

## Lung Surfactant Proteins A (SP-A) and D (SP-D) in Chronic Obstructive Pulmonary Disease (COPD)

Regina Hasa<sup>1</sup> Juliana Gjerazi<sup>2</sup> Eritjan Tashi<sup>2</sup> Donika Mema<sup>3</sup>  
Sofiel Telo<sup>3</sup> Jul Bushati<sup>2</sup> Anyla Bullo<sup>4</sup>  
1Sherbimi Laboratorit Biokimik-klinik. Spitali Universitar “Shefqet Ndroqi”  
2Sherbimi i Pneumologjise. Spitali Universitar “Shefqet Ndroqi”  
3Stafi Epidemiologjik & Statistikes. Spitali Universitar “Shefqet Ndroqi”  
4Departamenti i Laboratoreve, UMT, QSUT

### Abstract

**Introduction:** The biomarkers used in COPD are many, but studies of last decades are focused on the pulmonary surfactant, which plays a crucial role in normal lung function.

**Aim:** Evaluation of the levels of SP-A, SP-D and other markers in patients with SPOK and their relation to inflammation and smoking.

**Material :** We studied 118 patients with COPD . 10 cases were in stage B, 24 cases in stage C and 84 cases in stage D; 113 males and 5 females , mean age  $69 \pm 8$  years and  $70 \pm 8$  years respectively; **Methodology:** The SP-A and SP-D levels were measured on admission during acute exacerbation and in the day of discharge from hospital in remission of disease. We included a healthy control group.

**Results:** In the healthy control group mean SP-A =  $22.2 \pm 16.3$  ng / ml and mean SP-D =  $90 \pm 36.8$  ng / ml . In our study group ,on admission we found mean SP-A levels  $46.8 \pm 35.2$  ng / ml and SP-D  $175 \pm 99$  ng / ml. In COPD Stage B mean SP-A was  $33.78 \pm 19.7$  ng / ml, Stage C  $40.7 \pm 19.5$  ng / ml and stage D  $50.2 \pm 39$  ng / ml ( $p = 0.0001$ ). Mean SP-D at Stage B was  $168.3 \pm 121$  ng / ml, Stage C  $160 \pm 78$  ng / ml, and Stage D  $181 \pm 102$  ng / ml ( $p = 0.0001$ ). On the last day (remission) mean SP-A  $38 \pm 23.5$  ng/ml and mean SP-D  $147 \pm 91$  ng / ml. In COPD Stage B mean SP-A was  $42.7 \pm 26.2$  ng / ml, Stage C  $33 \pm 15.6$  ng / ml and stage D  $38.5 \pm 25$  ng / ml ( $p = 0.003$ ). Mean SP-D at Stage B was  $196 \pm 157$  ng / ml, Stage C  $130 \pm 53$  ng / ml, and Stage D  $137 \pm 81$  ng / ml ( $p = 0.002$ ). In *exacerbation* period smoking status has significant correlations with all biomarkers presented with the Spearman coefficient: SP-A ( $R_s = 0.249$ ,  $p = 0.002$ ); SP-D ( $R_s = 0.264$ ,  $p = 0.001$ ), IL6 ( $R_s = 0.255$ ,  $p = 0.002$ ) and CRP ( $R_s = 0.231$ ,  $p = 0.004$ ). In *remission* state smoking has significant positive correlation with SP-D ( $R_s = 0.282$ ,  $p = 0.002$ ) and SP-A ( $R_s = 0.273$ ,  $p = 0.003$ ). In both evaluations the mean values of SP-A and SP-D were significantly higher in smokers compared to nonsmokers ( $p < 0.05$ ). Levels of IL6 measured in first day were significantly higher in smokers  $p = 0.025$ , mean CRP levels had no significant differences between two groups.

**Conclusion:** SP-A and SP-D levels were higher in COPD patients compared to healthy control group. Their level were significantly higher AECOPD than in stable state of COPD and they correlated with the gravity of the disease. In remission state SP-A and SP-D levels reflects better the gravity of COPD. SP-A and SP-D levels are better related with smoking state and their levels are more increased in smokers .

