„LOW-GRADE SYSTEMIC INFLAMMATION IN PATIENTS WITH STABLE CHRONIC OBSTRUCTIVE PULMONARY DISEASE"

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Summary

Introduction: Chronic obstructive pulmonary disease (COPD) is a multicomponent disease with extrapulmonary effects. Systemic aspects of COPD include oxidative stress and altered circulating levels of inflammatory mediators and acute-phase proteins. Airflow limitation is associated with an abnormal inflammatory response mainly initiated by smoke inhalation. Even though chronic inflammation is a characteristic phenomenon of the disease, so far little is known about underlying pathogenetic mechanisms.

Aim: To evaluate circulating C-reactive protein (CRP) level as a biomarker of systemic inflammation, leukocyte count, lipid profile and smoking exposure in patients with stable COPD and their correlation with the severity of the disease.

Material and methods: Cross sectional study was conducted at 60 patients with COPD (age 40-75) and 30 subjects from general population without COPD, matched by age, gender and body mass index. All patients underwent laboratory testing and pulmonary function tests. The severity level in patients with COPD was determined according to GOLD (Global Initiative for Chronic Obstructive Lung Disease) criteria.

Results: We found statistically significant difference between mean serum CRP level in stable COPD than control group (10.2 vs. 5.9, P = 0.04, P< 0.05). The Pearson correlation between leukocytes count and CRP value in stable COPD patients, compared to control group, showed statistically significant correlation (r=0.358, P=0.005, P < 0.01). According to lipid profile, comparison was made between mean values of total cholesterol, triglycerides, low-density lipoprotein (LDL) and high-density lipoprotein (HDL) in both groups, but statistically significant difference was not found. Number of patients with leukocyte count >109/L was significantly higher in stable COPD than control group (45% vs. 26.7%, P= 0.01, P < 0.05). The degree of airflow limitation in COPD patients was significantly related to smoking exposure expressed by number of pack-years (Brinkman Index), Pearson correlation, (r= -0.525, P=0.000, P < 0.01), as well as to the serum CRP level (r= -0.324, P=0.012, P < 0.05).

Conclusion: The present study confirms that circulating CRP levels and total leukocyte count are higher in stable COPD patients. Serum CRP may be regarded as a valid biomarker of low-grade systemic inflammation which is the leading point to atherosclerosis.

Keywords: *COPD, low-grade systemic inflammation, C-reactive protein.*

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a multicomponent disease with extrapulmonary effects like: cardiovascular disease (CVD), anemia, polycythemia, malnutrition, muscle disorder, osteoporosis, metabolic syndrome, diabetes, gastroesophageal reflux, anxiety, depression, hormonal imbalance, infections, lung cancer, thrombosis (1-5). COPD is the fourth most common cause of death, after myocardial infarction, malignant diseases and cerebrovascular incidents

(2). Extrapulmonary manifestations are much more common in patients with COPD compared to smokers without COPD, suggesting that COPD is an independent risk factor for these manifestations (6,7). In Lung Health Study performed at 6000 smokers in 2000, it was found that FEV1 (forced expiratory volume in 1st second) reduction of 10%, increases the risk of fatal cardiovascular events (arrhythmias, heart failure, cerebrovascular ulcer, thromboembolism, cardiac death) by 28% and 20% for non-fatal coronary events between subjects with mild to moderate COPD (8). The pathophysiological mechanism as COPD increases the risk of CVD is

