Research Article

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Evaluation of the relationship between surfactant protein D levels and COVID-19 clinical severity: a case-control study

Sürfaktan protein D düzeyleri ile COVID-19 klinik şiddeti arasindaki ilişkinin değerlendirilmesi: bir vaka kontrol çalışması

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Abstract

Purpose: Serum surfactant protein D (SP-D) plays roles in the body such as protection against viral infection, bacterial and fungal clearance, clearance of apoptotic cells and suppression of inflammation. This study aims to examine the relationship between SP-D level and coronavirus disease (COVID-19) severity.

Methods: 80 patients (30 with mild disease and 50 with severe/critical COVID-19), and 50 healthy volunteers were enrolled in the study. SP-D levels were analyzed by ELISA in serum samples.

Results: The median of SP-D was found to be 2.47 (1.67-7.79) ng/ml in mild disease and 5.65 (3.09-16.55) ng/ml in severe/critical disease groups, while 2.89 (10.8-6.24) ng/ml in the healthy controls. The differences in SP-D levels between the severe/critical disease group compared to both mild disease and control groups were found statistically significant (p=0.007 and 0.001, respectively). ROC analysis showed greater AUC for the serum SP-D levels of the severe/critical COVID-19 patients compared to mild COVID-19 disease patients (AUC=0,691, 95% CI=0.56-0,822; p=0.004). Furthermore, SP-D levels were 86% sensitive and 51.6% specific at 2.44 ng/ml level (p=0.004) to detect severe/critical patients.

Conclusion: SP-D levels is useful for COVID-19 patients in the prediction of clinical severity and prognosis. SP-D is a valuable biomarker for predicting the clinical severity and prognosis.

Key words: Serum surfactant protein D, COVID-19, pneumonia.

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Öz

Amaç: Serum surfaktan protein D (SP-D) vücutta viral enfeksiyona karşı koruma, bakteriyel ve fungal klirens, apoptotik hücrelerin temizlenmesi ve inflamasyonun baskılanması gibi roller oynar. Bu çalışma, SP-D düzeyi ile koronavirüs hastalığı (COVID-19) şiddeti arasındaki ilişkiyi incelemeyi amaçlamaktadır.

Gereç ve yöntem: Çalışmaya 80 hasta (30 hafif hastalığı ve 50 ağır/kritik COVID-19) ve 50 sağlıklı gönüllü dahil edildi. Serum örneklerinde SP-D seviyeleri ELISA ile analiz edildi.

Bulgular: SP-D düzeyi ortanca değeri hafif hastalıkta 2,47 (1,67-7,79) ng/ml, ağır/kritik hastalık gruplarında 5,65 (3,09-16,55) ng/ml iken, sağlıklı control grubunda 2,89 (10,8-6,24) ng/ml olarak bulundu. Ağır/kritik hastalık grubu hem hafif hastalık hem de kontrol grubu ile karşılaştırıldığında SP-D düzeylerindeki farklılıklar istatistiksel olarak anlamlı bulundu (sırasıyla p=0,007 ve 0,001). ROC analizi, hafif COVID-19 hastalığı hastalarına kıyasla şiddetli/kritik COVID-19 hastalarında serum SP-D seviyeleri için eğri altında kalan alan daha yüksek saptandı (AUC=0.691, %95 CI=0,56-0.822; p=0,004). Ayrıca, ağır/kritik hastaları saptamak için SP-D düzeyleri 2,44 ng/ml düzeyinde (p=0,004) %86 duyarlı ve %51,6 özgül saptandı.

Sonuç: SP-D seviyeleri, COVID-19 hastaları için klinik şiddeti ve prognozu tahmin etmede faydalıdır. SP-D, klinik şiddeti ve prognozu tahmin etmek için değerli bir biyobelirteçtir.

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Anahtar kelimeler: Sürfaktan protein D, COVID-19, pnömoni.

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Introduction

SARS-CoV-2 virus infection was reported by World Health Organization (WHO) in the beginning of March 2020 as a world-wide pandemic and SARS-CoV-2 virus infection were called as coronavirus disease (COVID-19) [1, 2].

Studies on COVID-19 disease have documented that COVID-19 patients can develop symptoms of mild or severe acute respiratory infection. Mild symptoms include upper respiratory tract symptoms such as fever, dry cough, and fatigue. Severe symptoms include dyspnea, diarrhea, clinical onset of serious pneumonia, acute respiratory distress syndrome (ARDS) or multiple organ dysfunction [3, 4].