**Keywords:** COPD, acute exacerbation of COPD (AECOPD), SP-A and SP-D

### INTRODUCTION

Chronic Obstructive Pulmonary Disease (COPD) is characterized by chronic pulmonary inflammation, progressive airway obstruction and extrapulmonary systemic effects. [1] Chronic inflammation in COPD causes structural changes, progressive and not fully reversible obstruction of airways and with airflow limitation. [2] Tobacco smoke contaminates the airways [3.4] changes the macrophages and neutrophil phagocytic activity [5]. Smoking disrupts mucociliary activity, promotes the release of mediators such as IL8, IL6 and TNF- $\alpha$  [6] and cytotoxic enzymes that further potentiate inflammation and deepen pulmonary damage [4] causing progression to more severe COPD stages. [7]. The most commonly used biomarkers in COPD are IL6, IL8, TNF $\alpha$ , CRP and PCT. PCT is a very specific marker for the diagnosis of relevant bacterial infections and sepsis. Serum levels increase rapidly during infective processes. [8] PCT levels evidently correlate with hospital length of stay of patients with AECOPD. [9] CRP has pro-inflammatory and anti-inflammatory properties. Its levels increase during infections, and in acute and chronic inflammation. [10] A great number of studies have revealed the presence of chronic inflammation in the stable COPD. [11] It has been reported that IL6 levels reflect the chronic inflammation present in COPD and independently correlate with the reduction of FEV1 and FVC. [12] Studies of the last decades have been focused on the pulmonary surfactant, which plays a key role in pulmonary physiology. Its major functions mainly consist on preventing the alveolar collapse in low volume of lungs during exhalation and in the opening of bronchial airways during normal and forced respiration. Its non-biophysical, immunologic role is the protection of pulmonary tissue from the damage and infections caused by microparticles and inhaled microorganisms. This role is primarily attributed to surfactant protein A and surfactant protein D.

[13] SP-A and SP-D are two hydrophilic proteins produced by Type 2 pneumocytes [15] and Clara cells where the gene encoding their synthesis is found in chromosome 10 [13]. They belong to the proteins C type collectin family, similar to collagen that is directly related to the innate immune activity in the pulmonary tissue [14].

The aim of this study is to evaluate the levels of SP-A and SP-D and some other markers in COPD. This will be accomplished by measuring, analysing and comparing their behaviour in acute exacerbation and in remission period of the disease, by evaluation of their relations to COPD gravity of and to the smoking status.

## MATERIALS AND METHODS

**Subjects** - We recruited 118 COPD patients in the hospital Center “Shefqet Ndroqi” Tirana. Mean age was 69.2±/8.5 yrs. 113 were males, mean age 69±/8 years old and 5 were females mean age 70±/8 years old. In the study group 22 cases were nonsmokers(NS) and 96 smokers (S). Diagnosis was based on GOLD criteria [1]. 10 cases in stage B mean age 64±/7yrs, 24 cases in stage C mean age 70±/8yrs and 84 cases in stage D mean age 69±/9. Exclusion criteria are asthma, allergies and respiratory diseases except COPD.

**Samples** - The first blood samples were taken in the morning within the first 48 hours of hospital admission (in acute exacerbation period), and the second samples on the day of discharge from hospital (in remission period). Blood samples were taken to evaluate routine biochemical tests, inflammatory markers such CRP together with bloodgas analysis and cell blood count (CBC). Serum samples were stored at -20 ° C for the SP-A, SP-D and IL6 determination.

**Laboratory Tests** *Surfactant Protein A*: Human Surfactant Protein A ELISA (BioVendor). An immunoassay for the quantitative determination of human surfactant protein A. Sensitivity of the method is up to 0.16 ng / ml. The normal range interval of healthy individuals: 13-65ng / ml. *Surfactant protein D*: Human Surfactant D ELISA protein (BioVendor) –immunohistochemical assay for quantitative determination of human surfactant protein D. Sensitivity of the method is up to 0.01 ng / ml. The kit provides the healthy individuals mean serum value of 79.7ng / ml, and mean heparin plasma of 90.3ng / ml. CRP is estimated by the turbidimetry method. Human CRP agglutinates with anti-CRP coated latex particles monoclonal antibodies. The Interval of normal values for adults <5mg / L. *IL6*- Electrochemiluminescence (ECLIA) immunologic test with 1.5 to 5000 µg/ml measuring interval. Normal range <7pg / ml.

**Statistical Analysis** - Performed with SPSS 16 and 21. The results are given as mean ± deviation standard (DS). Pearson and Spearman correlation coefficients were used respectively between SPA, SP-D and inflammatory markers, SPOK stages and smoking state. ANOVA variance analysis, ROC curve, T-test and linear regression are used to evaluate statistical relations between independent groups in order to analyse the behavior of SP-A, SP-D CRP and IL6 and other markers according to COPD gravity in both acute exacerbation and in stable period. P <0.05 was called statistically significant.