still an underdeveloped field that will be studied in future (4). Cigarettes are a common risk factor for both diseases, but there are other predictors such as inflammation, oxidative stress, disrupted gas exchange leading to hypoxia, altered vascular biology, endothelial dysfunction, accelerated aging, prosthesis / antiprotease imbalance (9,10). In the analysis of 14 relevant studies, Gan et al. demonstrated that the level of systemic inflammatory markers such as: total number of leukocytes, C-reactive protein, interleukin 6 and fibrinogen are increased in patients with COPD compared to smokers without COPD, even in mild degree of obstruction (FEV1 50-80%) (9,11). Elevated CRP level is present in stable COPD and in exacerbations (12). Systemic inflammation increases with the severity of the disease and is associated with an increased mortality (13-15). Oxidative stress is present in COPD and in ischemic heart disease. Free oxygen radicals cause atherosclerosis through: proliferation of vascular smooth muscle, endothelial apoptosis, lipid oxidation, activation of matrix metalloproteinases (MMP) and altered vasomotor activity (9). Carotid artery disease as a risk factor for cerebrovascular incidents is considered one of the cardiovascular complications of COPD, because of the persistence of chronic, low-grade, systemic inflammation (16).

AIM

1. To evaluate circulating CRP level as a biomarker of systemic inflammation in patients with stable COPD and its correlation with the severity of the disease;
2. To determine the correlation between degree of airflow limitation in COPD patients and smoking exposure expressed by number of pack-years;
3. To compare dependence between leukocyte count and lipid profile and airflow limitation;

MATERIAL AND METHODS

Study design and setting
Cross sectional study, performed at the General Hospital „8-th September", Skopje, Macedonia, approved by the Ethics Committee of the Medical Faculty at University "Sts.Cyril and Methodius".

Study subjects
Study population included 60 patients with COPD diagnosed according to the actual Global Initiative for Chronic Obstructive Lung Disease (GOLD) criteria and 30 subjects without COPD matched to the COPD group by sex, age, body mass index (BMI), and smoking status. All enrolled subjects gave their written informed consent before entering the study.

Inclusion criteria for Investigated Group
Both genders, age 40-80 years, diagnosed COPD according the actual GOLD (Global Initiative for Chronic Obstructive Lung Disease) criteria, and history of current or former smoking (equal or more than 10 pack-years), clinically stable condition at least 6 weeks prior to involvement.

Exclusion criteria for Investigated Group
Age less than 40 years and more than 80 years, patients with other chronic respiratory diseases (asthma, bronchiectasis, active tuberculosis, sarcoidosis, lung carcinoma, pulmonary fibrosis, sleep apnea syndrome), body mass index (BMI) >35kg/m^2, diabetes mellitus; valvular heart disease; congenital heart disease, electrolyte imbalance, hepatic, renal failure, anemia, muscular skeletal disease, patients with immunosuppressive therapy, acute infectious disease, patients who do not agree to participate.

Inclusion criteria for the Control Group
Age over 40-80 years, smoking history >= 10 pack-years, current or former smokers, normal spirometry, stable clinical condition, without significant difference in sex, age, BMI and signed consent for participation.

Methods

All patients underwent laboratory testing and pulmonary function tests. The severity level in patients with COPD was determined according to GOLD (Global Initiative for Chronic Obstructive Lung Disease) criteria.

The BMI as a measure of body fat based on height and weight that applies to adult population was determined in all study subjects by computed calculation using BMI calculator (17).

Classification of smoking status was done by the World Health Organization (WHO) recommendations Brinkman Index as a clinical quantification of cigarette smoking is

used to measure a person's exposure to <u>tobacco</u>, and is calculated as number of pack-years = (number of cigarettes smoked per day / 20) × number of years smoked (18).

Pulmonary evaluation

Pulmonary evaluation included: dyspnea severity assessment, baseline and post-bronchodilator spirometry, arterial gas analysis and chest X-ray (in order to exclude respiratory disease other than COPD).

Assessment of the severity of dyspnea according to the Medical Research Council (MRC) Dyspnea Scale: I degree - I feel hunger for air only with a strong effort, II degree - I feel hunger for air when I rush flat or when I climb a mild uphill; III degree - I go slower than people of the same age on flat because of difficulty breathing or I need to stop to get bored when I go to my room on a flat; IV degree - I stop to go out after walking 100 meters or after a few minutes walking on a flat; V degree - I breathe too hard to leave my home (19).