In lung tissue, type II alveolar cells produce functional substances such as surfactants and release them onto the surface, thereby helping with gas exchange [5]. Lung surfactant is a unique compound enriched with phospholipids and four surfactant protein (SP) types. Two of these proteins hydrophilic are the surfactant protein D (SP-D) and surfactant protein A (SP-A), also called as collectins, and the other two have lipophilic structure named as surfactant protein B (SP-B) and surfactant protein C (SP-C) [6]. Being a member of collagen-containing C-type lectins, serum SP-D is a hydrophilic structured protein [7]. SP-D is secreted from alveolar type II pneumocytes and secretory bronchiolar epithelial cells named as Clara cells [8].

SP-D is preliminarily produced inside the respiratory system, thus it has been assessed as a potential biomarker in some subjects having allergic bronchopulmonary aspergillosis in pneumonia, drug-induced lung disease, cystic disease and interstitial fibrosis [9-11]. It has been also shown to decrease in bronchoalveolar lavage in individuals having chronic obstructive pulmonary disease (COPD) [12].

Our study aimed to enlighten the variations of levels of SP-D protein in cases diagnosed with mild COVID-19 disease and severe/critical COVID-19 (+) disease compared with the healthy population within a high number of individuals. The secondary aim of this study was to determine the diagnostic and prognostic features of SP-D as a biomarker.

Materials and methods

Study population

The ethics approval for our prospective case-control study was received from the Pamukkale University Non-Invasive Clinical Trials Ethics Committee. The study was carried out between 01.11.2020 and 15.12.2020. After being informed concerning with the study, all of the individuals were asked to give written consent to be admitted in the study. The individuals were assessed by means of inclusion and exclusion criteria. COVID-19 infection was diagnosed and assessed clinically according to WHO guideline as a result of clinical assessment in the emergency department (ED). As the diagnosis of the patients were confirmed by reverse transcription polymerase chain reaction (RT-PCR) [13]. 80 patients (30 patients mild COVID-19 disease; 50 patients severe/critical COVID-19 disease) and 30 healthy controls without symptoms were included in the study.

Mild COVID-19 disease group

This group involved the patients (a) who admitted to the COVID-19 outpatient clinic (b) whose CT imagings were normal and PCR tests were positive.

Severe/Critical COVID-19 disease group

This group involved the patients (a) who admitted to the ED with symptoms and was diagnosed with severe/critical COVID-19 infection according to WHO guideline [13] (b) who had positive RT-PCR nasopharyngeal swab samples collected in ED included in the study.

Control group

This group involved the volunteers who not having a history of acute, subacute or chronic

disease; no infection in the last fortnight; no particular medication and have no contact with people infected with COVID-19 admitted to ED with non-infectious complaints.

The exclusion criteria for the present study were kidney failure, liver failure, chronic inflammatory disease history, confirmation of any cancer onset, history of cerebrovascular disease, respiratory diseases such as acute pulmonary embolism, asthma disease and COPD, and pregrancy. Moreover, the patients having CT imaging results evaluated as COVID-19 pnemonia and negative PCR tests were not involved in the study.

Data collection

Medical history, demographic data, vital findings, biochemical parameters (hemogram, C-reactive protein (CRP), ferritin, D-dimer, and high-sensitivity troponin T (hsTnT)) and radiological findings, time to onset of symptoms, CT severity scores were collected for statistical analysis.

Computerized Tomography (CT) evaluation

The thoracic CT severity scores were calculated, as cited in the literature, by an emergency medicine specialist blinded to the study [14].

Clinical evaluation and management

The individuals were evaluated clinically based on guidelines of WHO used for COVID-19 diagnosis and treatment [13]. On the basis of the up-date of this guide, the algorithm used for patient management was also edited.

The complications of the patients after 6 months and the drugs used in the treatment of COVID-19 infection were also screened. It was also recorded whether they received non-macrolide antibiotic treatment due to superinfection.

It was observed that Hydroxyqloroquin, Favipiravir, Clarithromycin (for patients who had pneumonia findings) and Enoxaparin treatment were started at ED in all patients according to the current treatment guideline of the Turkish Ministry of Health [15], and pulse steroid therapy was given in addition to this treatment in severe/critical patients.