## RESULTS

In control group SP-A = 22.2 ±/ 16.3 ng / ml, SPD = 90 ±/ 36.8 ng / ml, IL6 = 5.86 ±/ 2.11pg / ml and CRP = 5.2 ±/ 2.2mg / L. On admission: SP-A = 46.8 ±/ 35.2ng / ml, SP-D = 175 ±/ 99 ng / ml, IL6 = 36.7 ±/ 95.5pg / ml and CRP = 60.7 ±/ 81.4mg / L. On last day of hospital stay: SP-A = 38 ±/ 23.5 ng / ml, SP-D = 147 ±/ 91 ng / ml, IL6 = 18.7 ±/ 35.4 pg / ml and CRP = 7.42 ±/ 35.4mg / L. The SP-A values have positive correlation with the number of leukocytes ( $R_p = 0.436$   $p = 0.0001$ ), neutrophils (%) ( $R_p = 0.207$   $p = 0.035$ ), fibrinogen concentration ( $R_p = 0.414$   $p = 0.0001$ ), CRP ( $R_p = 0.374$   $p = 0.0001$ ) and with IL6 ( $R_p = 0.299$   $p = 0.0001$ ). SP-D has positive correlation with SP-A ( $R_p = 0.424$ ,  $p = 0.0001$ ). IL6 is significantly positively correlated with CRP, pearson coefficient ( $R_p = 0.647$   $p = 0.0001$ ). SP-A, SP-D and IL6 are not correlated with obstruction parameters, while CRP has a negative correlation with FEV1.  $R_p = -0.279$   $p = 0.016$ .

*Evaluation of SP-A, SP-D and other markers relations to COPD gravity (stages).*

*During AECOPD* -In GOLD stage B, stage C and stage D patients the SP-A values resulted 33.78 ±/ 19.7 ng / ml, 40.7 ±/ 19.5ng / ml and 50.2 ±/ 39 ng / ml respectively ( $p = 0.0001$ ) (fig 1.a). In GOLD Stage B, Stage C and Stage D patients the SP-D values were 168.3 ±/ 121ng / ml, 160 ±/ 78 ng / ml, and 181 ±/ 102ng / ml respectively ( $p = 0.0001$ ) (fig.1.b). In GOLD stage B, stage C and stage D group the CRP values were 10.2 ±/ 9 mg / ml, 42.6 ±/ 45mg / L and 69.2 ±/ 84mg / L respectively ( $p = 0.0001$ ). COPD stage B, stage C and stage D groups had mean IL6 = 16.8 ±/ 20pg / ml, 15.8 ±/ 14.3pg/ml and 30.3 ±/ 49pg/ml respectively ( $p = 0.003$ ). *After remission values* in COPD stage B, stage C and stage D patients the SP-A values resulted 42.7 ±/ 26.2ng/ml, 33 ±/ 15.6ng/ml and 38.5 ±/ 25ng/ml ( $p = 0.003$ ) (fig.1.c). SP-D in GOLD Stage B patient was 196 ±/ 157ng / ml, Stage C 130 ±/ 53 ng/ml, Stage D 137 ±/ 81ng/ml ( $p = 0.002$ ) (fig1.d). CRP mean levels in GOLD stage B, stage C and stage D group were 7.8 ±/ 6.4 mg/ml, 9.6 ±/ 9.9mg /L and 6.5 ±/ 8.1mg/L respectively ( $p = 0.353$ ). Mean IL6 levels in stage B, stage C and stage D were 7.6 ±/ 2.5pg/ml, 29.7 ±/ 60pg/ml and 15.7 ±/ 26pg/ml respectively ( $p = 0.048$ ) (FIG.1).