The baseline spirometry, including measures of forced vital capacity (FVC), forced expiratory volume in one second (FEV_1), FEV_1/FVC, and maximal expiratory flow at 75%, 50%, 25%, and 25-75% of FVC (MEF_{75}, MEF_{50}, MEF_{25}, and MEF_{25-75}, respectively), was performed in all subjects using electronic spirometer Spirobank G USB Spirometer (Medical International Research, Roma, Italy) with recording the best result from three measurements the values of FEV_1 of which were within 5% of each other. The results of spirometry were expressed as percentages of the predicted values according to the actual recommendations of European Respiratory Society (ERS) and ATS (20).

Bronchodilator test was performed by spirometric measurements before and 20 minutes after administration of 400 μg salbutamol by metered dose inhaler through spacer. Post-bronchodilator value of the FEV_1/FVC ratio less than 0.70 indicated persistent airflow limitation (20).

According to the actual GOLD recommendations, COPD was considered by finding of a post-bronchodilator FEV_1/FVC ratio less than 0.70 in symptomatic subjects (dyspnea, chronic cough and/or sputum production) with a history of

exposure to risk factors for the diseases (noxious particles and gases). In addition, according to the FEV_1 value, airflow limitation in the subjects with COPD, i.e. severity of the disease, was classified as mild (FEV_1 value higher than 80% of the predicted value), moderate (FEV_1 value higher than 50% but lower than 80% of the predicted value), severe (FEV_1 value higher than 30% but lower than 50% of the predicted value), and very severe (FEV_1 value lower than 30% of the predicted value) (1).

Gas analysis was performed with SIEMENS RAPIDPOINT 405 System (Siemens Healthineers, Australia).

Laboratory evaluation

Analysis of venous blood taken in the morning after a minimum of eight hours of night starvation): sedimentation rate, complete blood count, biochemistry with lipid status - total cholesterol, triglycerides, HDL, LDL. Subjects with serum cholesterol values greater or equal to 5.2 mmol/L and / or serum triglyceride values greater than or equal to 1.7 mmol/L, or those giving data for the use of statins are in the hyperlipidemia group.

Serum CRP measurements - All study participants underwent blood sampling and measuring of CRP in serum by latex-enhanced immunonephelometric assay (ABX Pentra CRP CP, HORIBA GROUP, Montpellier, France), reference value 0-10mg/L.

RESULTS

Demographic and other characteristics of the study subjects are given in Table 1. The two groups were similar regarding the sex and age distribution of the included subjects, as well as regarding their smoking status and mean BMI. The mean values of spirometric parameters (FVC, FEV_1 and FEV_1/FVC ratio) were significantly lower in COPD patients than in non-COPD controls.

Table 1. Demographics and other characteristics of the study subjects.

Characteristic	COPD patients (n = 60)	Non-COPD subjects (n = 30)
Sex Males Females	52 (86.7%) 8 (13.3%)	23 (76.7%) 7 (23.3%)

Mean age (years) Males Females	65.9 ± 7.5 67.9 ± 6.1	64.8 ± 8.6 66.7 ± 7.8
Smoking status Active smokers Former smokers Pack-year smoked	35 (58.3%) 25 (41.7%) 66.1 ± 25.8	18 (60%) 12 (40%) 67.4 ± 25.5
Mean BMI value	25.8 ± 4.9	24.9 ± 2.1
Mean baseline values of spirometric parameters FVC (% pred) FEV_1 ((% pred) FEV_1/FVC ratio	78.8 ± 12.3 47.5 ± 17.9 0.6 ± 0.07	115.2 ± 16.8 92.3 ± 14.7 0.8 ± 0.05
Level of serum CRP < 3,14 mg/L 3,14 - 10mg/L >10mg/L	10 (16.7%) 27 (45%) 23 (38.3%)	15 (50%) 10 (33.3%) 5 (16.7%)

COPD: chronic obstructive pulmonary disease; BMI: body mass index; FVC: forced vital capacity; FEV_1: forced expiratory volume in one second; %pred: percentage of the predicted value; CRP: C-reactive protein; mg: milligram; L: liter.

