 [0-5] | |

NE=non-exacerbator phenotype; AE= exacerbator phenotype ; C/A=COPD with asthma
F-One way ANOVA Kruskal-Wallis H test * significant p<0,05

There were 16 patients (53.33%) with the clinical phenotype of NE, and 7 (23.33%) patients with AE and C/A each.

A significantly higher association of the female gender with the clinical phenotype C/A was determined, that is, a significantly higher association of the male gender with the clinical phenotypes NE and AE (Pearson Chi-square test=9.363; df = 2; p=0.0093).

6 (20%) of the patients had a history of allergies, 1 (6.25%), 5 (71.43%) in the C/A group, and none in the AE group.

The analysis of the three phenotypes (NE, AE and C/A) according to selected clinical parameters indicated a non-significant difference in relation to FEV1 (p=0.6151) and BODE (p=0.2845) and a significant difference in relation to CAT (p =0.0486) in addition to the significantly lowest value in NE, as well as in relation to mMRC (p=0.0463) in addition to the significantly highest value in AE (Table 1a).

**Table 1b.** Analysis according to phenotypes and selected clinical parameters

| Clinical parameters | NE | AE | C/A | p |
|---|---|---|---|---|
| **GOLD** | | | | |
| **GOLD 1** | 1 [6,25%] | 0 [0%] | 0 [0%] | - |
| **GOLD 2** | 6 [37,50%] | 2 [28,57%] | 3 [42,86%] | |
| **GOLD 3** | 9 [56,25%] | 3 [42,86%] | 3 [42,86%] | |
| **GOLD 4** | 0 [0%] | 2 [28,57%] | 1 [14,29%] | |
| **CB** | | | | |
| **no** | 7 [43,75%] | 2 [28,57%] | 1 [14,29%] | p=0,3687 |
| **yes** | 9 [56,25%] | 5 [71,43%] | 6 [85,71%] | |
| **Emphysema** | | | | |
| **no** | 6 [75,50%] | 1 [14,29%] | 1 [14,29%] | p=0,3574 |
| **yes** | 10 [62,50%] | 6 [85,71%] | 6 [85,71%] | |
| NE=non-exacerbator phenotype; AE= exacerbator phenotype ; C/A=COPD with asthma<br>Fisher Freeman Halton test * significant p<0,05 | | | | |

Additionally, the analysis of NE, AE and C/A phenotypes in relation to GOLD class indicated that: a) GOLD 4 was absent in NE, and GOLD 3 - 9 (56.25%) was the most prevalent; b) AE was absent in GOLD 1, and the most prevalent was GOLD 3 - 3 (42.86%); and c) C/A GOLD 1 was absent, and GOLD 2 and GOLD 3 - 3 were equally represented (42.86%).

There was no significant association of any of the three phenotypes (NE, AE and X/A) with the presence of chronic bronchitis (p=0.3687) and with the presence of emphysema (p=0.3574) (Table 1b).

The analysis of the phenotypes NE, AE and C/A in relation to selected inflammatory parameters indicated a significant difference in relation to: a) CRP (p=0.0499) with significantly lower value in NE; b) Le (p=0.0460) with a significantly highest value in AE; c) Eo (p=0.0015) with significantly highest value in C/A; d) Il-4 (p=0.0051) with a significantly highest value in C/A; and e) Il-8 (p=0.0166) with a significantly lowest value in NE.

There was no significant difference between the three phenotypes (NE, AE and C/A) in terms of Ne and Il-18. At this stage, no Il-6 was detected in any of the research subjects (Table 2).