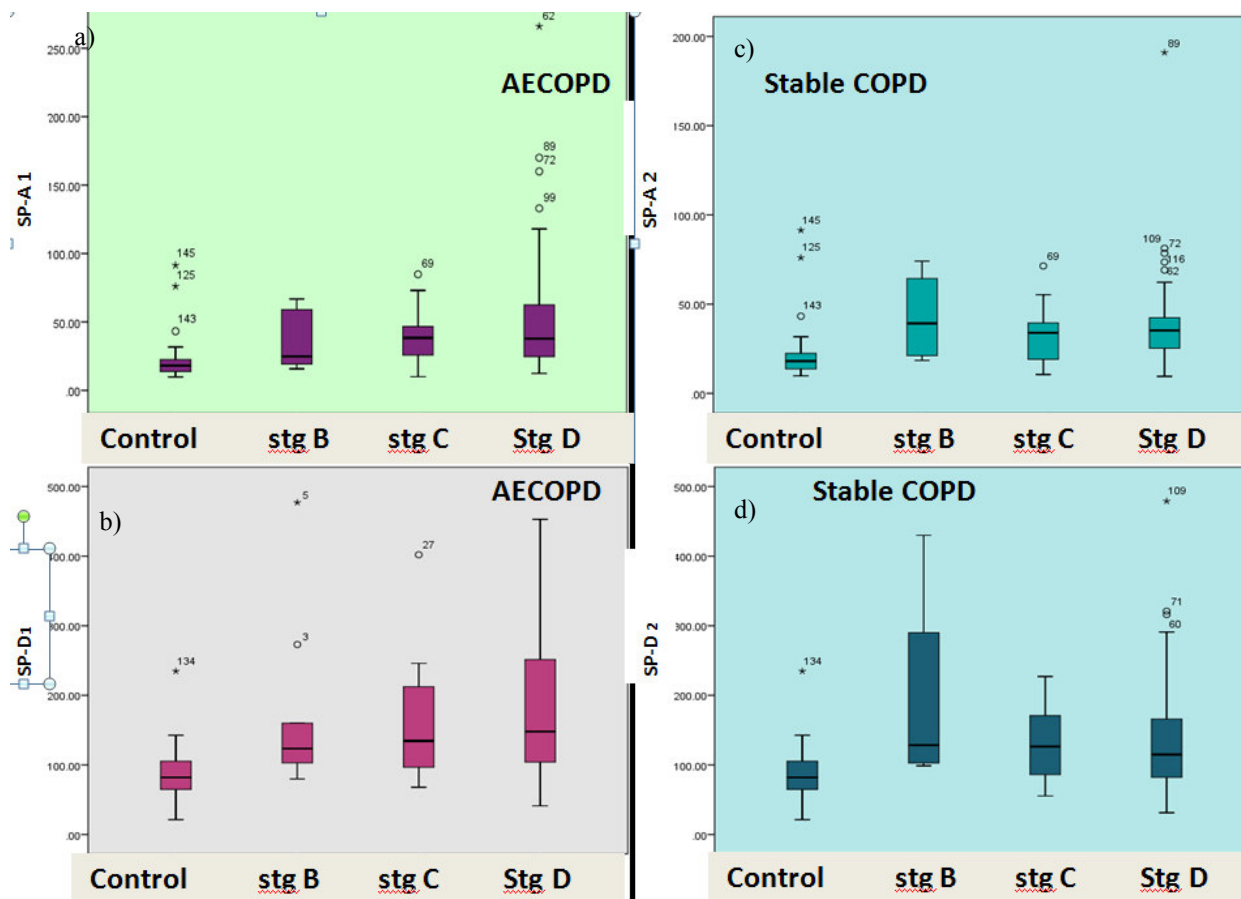


Figure 1.: a) SP-A values in AECOPD, b) SP-D values in AECOPD, c) SP-A in stable COPD (remission) and d) SP-D values in stable COPD (remission).

In AECOPD the SP-A, SP-D, CRP and IL6, values are positively correlated with COPD stages. We found Spearman correlation coefficients  $R_s = 0.461$ ,  $p = 0.0001$ ;  $R_s = 0.397$ ,  $p = 0.0001$ ;  $R_s = 0.597$ ,  $p = 0.0001$ ;  $R_s = 0.524$ ,  $p = 0.0001$ , respectively. In remission state the SP-A, SP-D, IL6 and CRP values positively correlated with the GOLD COPD stages:  $R_s = 0.442$ ,  $p = 0.0001$ ,  $R_s = 0.271$ ,  $p = 0.003$ ,  $R_s = 0.305$ ,  $p = 0.001$  and  $R_s = 0.260$ ,  $p = 0.005$  respectively.

In exacerbation we also used ROC curve analysis to evaluate the sensitivity (SE), specificity and diagnostic accuracy of the serum concentrations of SP-A, SP-D, CRP and IL-6 to discriminate COPD patient from the health control group (fig.2): SP-A AUC 0.844, 95% CI (0.773 - 0.913);  $p < 0.001$ . SP-D AUC 0.790, 95% CI (0.715 - 0.866);  $p < 0.001$ . CRP AUC 0.867, 95% CI (0.820 - 0.924);  $p < 0.001$ . IL6 AUC 0.849, 95% CI (0.788 - 0.910);  $p < 0.001$ . CRP and IL6 have higher SE to distinguish COPD from the control group during exacerbation. SP-A has higher SE and diagnostic accuracy than SP-D.