In addition, the mean value of serum CRP was significantly higher in COPD patients than in non-COPD (10.2 *vs.* 5.9; *P = 0.04*) suggesting a low-grade systemic inflammations in these patients.

Laboratory findings of lipid profile and total leukocyte count are presented in table 2. Number of patients with leukocyte count $>10^9$/L was significantly higher in stable COPD than control group (45% *vs.* 26.7%; *P = 0.01*).

Table 2. Presentation of lipid profile and total leukocyte count in COPD and non-COPD subjects.

Characteristic	COPD patients (n = 60)	Non-COPD subjects (n = 30)
Lipid profile		
Normal lipid status (1)	33 (55%)	15(50%)
Hypercholesterolemia (>=6.2 mmol/L) (2)	21 (35%)	10(33.3%)
Hypertrygliceridemia (>=1,7mmol/L) (3)	5 (8.3%)	4(13.3%)
Hypercholesterolemia + Hypertrygliceridemia (4)	1 (1.7%)	1(3.3%)
Leukocyte count (white blood cells (WBC) – ref. value 3.5-10 x 109 /l)		
WBC <=109 (1)	33 (55%)	22(73.3%)
WBC >109 (2)	27 (45%)	8(26.7%)

According to the severity of airflow limitation, i.e. to the post-bronchodilator value of FEV_1, COPD patients were categorized in four stages: mild, moderate, severe and very severe COPD.

Table 3. Distribution of the COPD patients by degree of airflow limitation.

COPD severity	COPD patients (n = 60)
GOLD 1 - mild (FEV1 ≥ 80% pred)	4 (6.7%)
GOLD 2 – moderate (FEV1 = 50% – 79% pred)	21 (35%)
GOLD 3 – severe (FEV1 = 30% – 49% pred)	22 (36.7%)
GOLD 4 – very severe (FEV1 < 30% pred)	13 (21.7%)

COPD: chronic obstructive pulmonary disease; GOLD: Global Initiative for Chronic Obstructive Lung Disease; FEV_1: forced expiratory volume in one second; %pred: percentage of the predicted value.

The Pearson correlation test was performed to determine dependence between leukocyte count and CRP value in stable COPD patients, compared to control group, presented in table 4, showing statistically significant correlation (*r=0.358, p=0.005, P < 0.01*).

Table 4. Correlation between leukocyte count and CRP value in stable COPD patients compared to control group.

Correlations		WBC	CRP
WBC	Pearson Correlation	1	,358**
	Sig. (2-tailed)		,005
	N	60	60
CRP	Pearson Correlation	,358**	1
	Sig. (2-tailed)	,005	
	N	60	60
**. Correlation is significant at the 0.01 level (2-tailed).			

According to smoking exposure and lung functional capacity, Pearson correlation test showed that the degree of airflow limitation in COPD patients was significantly related to smoking exposure expressed by number of pack-years (Brinkman index), Pearson correlation, (r= -0.525, p=0.000, P < 0.01), Table 5.

Table 5. Correlation between smoking exposure and airflow limitation (decline of FEV1%).

Correlations

		FEV1%pred	Brinkman index
FEV1%pred	Pearson Correlation	1	-,525)**
	Sig. (2-tailed)		,000
	N	60	60
Brinkman index	Pearson Correlation	-,525)**	1
	Sig. (2-tailed)	,000	
	N	60	60
**. Correlation is significant at the 0.01 level (2-tailed).			

The degree of airflow limitation in COPD patients was significantly related to serum CRP level (r= -0.324, p=0.012, P < 0.05), presented in Table 6.

Table 6. Correlation between serum CRP level and airflow limitation (decline of FEV1%).