**Table 2.** Analysis according to phenotypes and selected inflammatory parameters

| Inflammatory parameters | Mean±SD | Min/Max | Median [IQR] | p |
|---|---|---|---|---|
| **CRP [mg/L]** | | | | |
| **NE** | 3,36±2,32 | 0,5/ 8 | 2,3 [1,5-5,8] | $X^2$ [2]=5,954; p=0,0499* |
| **AE** | 8,31±5,23 | 1,9/ 15 | 8,4 [2,1-14] | |
| **C/A** | 6,90±4,97 | 1/ 17 | 6 [4,6-8] | |
| **Total** | 5,34±4,97 | 0,5/ 17 | 4,7 [4,6-6,8] | |
| **Le** x$10^9$/L | | | | |
| **NE** | 7,32±1,90 | 4,3/10,2 | 7,1 [5,6-8,9] | F=3,128; df=2; p=0,0460* |
| **AE** | 9,56±2,62 | 12/ 6,6 | 10,8 [6,6-11,9] | |
| **C/A** | 7,81±1,32 | 9,5/ 6,3 | 7,9 [6,3-9,1] | |
| **Total** | 7,56±2,12 | 12/ 6,3 | 7,9 [6,3-9,5] | |
| **Ne** x$10^9$/L | | | | |
| **NE** | 4,69±1,59 | 2,6/ 6,9 | 4,5 [3,3-6,0] | F=1,471; df=2; p=0,2476 |
| **AE** | 5,89±1,85 | 2,6/ 7,6 | 6,6 [4,2-7,2] | |
| **C/A** | 5,00±0,91 | 3,8/ 6,2 | 5,3 [4,0-5,7] | |
| **Total** | 5,04±1,56 | 2,6/ 7,6 | 5,4 [3,6-6,2] | |
| **Eo** x$10^9$/L | | | | |
| **NE** | 0,86±1,08 | 01/ 4,5 | 0,6 [0,1-0,9] | $X^2$ [2]=12,973; p=0,0015* |
| **AE** | 0,50±0,28 | 0,1/ 1,0 | 0,5 [0,3-0,6] | |
| **C/A** | 2,00±0,39 | 1,3/ 2,5 | 2,1 [1,7-2,3] | |
| **Total** | 1,04±0,98 | 0,1/ 4,5 | 0,7 [0,4 [1,7] | |
| **Il-4** | | | | |
| **NE** | 0,31±0,51 | 0/ 1,16 | 0 [0-0,8] | Z=-2,806; p=0,0051* |
| **AE** | 0,00±0,00 | 0/ 0 | 0 [0-0] | |
| **C/A** | 1,16±0,00 | 1,16/ 1,16 | 1,16 [1,16-1,16] | |
| **Total** | 0,44±0,56 | 0/ 1,16 | 0 [0-1,16] | |
| **Il-6** | | | | |
| **NE** | 0,00 | 0,00 | 0,00 | - |
| **AE** | 0,00 | 0,00 | 0,00 | |
| **C/A** | 0,00 | 0,00 | 0,00 | |
| **Total** | 0,00 | 0,00 | 0,00 | |
| **Il-8** | | | | |
| **NE** | 16,81±8,98 | 2,2/ 36,24 | 15,4 [11,6-22,7] | $X^2$ [2]=8,194; p=0,0166* |
| **AE** | 29,78±13,17 | 19,2/ 53,8 | 24,4 [20,7-42,3] | |
| **C/A** | 30,84±16,69 | 20,4/ 66,7 | 22,7 [20,9-35,9] | |
| **Total** | 28,37±30,68 | 2,2/ 174,6 | 20,9 [15,4-26,9] | |
| **Il-18** | | | | |
| **NE** | 201,76±84,88 | 34,9/ 354,9 | 186,6 [155,9-254,7] | F=2,023; df=2; p=0,1519 |
| **AE** | 280,56±155,2 | 118/ 552,2 | 240,6 [134,6-418,5] | |
| **C/A** | 274,66±76,33 | 164/ 361,1 | 293,6 [208,9-354,9] | |
| **Total** | 237,15±106,78 | 34,9/ 552,2 | 216,9 [164,0-315,2 | |