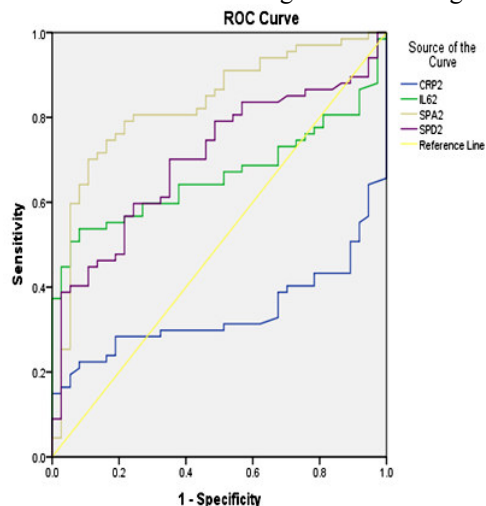


Figure 2.

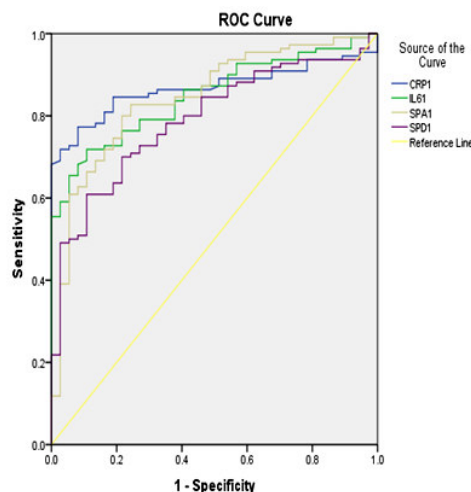


Figure 3.

Figure 2.: ROC curve analysis .The biomarkuers on admition. CRP and IL6 curves have higher left position than others . This confirms the higher SE these two tests posses to discriminate COPD patients from control group. SP-A is a specific lung marker that has quite the same SE as those of CRP and IL6

*In remission period* values , ROC curve analysis showed that serum SP-A has higher SE and better diagnostic accuracy to discriminate COPD patients from the control group: SP-A AUC 0.829 ,95% CI (0.745 - 0.912);  $p < 0.001$ . SP-D AUC 0.734 ,95% (0.603-0.803);  $p = 0.001$ . IL6 AUC 0.664 ,95% CI (0.562-0.766);  $p = 0.006$ , CRP AUC 0.338 ,95%CI (0.234-0.442)  $p = 0.007$ (fig.3). when Measured in remission period of COPD, CRP and IL6 did not show significant sensitivity to differ COPD patients from the control group.

Figure 3.: ROC curve. Biomarkers on the last day of hospital stay . SP-A and SP-D curves are positioned higher on the left than the curves of CRP and IL6. That means SP-A and then SP-D have higher SE then CRP and IL6 to differ COPD patients from the control group.

*In AECOPD* - multiple linear regression of CRP, IL6, SP-A and SP-D, showed these variables can positively significantly predict the COPD stages:  $F(4,142) = 14.978$   $p = 0.0001$ .  $R = 0.545$ ,  $R^2 = 0.297$ . CRP ( $p = 0.004$ ) and SP-D ( $p = 0.0001$ ). *On the day of discharge(remission)* - the multiple linear regression of biomarkers CRP, IL6, SP-A and SP-D showed these variables can predict the COPD stage significantly  $F(4,99) = 6.894$ ;  $p = 0.0001$ ,  $R = 0.467$ ,  $R^2 = 0.218$ . SP-A ( $p = 0.001$ ) and SP-D ( $p = 0.011$ ). Multiple linear regression analysis of the SP-A and SP-D , found that these two variables can predict the COPD stages significantly  $F(2,118) = 11.3$   $p = 0.0001$ ,  $R = 0.401$ ,  $R^2 = 0.161$ . SP-A ( $p = 0.001$ ) and SP-D ( $p = 0.038$ ).

Evaluation of SP-A , SP-D and other biomarkers related to smoking.