Correlations

		CRP	FEV1%pred
CRP	Pearson Correlation	1	-,324)*
	Sig. (2-tailed)		,012
	Sum of Squares and Cross-products	6708,009	-3650,411)
	Covariance	113,695	-61,871)
	N	60	60
FEV1%pred	Pearson Correlation	-,324)*	1
	Sig. (2-tailed)	,012	
	Sum of Squares and Cross-products	-3650,411)	18944,983
	Covariance	-61,871)	321,101
	N	60	60
*. Correlation is significant at the 0.05 level (2-tailed).			

DISCUSSION

COPD is not only a pulmonary disease, it is a systemic disease with numerous extrapulmonary manifestations. Particular emphasis is placed on the association with cardiovascular morbidity, which is why it is one of the biggest causes of morbidity and mortality in the world today. From the sixth place in the 1990s. now is at fourth place, and is estimated to be third in 2020 (1). It is associated with many comorbidities that further increases hospital costs and reduces lifespan. Therefore it is very important to evaluate these patients for comorbidities with particular reference to cardiovascular. In our study dominated male patients, which is probably due to the fact that men are larger cigarette consumers as the main risk factor. Their presence was 86.7% of men, and 13.3% of women, out of 60 COPD patients, current smokers 58.3% and former smokers 41.7%. Mean age of patients with COPD, 65.9 ± 7.5 for men and 67.8 ± 6.1 for women. Average intensity of cigarette smoking expressed through the Brinkman Index, 66.1 ± 25.8 pack/year. According to the GOLD criteria, they were predominantly in GOLD stage 2 and 3 according to spirometry (FEV1%pred 30-80%) 43 patients (71.7%), mean FEV 1%pred was 47.5 ± 17.9. There was a positive correlation between leukocyte and CRP values in the examined group. There was a significant difference in the median value of CRP between the examined and the control group 10.2 ± 11.1 *vs.* 5.9 ± 4.3, which confirms the existence of low-grade systemic inflammation in these patients. De Torres and associates, Dahl and associates, indicated the CRP level as predictor of prognosis in patients with COPD (14,15). In our analysis, there was a correlation in negative direction between the leukocyte count and airflow limitation (FEV1%pred), which means that with the decrease in FEV1%, elevates the number of leukocytes as participants in the systemic inflammation cascade with p < 0.05 (9,21). The investigated correlation between FEV1% and CRP showed statistically significance, p < 0.05 in negative direction, decline in FEV1% was associated with higher CRP value. Smoking cigarettes as a major risk factor for impaired pulmonary function was confirmed by obtaining a high significance p<0.01 in a negative direction with Pearson Correlation Test, which means that higher Brinkman Index is associated with decline in FEV1% pred. Mohesh and associates in 2016 in the International Journal of Medical Research & Health Sciences reported a study that also shows a negative correlation between nicotine dependence and spirometric values (22).

CONCLUSIONS

1. COPD dominates in males due to the greater prevalence of cigarette smoking in men;
2. In the analyzed population, patients were predominantly in GOLD 2 and 3 stage of disease;
3. The mean value of the CRP was significantly higher in the investigated group compared to the controls.

4. The number of subjects with leukocyte count > 109/L was significantly higher in the examined vs. control group.

5. There is a significant dependence $p < 0.01$ in positive direction between the value of leukocytes and CRP in patients with COPD.

6. The correlation between smoking exposure expressed through Brinkman Index (pack / years) and spirometric values (FEV1% pred) showed significant dependence ($p < 0.01$) between the two variables in negative direction.

7. Correlation between FEV1% and CRP showed significance with $P = 0.012 < 0.05$ in a negative direction, elevation in the value of one variable means a decrease in the second.

REFERENCES

1. Global Initiative for Chronic Obstructive Lung Disease (GOLD). Executive Summary: Global Strategy for Diagnosis, Management, and Prevention of COPD - Updated 2018. Available at: www.goldcopd.org (Accessed 20.04.2019).

2. Budev MM, Arroliga AC, Wiedemann HP, Matthay RA. Cor Pulmonale: An Overview. Semin Respir Crit Care Med. 2003;24(3):233-44.