NE=non-exacerbator phenotype; AE= exacerbator phenotype ; C/A=COPD with asthmaF-One way ANOVA $X^2$=- Kruskal-Wallis H test Z=Mann-Whitney U Test
* significant p<0,05

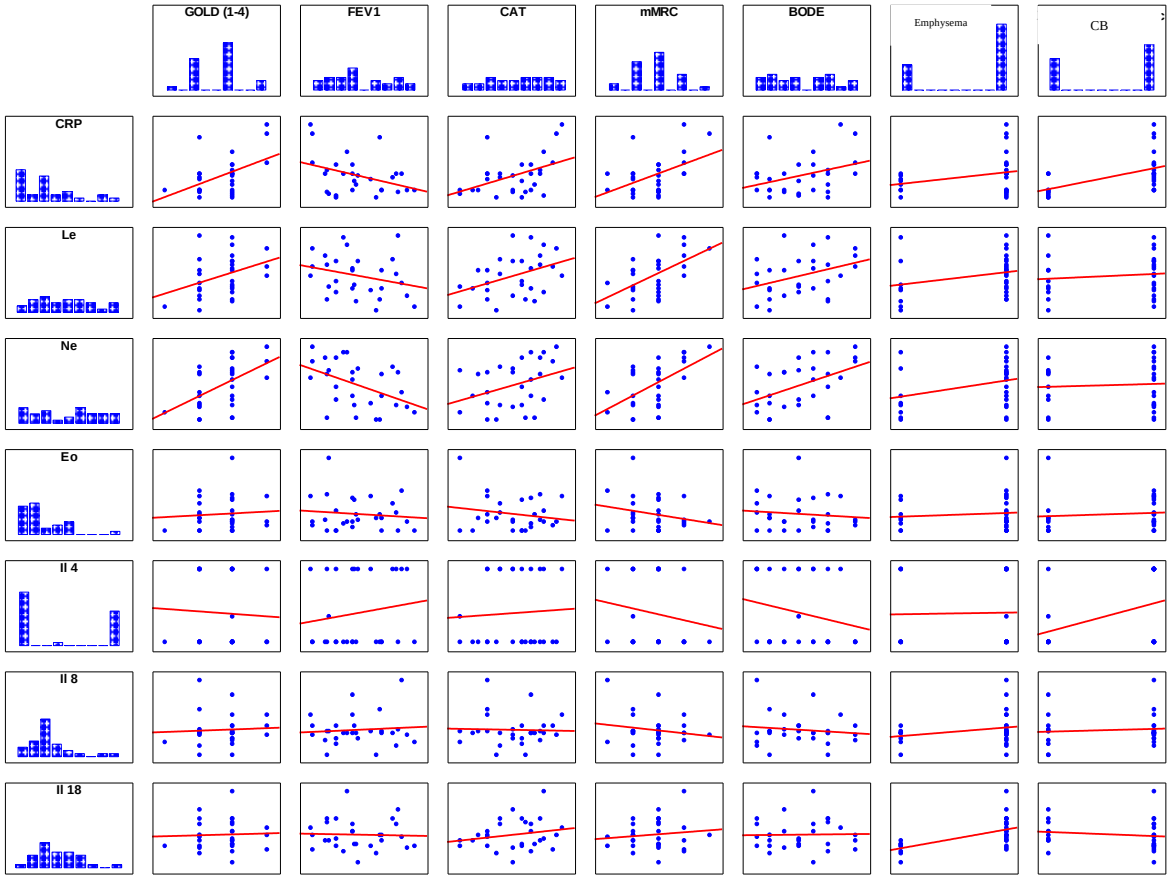
For the entire sample it was observed for: a) CRP - a significant linear moderate/strong positive correlation between CRP and GOLD, CAT, mMRC and chronic bronchitis, as well as a significant linear moderate negative correlation between CRP and CAT.