Blood samples and laboratory parameters

Routinely checked parameters of hemogram, and serum CRP, ferritin, creatinine, urea and D-dimer during their examination in ED were evaluated. Blood sample of 2 cc drawn from antecubital vein into a dry test tube was centrifugated for 15 minutes at 5000 rpm and its serum component was separated for measurement of SP-D level. Then, serum SP-D level was meassured using the Enzyme-Linked Immunosorbent Assay (ELISA) method. The SP-D levels was measures via same protocol in that laboratory. The laboratory results obtained from the patients admitted to ED were recorded for statistical analysis.

Surfactant Protein D (SP-D) level measurement

A commercial ELISA kit (Human SP-D ELISA Kit, Elabscience, E-EL-H1269, USA), was used to measure serum SP-D levels.

Data analysis

As a planned reference study was not established in the literature, a power analysis was conducted prior to the present study. The power analysis presented that minimum 112 individuals (minimum 28 for each group) were required to obtain 95% power at 95% confidence interval, predicting a medium-high effect size (f=0.4). SPSS software program was used for statistical analysis of the data set. The continuous variables were expressed as median (IQR), while numbers and percentages were used for the categorical variables. Kolmogrov-Smirnov test was used for calculation of parametric distribution for the continuous data Mann-Whitney U test and Kruskal-Wallis variance analysis and were conducted to compare the differences in independent groups. The relationships among continuous variables were evaluated with Spearman correlation. Chi-square test was used for the analysis of categorical variables. To measure the discriminant performance of SP-D levels ROC curve analysis was conducted. Binary logistic regression analysis was performed for evaluating of effects of the parameters on disease severity. $P<0.05$ was evaluated as statistically significant.

Results

The gender distribution and mean age were similar in the groups (p=0.491 and p=0.609 respectively).

Vital parameters of the groups were given in Table 1.

SP-D level median values were 2.89 (10.8-6.24) ng/ml in control group, 2.47 (1.67-7.79) ng/ml in mild disease group, 5.65 (3.09-16.55) ng/ml in severe/critical disease group (Table 2). There was a statistical significance between the SP-D levels among the groups (p=0.001). Serum SP-D level was determined higher in the severe/critical disease group compared to both the control group and the mild disease group (p=0.007 and 0.001) (Table 1) (Figure 1).

Hemogram, biochemical and blood gas analysis parameters in the study groups are given in Table 2.

The correlations between serum surfactant associated protein D level and clinical and laboratory parameters were examined in patients who have COVID-19 infection. SP-D level and sPO_2 level were found mild negative correlated (rho=-0.248 and p=0.026).

CT severity and SP-D levels were found moderate positive correlated (rho=0.42 and p=0.002) (Table 3)

Table 1. Clinical and demographical datas of the groups

		Control (N=30)	**Mild Disease** (N=30)	**Severe-Critical Disease** (N=50)	***p*-Value**
		Median (IQR)	*Median (IQR)*	*Median (IQR)*	
Symptom Duration (Day)			2 (0-3)	3 (2-6.25)	***[1]p=0.003***
Fever (^{0}C)		36.6 (36.5-36.75)	36.7 (36.5-37.1)	36.7 (36.5-37.2)	*p=0.071*
sPO_2		96 (95-98)	97.5 (96-98)	95 (93.75-96)	***p=0.0001***
SBP (mm/Hg)		120 (112-150)	120 (110-137)	125 (110-140)	*p=0.418*
DBP (mm/Hg)		80 (70-85)	80 (70-80)	80 (70-80.5)	*p=0.503*
Age (Year)		57 (40.75-46.5)	50.5 (46-56)	54 (37.75-68)	*p=0.428*
Gender	**Male**	14 (46.7%)	16 (53.3%)	21 (42%)	
	Female	16 (53.3%)	14(46.7%)	29 (58%)	*[5]p=0.616*
SP-D (ng/mL)		2.89 (1.08-6.24)	2.47 (1.67-7.79)	5.65 (3.09-16.55)	***p=0.001 [1]p=0.007 [3]p=0.387 [4]p=0.001***
Comorbidities					
Hypertension N(%)			12 (40%)	21 (42%)	*[5]p=1*
Diabetes Mellitus, N(%)			9 (30%)	19(38%)	*[5]p=0.629*
Coronary Artery Disease, N(%)			10 (33.3%)	22 (36%)	*[5]p=0.48*

p-values are derived from Kruskal Wallis test, [1]*p*-values are derived from Mann Whitney U test
[3]*p*-value is derived from Mann Whitney U test and refers to the comparison between Control and mild disease groups
[4]*p*-value is derived from Mann Whitney U test and refers to the comparison between Control and severe-critical disease groups
[5]*p*-values are derived from chi square test
IQR: Interquartile range, SBP: Systolic Blood Pressure, DBP: Diastolic Blood Pressure
SP-D: surfactant proten D