3. Sabit R, Bolton C. Sub-clinical left and right ventricular dysfunction in patients with COPD. Respir Med. 2010;104(8):1171-1178.

4. Don DS, Man P. Chronic obstructive pulmonary disease: a novel risk factor for cardiovascular diseasel. Can J Physiol Pharmacol. 2005;83(1):8-13.

5. Anthony S, Fauci A, Braunwald E, Kasper D, Hauser S, Longo D et al. Principles of Harrison's Internal Medicine. 17th Ed. 2008;1635-1642.

6. Curkendall SM, Lanes S, de Luise C, Stang MR, Jones JK, She D, et al. Chronic obstructive pulmonary disease severity and cardiovascular outcomes. Eur J Epidemiol. 2006; 21(11): 803–813.

7. Stone IS, Barnes NC, Petersen SE. Chronic obstructive pulmonary disease: a modifiable risk factor for cardiovascular disease? Heart. 2012;98:1055–1062.

8. Athonisen NR, Connett JE, Enright PL, Manfreda J;Lung Health Study Research Group. Hospitalizations and mortality in the Lung Health Study. Am J Respir Crit Care Med. 2002;166(3):333-339.

9. Maclay JD, MacNee W. Cardiovascular disease in COPD: mechanisms. Chest. 2013 Mar;143(3):798-807.

10. Caicedo L, Rodríguez P, Morales MC, Maestu L, Alvarez-Sala WL. COPD as a cardiovascular risk factor. PubMed. 2012.

11. Don D. Sin, S.F. Paul Man. Why Are Patients With Chronic Obstructive Pulmonary Disease at Increased Risk of Cardiovascular Diseases? Circulation. 2003;107:1514-1519.

12. Jeremy A, Kadiev S, Criner G, Scharf S, Minai O, Diaz P. Cardiac disease in Chronic Obstructive Pulmonary Disease. Proc Am Thorac Soc. 2008; 5(4):543-548.

13. Gan WQ, Man S, Senthilselvan A, et al. Association between chronic obstructive pulmonary disease and systemic inflammation: a systematic review and a meta-analysis. Thorax. 2004; 59:574-580.

14. de Torres JP, Cordoba-Lanus E, Lopez-Aguilar C, et al. C-reactive protein levels and clinically important predictive outcomes in stable COPD patients. Eur Respir J. 2006;27(5):902-907.

15. Dahl M, Vestbo J, Lange P, Bojesen SE, Tybjaerg-Hansen A,Nordestgaard BG. C-reactive protein as a predictor of prognosis in chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 2007;175(3):250-255.

16. Kim SJ, Yoon DW, Lee EJ, Hur GY, Jung KH, Lee SY, Shin C, Shim JJ, Kang KH, Yoo SH, Kim JH. Carotid atherosclerosis in patients with untreated chronic obstructive pulmonary disease.NT J TUBERC LUNG DIS. 2011;15(9):1265–1270.

17. Calculate your Body Mass Index. Available at: https://www.nhlbi.nih.gov (Accessed 22.04.2019).

18. World Health Organization. Guidelines for controlling and monitoring the tobbaco epidemic. Geneva: WHO, 1998.

19. Fletcher CM. Standardised questionnaire on respiratory symptoms: a statement prepared and approved by the MRC Committee on the Aetiology of Chronic Bronchitis (MRC breathlessness score). BMJ 1960; 2: 1662.

20. Miller MP, Hankinson J, Brusasco V, et al. Standardisation of spirometry. Eur Respir J. 2005; 26: 319–338.

21. Don D. Sin, S.F. Paul Man. Why Are Patients With Chronic Obstructive Pulmonary Disease at Increased Risk of Cardiovascular Diseases? Circulation. 2003;107:1514-1519.

22. Mohesh MG, Sundaramurthy A, Prema Sembulingam P. The impact of cigarette smoking on lung function in smokers with differences in their nicotine dependency. International Journal of Medical Research & Health Sciences. 2016, 5, 7:36-41.

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