The value of CRP increased with increasing GOLD class, CAT, mMRC as well as with the presence of chronic bronchitis, and decreased with increasing FEV1; b) Le - significant linear moderate/strong positive correlation between Le and GOLD, CAT, mMRC and BODE. As these clinical parameters grew, so did the value of Le; c) Ne - significant linear moderate/strong positive correlation between Ne and GOLD, mMRC and BODE, as well as significant linear moderate negative correlation between Ne and FEV1.

The value of Ne increased with increasing GOLD class, mMRC and BODE, and decreased with increasing FEV1; d) Il-4 - significant linear moderate positive correlation between Il-4 and chronic bronchitis and with the presence of chronic bronchitis, the level of Il- 4 increased significantly; and e) ) Il-18- significant linear strong positive correlation between Il-18and emphysema and with the presence of emphysema, the level of Il-18increased significantly. Correlations between other inflammatory and clinical parameters did not show significance (Table 3 and Graph 1);

**Table 3.** Correlation between selected inflammatory and clinical parameters

| Inflam-matory | Clinical | | | | | | |
|---|---|---|---|---|---|---|---|
| | GOLD | FEV1 | CAT | mMRC | BODE | Emphysema | CB |
| CRP | $r_{[30]}$=0,469; p=0,010* | $r_{[30]}$=-0,368; p=0,049* | $r_{[30]}$=0,443; p=0,016* | $r_{[30]}$=0,463; p=0,011* | $r_{[30]}$=0,352; p=0,061 | $r_{[30]}$=0,277; p=0,145 | $r_{[30]}$=0,532; p=0,003* |
| Le | $r_{[30]}$=0,371; p=0,048* | $r_{[30]}$=-0,273; p=0,152 | $r_{[30]}$=0,411; p=0,027* | $r_{[30]}$=0,563; p=0,001* | $r_{[30]}$=0,368; p=0,050* | $r_{[30]}$=0,281; p=0,142 | $r_{[30]}$=0,115; p=0,554 |
| Ne | $r_{[30]}$=0,512; p=0,005* | $r_{[30]}$=-0,464; p=0,011* | $r_{[30]}$=0,361; p=0,054 | $r_{[30]}$=0,556; p=0,002* | $r_{[30]}$=0,464; p=0,001* | $r_{[30]}$=0,367; p=0,084 | $r_{[30]}$=0,061; p=0,751 |
| Eo | $r_{[30]}$=0,078; p=0,686 | $r_{[30]}$=-0,111; p=0,566 | $r_{[30]}$=-0,190; p=0,324 | $r_{[30]}$=-0,234; p=0,223 | $r_{[30]}$=-0,112; p=0,564 | $r_{[30]}$=0,102; p=0,598 | $r_{[30]}$=0,098; p=0,613 |
| Il-4 | $r_{[30]}$=-0,049; p=0,800 | $r_{[30]}$=0,155; p=0,421 | $r_{[30]}$=0,058; p=0,766 | $r_{[30]}$=-0,155; p=0,421 | $r_{[30]}$=-0,215; p=0,263 | $r_{[30]}$=0,021; p=0,914 | $r_{[30]}$=0,388; p=0,038* |
| Il-8 | $r_{[30]}$=0,058; p=0,765 | $r_{[30]}$=0,090; p=0,642 | $r_{[30]}$=-0,034; p=0,861 | $r_{[30]}$=-0,171; p=0,374 | $r_{[30]}$=-0,126; p=0,514 | $r_{[30]}$=0,255; p=0,182 | $r_{[30]}$=0,087; p=0,655 |
| Il-18 | $r_{[30]}$=0,043; p=0,823 | $r_{[30]}$=-0,037; p=0,848 | $r_{[30]}$=0,209; p=0,277 | $r_{[30]}$=0,124; p=0,521 | $r_{[30]}$=0,022; p=0,910 | $r_{[30]}$=0,577; p=0,001* | $r_{[30]}$=-0,134; p=0,489 |
| *significant p<0,05 | | | | | | | |




**Graph 1.** Correlation between selected inflammatory and clinical parameters

**Discussion**

The obtained results show a predominance of the non-exacerbator phenotype of 53.33%, and an equally lower representation of the frequent exacerbator phenotype and the COPD with Asthma phenotype with 23.33% each. The results of this study are consistent with previous studies phenotyping clinically relevant COPD phenotypes. Back in 2014, Vestbo was among the first to propose a division into 9 phenotypes [24].