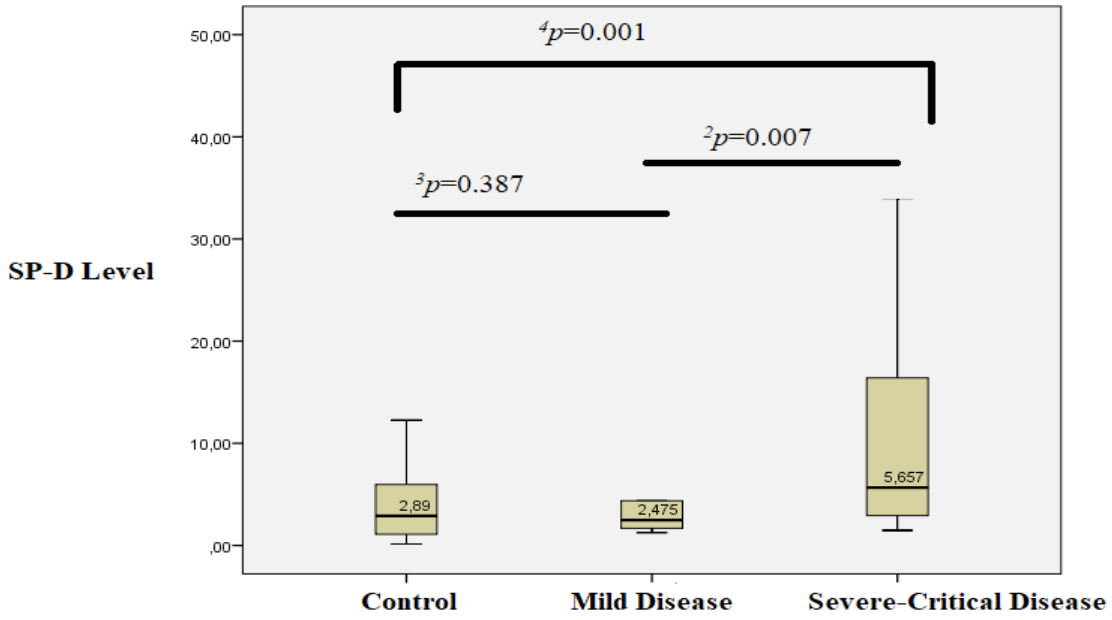

Figure 1. SP-D levels

Table 2. Laboratory parameters of the groups

	Control (N=30)	**Mild Disease** (N=30)	**Severe-Critical Disease** (N=50)	***p*-Value**
	Median (IQR)	*Median (IQR)*	*Median (IQR)*	
WBC (K/µL)	7.62 (5.23-11.24)	7.57 (5.39-10.04)	7.14 (4.74-10.79)	[1]*p*=0.681
Hb (g/dL)	11.7 (11.07-13.47)	14.2 (12.07-15.95)	13.85 (11.67-15.22)	[1]*p*=0.003
Neu. (K/µL)	4.49 (3.05-7.68)	3.97 (3.04-6.74)	4.54 (2.86-7.6)	[1]*p*=0.83
Lymph. (K/µL)	1.4 (0.84-1.94)	1.88 (1.46-3.26)	1.4 (1.09-2.14)	[1]*p*=0.015 [2]*p*=0.008
Plt. (K/µL)	211 (163.5-293.2)	254 (224.5-299.2)	228 (181.7-294.5)	[1]*p*=0.374
CRP (mg/L)	1.8 (1.08-2.91)	1.67 (0.38-6.74)	21.34 (4.71-65.99)	[1]*p*=0.0001 [2]*p*=0.0001
D-Dimer (ng/mL)		161 (56-288)	238 (141.5-599.25)	[2]*p*=0.015
Ferritin (ug/L)		55.59 (18.6-152.87)	170.3 (62.7-311)	[2]*p*=0.008
hsTnT (µg/L)		3.66 (3-5.49)	5 (3.12-13)	[2]*p*=0.054