There are 22 nonsmokers (NS) and 96 smokers (S) . The healthy control group consisted of 25 nonsmokers and 13 smokers. In both evaluations the mean values of SP-A and SP-D were significantly higher in smokers compared to nonsmokers ( $p < 0.05$ ). (tab.1). Levels of IL6 measured in first day were significantly higher in smokers  $p = 0.025$ . mean CRP levels had no significant differencies between two groups according to smoking state. (tab.1).

Biomarkers	NonSmokers M+-SD	Smokers , M+-SD	Oneway Anova ( p)
SP-A <sub>1</sub>	32.2+-22	46+-36.5	<b>0.017</b>
SP-D <sub>1</sub>	124+-74	173+-100	<b>0.002</b>
SP-A <sub>2</sub>	33+-14.4	38.6+-25	<b>0.044</b>
SP-D <sub>2</sub>	112+-68	144+-82	<b>0.011</b>
IL6 <sub>1</sub>	12.3+-12.6	30+-51	<b>0.025</b>
IL6 <sub>2</sub>	11.3+-8	19.7+-38.6	0.081
CRP <sub>1</sub>	30.5+-56	54.3+-75	0.054
CRP <sub>2</sub>	7.65+-8	7+-10	0.875

Table 1. Biomarkers' values according to smoking and nonsmoking groups. Numbers 1 and 2 are used as a symbols of the biomarker values on admition and in the last day of hospital stay respectively.

*In acute exacerbation* we analysed SP-A and SP-D biomarkers in three patients groups according to the amount of cigarettes in pakeyears. Group I that consumed <50 packyears cigarette , group II 50-100 packyears, and group III that has consumed >100 packyears. The values of SP-A in each group were 39.9+/-24ng/ml , 53.7+/-45.5 ng/ml and 61.9+/- 37.9ng/ml respectively ;( $p = 0.046$ ). SP-D values in each group were 164.8+/- 94 ng/ml, 171+/- 90 ng/ml and 237 +/-118ng/ml respectively;(  $p = 0.036$  ) . *In remission period* there were no significant differencies in SP-A and SP-D values between these three groups ( $p = 0.732$  dhe  $p = 0.735$ ).

*In acute exacebation* . We divided the smoking patients in two groups. *Group I* includes patients that have been smoking for <30 years and in group II are patients that have been smoking for > 30 years Their SP-A values resulted 37.7 +/- 20.6 ng / ml and 55.2 +/- 42.4 ng / ml respectively ( $p = 0.006$ ) . *During remission* state in the groups I and II SP-A values were 31.7 +/- 13.1 ng / ml and 42.4 +/- 13.1ng / ml respectively; ( $p = 0.036$ ) , IL6 values were 9.3 +/- 6.9pg / ml and 25.8 +/-46.3pg/ml ( $p = 0.041$ ). SP-A value positively correlates with the number of years of smoking (Pearson  $R_p = 0.233$ ,  $p = 0.012$ ).

*In exacerbation* period smoking status has significant correlations with all biomarkers presented with the Spearman coefficient: SP-A(  $R_s = 0.249$ ,  $p = 0.002$ ); SP-D(  $R_s = 0.264$   $p = 0.001$ ), IL6(  $R_s = 0.255$ ,  $p = 0.002$ ) and CRP (  $R_s = 0.231$ ,  $p = 0.004$ ). *In remission* state smoking has significant positive correlation with SP-D ( $R_s = 0.282$ ,  $p = 0.002$ ) and SP-A ( $R_s = 0.273$ ,  $p = 0.003$ ) .IL6<sub>2</sub> had significant positive correlation with the packyears(amount of cigarettes) ,Pearson coefficient ( $R_p = 0.243$ ,  $p = 0.035$ ). Multiple linear regression found that CRP and packyears are significant positive predictors for the values of SP-A { $F(2 ;111) = 9.8$  ( $p = 0.0001$ ),  $R = 0.387$ ,  $R^2 = 0.150$ }, CRP ( $p = 0.001$ ) and packyears ( $p = 0.009$ ). Smoking had a slight significant ability to predict SP-D values . { $F(1,154) = 9,584$  ( $p = 0.002$ ),  $R = 0.242$ ,  $R^2 = 0.059$ }. Inflammatory markers such as CRP or IL6 were not significant predictors for SP-D values.