Then in the first cluster analysis by Weatherall et al. 5 clinical phenotypes were distinguished [25].

The concept of phenotyping was developed by obtaining new data from large studies such as ECLIPSE [26] and resulted in the inclusion of the clinical phenotypes: non-exacerbator, asthma-COPD overlap (ACO), exacerbator with emphysema and exacerbator with bronchitis in the Spanish national GesEPOC recommendations and their wide acceptance in research and clinical purposes [22].

The extensive analysis of Miravitlles et al. showed the following distribution: 60.6% non-exacerbators, 15.9% COPD/Asthma patients (C/A), 19.3% chronic bronchitis exacerbators and 4.3% non-chronic bronchitis exacerbators. Patients with COPD /Asthma were more often women with better lung function [27].

In our subjects, a significant association of the female gender with the clinical phenotype C/A was also determined and a significantly higher association of the male gender with AE. Of the patients with the C/A phenotype, 71% had a history of allergies.

From the clinical parameters, CAT stood out with a significantly lowest value in NE and mMRC with a significantly highest value in AE. The CAT is considered an accurate and reliable measure of health-related quality of life in patients with COPD [28].

Dyspnea is the most common symptom in patients with COPD and the mMRC scale is commonly used to assess it [29].

The GOLD document recommends the use of both CAT and mMRC to assess symptoms [1]. Previous studies in Europe and beyond have shown that the frequent exacerbator phenotype is associated with high CAT and mMRC values, studies by Cosio et al. in Spain [30], Corlateanu et al. in Romania [31], Chai et al. in Malaysia [32] and several others.

Our study contributed to the available literature data indicating that patients with less than one exacerbation per year have fewer symptoms and less impact of the disease on daily life, while patients with frequent exacerbations have the greatest functional impairment due to dyspnea. The BODE index is considered a better predictor of COPD mortality than its components individually, and has been shown to have a higher value in certain phenotypes in several large studies. In the study by Golpe et al. in Spain the acute exacerbator phenotype showed a significantly higher risk of death [33], in the COPDGene study the combination of predominant emphysema and predominant airway disease was associated with the highest risk of all-cause mortality [34].

In this study, the values of the BODE index did not show a significant difference between the different phenotypes, which may be due to the small sample and the fact that we did not perform additional subanalysis according to the other clinical parameters.

Regarding the severity of obstruction expressed by postbronchodilator FEV1, the results showed no significant difference between the phenotypes. Although some studies show a milder degree of COPD in non-exacerbators and the most severe in frequent exacerbaters [30], larger trials phenotyping COPD patients have not found a strong association between phenotype and severity of obstruction. This is also reflected in the current GOLD recommendations, where the severity of the obstruction is not a decisive factor in the treatment and prognosis of the disease [1].

Yet the results of the distribution of GOLD classes by phenotype showed that in AE there is no mild degree (GOLD 1), while in NE there is no very severe degree (GOLD 4) of COPD.

No significant association of any of the phenotypes with the presence of chronic bronchitis or emphysema was determined. This was an expected result according to the available literature.

Several studies have shown elevated CRP values in COPD patients compared to healthy subjects and its association with FEV1, GOLD disease stage and BODE index [35,36], but there are fewer investigations of phenotypic differences in its values [37].

Our results provide further evidence that patients with an AE phenotype have elevated CRP values and in stable disease as an indicator of systemic inflammation.