[1]*p*-values are derived from Kruskal-Wallis test
[2]*p*-values are derived from Mann-Whitney U test and refers to the comparison between mild disease and severe-critical disease groups
WBC: White blood cell, hb: hemoglobin, Neu: neutrophil count, Lymph: lymphocyte count
Plt: platelet count, CRP: C-Reactive Protein, hsTnT: high sensitive troponin T

Table 3. Correlations between Surfactant Associated Protein (SP-D) levels and laboratory and clinical parameters in patients groups

		SP-D
Fever	*rho*	0.1
	p Value	0,301
sPO_2	*rho*	**-0.248**
	p Value	**0,026**
SBP	*rho*	-0.018
	p Value	0,873
DBP	*rho*	-0.08
	p Value	0,479
WBC Count	*rho*	-0.9
	p Value	0,425
Hb	*rho*	-0.036
	p Value	0,751
Neu.	*rho*	-0.005
	p Value	0,966
Lymph.	*rho*	-0.142
	p Value	0,205
Plt.	*rho*	-0.17
	p Value	0,128
CRP	*rho*	0.172
	p Value	0,124
D-Dimer	*rho*	0.043
	p Value	0,720
Ferritin	*rho*	0.139
	p Value	0,263
hsTnT	*rho*	0.026
	p Value	0,845
CT Severity Score	*rho*	**0.42**
	p Value	**0,002**
Symptom Duration	*rho*	0.02
	p Value	0,863

p and rho values are derived from Spearman Correlation test, SBP: Systolic Blood Pressure
DBP: Diastolic Blood Pressure, WBC: White blood cell, Hb: hemoglobin, Neu: neutrophil count
Lymph: lymphocyte count, Plt: platelete count, CRP: C-Reactive Protein, hsTnT: high sensitive troponin T

ROC analysis presented greater AUC for the serum SP-D levels of the severe/critical COVID-19 patients compared to mild COVID-19 disease patients (AUC=0.691, 95% CI=0.56-0.822; *p*=0.004). Furthermore, the SP-D level was evaluated to be 86% sensitive and 51.6% specific at 2.44 ng/ml level (*p*=0.004) (Figure 2) to detect severe/critical patients.

When the data whether the patients received antibiotherapy for superinfection after being evaluated at ED were considered, only 4 (13.3%) patients in the mild disease group and 21 (42%) patients in the severe/critical disease group were reported to have received antibiotherapy. The median level of serum SP-D was found to be higher in patients who needed antibiotic therapy than in patients who did not need antibiotics in the severe/critical disease group (Figure 3).

Regression analysis results were given in Table 4.