Biomarker	quitting smoke >10 years	Mean	Std.Dev	ANOVA p
SP-A <sub>1</sub>	+	46.3	28	0.686
	-	42.5	21	
SP-A <sub>2</sub>	+	35	14	0.224
	-	28	12	
SP-D <sub>1</sub>	+	<b>210</b>	109	<b>0.038</b>
	-	<b>135</b>	64	
SP-D <sub>2</sub>	+	<b>151</b>	76	<b>0.026</b>
	-	<b>81</b>	44.5	

Table.2. shows the values of SP-A and SP-D in exacerbation and in the period of stable COPD according to two groups. The first group has quit smoking more > 10 years ago and the other < 10 years ago.

SP-A and SP-D mean values are lower in the exsmokers' group that have quit smoking more than 10 years ago compared to the group of patients that quit smoking <10 years ago (tab.2).

The mean SP-D values in acute exacerbation and in stable COPD were significantly higher in cases that have quit smoking more 10 year ago.

## DISCUSSION

### *Biomarkers in acute exacerbation and in stable COPD*

Several studies reveal the importance of the use of inflammation biomarkers in AECOPD and stable COPD. The changing levels of these biomarkers reflects the inflammatory situation in COPD. Increased CRP values used as indicator of inflammation and acute exacerbation, have been reported by *D. Dev et al* since in 1998. [16] CRP levels are higher in acute exacerbation [17] especially those coming from bacterial infections. [18] Increased values of non-specific markers of inflammation such as fibrinogen, CRP and IL6 reflect the degree of inflammation of airways in COPD patients. [19] Persistence of raised of CRP and IL6 levels predicted recurrence of inflammation and further deterioration of airway obstruction [20]. IL-6 levels reflect chronic inflammation, typical feature of COPD[21,22]. The present study evaluates the serum values of SP-A, SP-D, CRP and IL6 measured in a group of COPD patients ,first during acute exacerbation period and then in stable state of COPD. Serum levels of biomarkers in COPD patients were higher compared to the serum values measured in a healthy control group. *Kateryna.G. et al* and *Helen Ilumets et al* also have found that the surfactant protein A and D levels were more elevated in COPD patients compared to healthy control group [23,24]. In this study SP-A,SP-D, IL6 and CRP values in AECOPD were found higher compared to their values in stable COPD. In a similar study, *Tania A.Shakoor et al.* evaluated SP-D in acute exacerbation, in stable state and in control group cases. Similarly to us they found the highest values of SP-D in the period of exacerbation compared to stable COPD values and the control group had lower values compared to those of COPD patients. [25]

In an ECLIPSE analysing –review study, SP-D values in the serum of COPD patients were significantly lower after treatment compared to those before to treatment with corticosteroids [26, 27]. In our study ,in the exacerbation period of SP-A values were significantly correlated with CRP and IL6 and with routine inflammatory markers such as leukocytes, neutrophils and fibrinogen. It seems that SP-A values reflect the inflammatory changes of pulmonary tissue. The SP-D values are not correlated with inflammatory markers.

### *Biomarkers and the gravity of COPD*

In this study biomarkers levels have significant positive correlation with the severity of COPD (disease stage). In AECOPD period mean values of SP-A, SP-D, CRP and IL6 were significantly different in each stage group. The highest values were found in stage D patients. These markers reflected the inflammation degree of exacerbation and the disease .During exacerbation CRP and IL6 provide better information than SP-A and SP-D about the inflammation degree and COPD gravity. Despite the significant correlations they have with the gravity of COPD,in the present study SP-A and SP-D have no correlations with respiratory function tests. These findings are similar to the *Kateryna.G et al* study's results who concluded that SP-D values increase in all COPD patients with but not significant correlation to COPD stages, FEV1 and FEV1 / FVC values. This implies that may be SP-D does not reflect the degree of airway obstruction. [24] Meanwhile in the study of *Helen Ilumets et al*, the values of SPA and SP-D resulted with significant negative correlation with respiratory function tests [23]. In the present study only CRP levels have significant negative correlation with FEV1 during acute exacerbation . Similarly to our results *they also* found that SP-A values in stable COPD had increased SE to distinguish the COPD patients from the control cases[23]. According to our findings SP-A and SP-D reflect better COPD gravity. In remission period we found that the biomarkers levels tend to decrease but still not within the normal range .This may be because they reflect the degree of chronic inflammation injury of pulmonary tissue, or because the exacerbation remission may not be fully completed. During exacerbation CRP and SP-D have

positive predictive ability for the COPD stage. On the other hand, SP-A and SP-D revealed better ability than CRP and IL6 to reflect and predict the gravity of COPD in stable condition of the disease. SP-A and SP-D resulted significant positive predictors for the more severe COPD stages. This findings are also supported by *Papaioannou A.I et al* [28] and *Doaa S.E. Zaky et al*, whose studies showed that in the more advanced stages (C-D) of COPD the SP-D values are higher compare to the earlier stages (stage A-B). [29,30].