We also showed that the value of CRP increased with increasing GOLD class, CAT, mMRC as well as with the presence of chronic bronchitis, and decreased with increasing FEV1. Peripheral blood leukocytosis has been investigated as a possible inflammatory biomarker for COPD, but has not shown a definitive correlation with decline in lung function [38], risk of acute exacerbation and mortality [39].

In our patients, peripheral leukocytosis is a significant parameter for AE phenotype. We also found a strong positive correlation between Le and GOLD, CAT, mMRC and BODE; and positive correlation between Ne and GOLD, mMRC and BODE. Elevated concentrations of eosinophils and their proinflammatory factors (including Il-4) have been found in the airways and blood of COPD patients, indicating that eosinophils actively contribute to the inflammatory process in these patients [40].

Although the presence of elevated sputum eosinophils has been noted as a common feature of the COPD and Asthma phenotype, no consistent recommendations have been made, and some clinical studies include eosinophils as part of the definition of C/A, but not as a requirement [1].

Elevated concentrations of eosinophils and their proinflammatory factors (including Il-4) have been found in the airways and blood of COPD patients, indicating that eosinophils actively contribute to the inflammatory process in these patients [40].

The results of our research showed a significantly higher value of Eo and Il-4 in C/A, which confirms the data in the literature on eosinophilic inflammation and appoints it as a potential therapeutic target. In patients with chronic bronchitis of all phenotypes there was a significant increase in Il-4 values, a finding that confirms the research of Zhu et al. to increase bronchial plasma cells that release Il-4 and stimulate mucus hyperproduction. Agusti et al., identified six inflammatory biomarkers (including Il-8)

associated with systemic inflammation and reported that patients with COPD and elevated biomarkers are at high risk for increased all-cause mortality and frequent exacerbations. and during the three-year follow-up [41].

Our results are in agreement with published literature data of significantly higher Il-8 values in AE and C/A phenotypes. Studies provide strong evidence for the involvement of Il-8 in the pathogenesis of COPD [10]. Clinically, significantly higher serum levels of Il-8 have been found in patients with COPD and smokers. Serum levels of Il-8 in patients with GOLD stage III and IV were also found to be significantly higher [42].

Studies in experimental life models have shown that overexpression of Il-18 results in emphysematous lesions in mice [43].

In this research, we found a strong positive correlation between Il-18 and the presence of emphysema that supports the results of experimental studies.

A weakness of this research is that it is a cross-sectional study aimed at estimating the prevalence of predefined phenotypes, without being able to validate these phenotypes prospectively based on outcomes. Also, the small sample may affect the statistical significance of the results. However, it is of great value as the first study to perform phenotyping in our COPD patients and the first comprehensive assessment of their clinical and inflammatory parameters.

**Conclusion**

In conclusion, the three clinical phenotypes: non-exacerbator phenotype, exacerbator phenotype and COPD with asthma phenotype have their own specific clinical and inflammatory characteristics that have both clinical, prognostic and therapeutic implications.

The non-exacerbator phenotype is the most common, more common in males, patients are distinguished by fewer symptoms than the rest and less severe and very severe forms of the disease. They have lower values of most systemic inflammatory parameters, which indicates less local and systemic inflammation. The exacerbator phenotype is also more common in males, with highly symptomatic disease and a higher proportion of patients with severe and very severe disease.

The systemic inflammatory profile in frequent exacerbators in the stable phase is characterized by elevated CRP, Il-8 and total Le, while Il-6 can be considered a biomarker of acute exacerbation.

The phenotype of COPD and asthma is more common in women, with the highest percentage of allergies history. Their systemic inflammatory profile is dominated by elevated eosinophils and Il-4.

The positive correlation between selected inflammatory and clinical parameters provides additional data that may help to elucidate pathogenetic mechanisms and determine potential therapeutic targets that would personalize the approach to the patient.

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