#### *Biomarkers in relation to smoking*

Smoking effects on the lungs of COPD patients have especially become the subject of many studies [31]. In the present study *SPA* and *SP-D* levels are evaluated both in acute exacerbation and in remission. *SP-A* and *SP-D* are higher in smokers compared to nonsmoker cases. Their levels are especially higher in the group of cases that consumes > 100 packyears cigarettes compared to the values of the patients group that have been smoking for more than 30 years continuously. The fact that in our findings *SP-A* has positive correlation with inflammation markers and has significant regression coefficients with CRP and smoking, suggests that *SP-A* levels are affected by both inflammation and smoking. *SP-A* and *SP-D* values are more increased in the smokers group during acute exacerbation state of COPD. This may strengthen confirmations from other researchers that it very often is difficult to distinguish the true pathological changes caused by smoking from those ones caused by the progression of disease itself. It is not excluded the fact that smoking, inflammation and the changes in the disease overlap and potentiate one another [32]. Anyway these specific markers are released by the inflammation or the injury of pulmonary tissue [33]. In our study, *SP-D* levels even though increased in acute exacerbation, they don't correlate with inflammatory markers and its values are only influenced by smoking. As a matter of fact the ECLIPSE study concluded that the decline of *SP-D* values after anti-inflammatory treatment is not related to the fact that anti-inflammatory drugs inhibit *SP-D* production, but that the inhibition of inflammation corrects the permeability of the alveolus-capillary barrier, preventing so the *SP-D* flow into circulation [26].

The levels of *SP-A* and *SP-D* were also evaluated in the studies of *K.Kida et al 1997*, *Helen I. et al 2011* and *Tetyana P et al 2014*. They showed that *SPA* [23, 41] and *SP-D* [34] were significantly increased in smokers. According to *Hideo Kabayashi et al* *SP-A* values positively correlated with the amount of cigarettes and negatively correlated with FEV1 values [35]. When evaluated in acute ecaxerbatation, we found higher *SP-A* and *IL6* values in the smokers group who have smoked > 30 years continuously, compared to those who smoked <30 years. It seems that *SP-A* and *IL6* values in exacerbatation period, are affected from the long-term smoking. This means that inflammation, which is reflected in *IL6* values [36], and smoking injury [37] may potentiate each-other damages in the pulmonary tissue. This changes are reflected with higher *SPA* and *SP-D* values. In our findings it seems that *IL6* level are affected from the long term smoking and the amount of cigarettes. We found significantly higher *IL6* levels in smokers during acute exacerbation, but no significant CRP changes were found between smokers and nonsmoker cases. *Ardestani ME et al*, also have evaluated *IL6* and CRP in smokers and nonsmoker groups of COPD patients. They concluded *IL6* had higher sensitivity as a predictor of COPD in smokers and that *IL6* and *CRP* were increased in COPD smokers compared to non-smokers and healthy individuals. [36]. In our study *SP-A* and *SP-D* values tend to be lower in the exsmokers group that have quitted smoking more 10 years ago compared to the group of patients that quitted smoking <10 years ago. The mean *SP-D* values in AECOPD and in stable COPD were significantly different between this two groups. This may imply that *SP-D* values reflect the repairing process and the regeneration of the damaged pulmonary tissue [38], re-emphasizing the importance of community awareness to stop smoking [39,40].

#### CONCLUSION

- *SP-A*, *SP-D*, CRP and *IL6* levels were higher in acute exacerbation period compare to those in COPD remission period, but yet they were not related to airway obstruction.
- When measured in stable condition of the disease *SP-A* and *SP-D* values correlated better with COPD gravity.
- *SP-A* and *SP-D* level are higher in patients with more severe COPD stages.
- *SP-A* values reflect the gravity of COPD better than *SP-D*.
- Using these relatively new markers together with the functional respiratory tests can give us a clearer picture of the COPD severity.
- *SP-A* and *SP-D* levels were higher in smokers than non-smokers and their values have significant positive correlations with smoking state.
- *SP-A* levels are affected from inflammation and smoking
- *SP-D* levels are more affected by smoking and they tend to decrease, if smoking is stopped for a long period of time.
- *IL6* level are also affected from the long term smoking and the amount of cigarettes

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