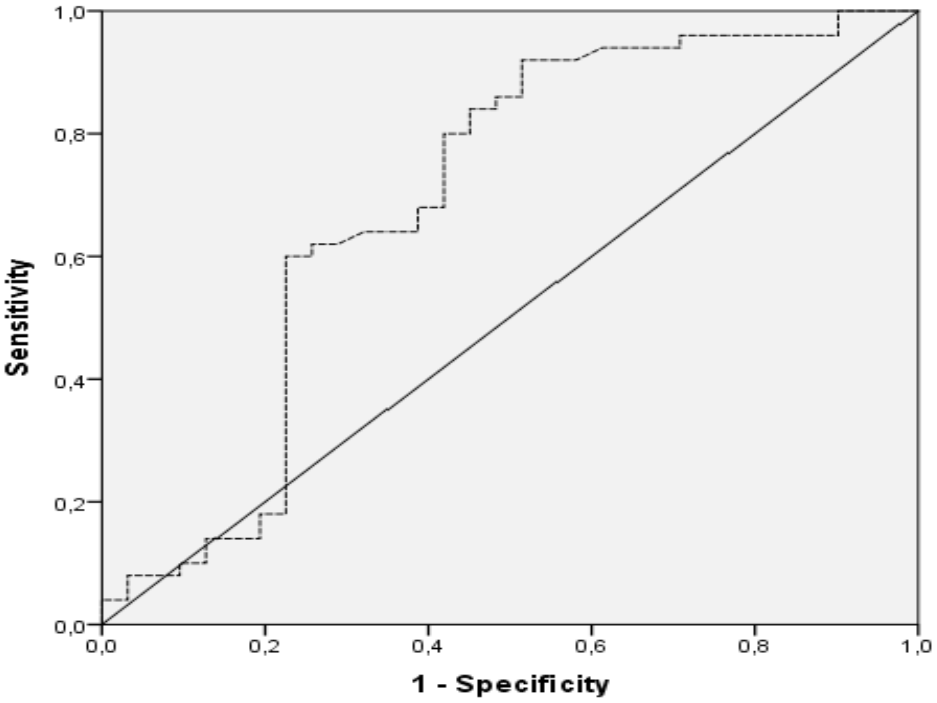

Figure 2. ROC curve analysis for predicting clinical severity

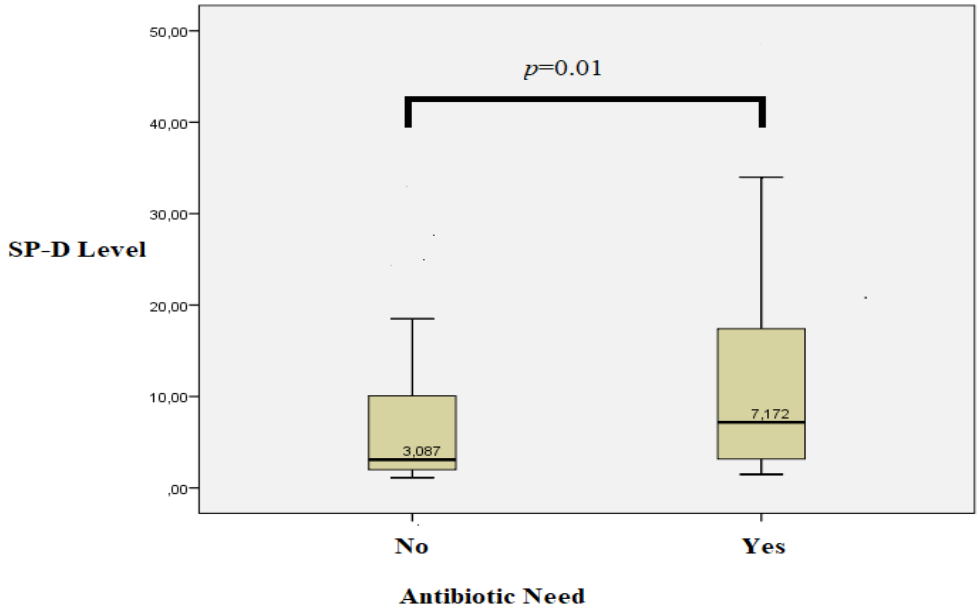

Figure 3. Serum SP-D levels in patient who have antibiotic need or not in severe-critical group

Table 4. Regression analysis between the parameters and disease severity of the patients

	B	S.E	Wald	*p*-Value	OR	95% CI	
						Lower	Upper
SP-D Level	0.067	0.034	3.885	0.049	1.069	1.000	1.142
Fever	0.148	0.475	0.097	0.755	1.160	0.457	2.944
SpO_2	-0.161	0.075	4.609	0.032	0.852	0.735	0.986
Systolic BP	0.001	0.017	0.001	0.972	1.001	0.968	1.034
Diastolic BP	0.021	0.030	0.495	0.482	1.021	0.964	1.082
WBC	0.435	0.504	0.747	0.388	1.545	0.576	4.146
Hemoglobin	-0.037	0.117	0.100	0.752	0.964	0.767	1.212
Neutrophil counts	-0.305	0.578	0.278	0.598	0.737	0.238	2.289
%Neu	0.149	0.103	2.108	0.147	1.161	0.949	1.421
Lymphocyte counts	-1.552	0.675	5.286	0.022	0.212	0.056	0.795
%Lymphocyte	0.276	0.115	5.726	0.017	1.317	1.051	1.651
Platelete	0.000	0.001	.012	0.912	1.000	0.998	1.002
Monocyte counts	-0.183	0.372	.243	0.622	0.833	0.402	1.725
Gender	1.082	0.564	3.684	0.055	2.951	0.977	8.910
Comorbidity existence	-2.173	0.586	13.775	0.0001	0.114	0.036	0.359

WBC: White blood cell

Discussion

SP-D, possesses a key role in lungs, in the system of humoral and innate immunity; binds a wide range of pathogenic microorganisms, inhibits the growth of microorganisms, is involved in damage bacterial membrane, stimulates phagocytosis, chemotaxis, regulates the expression of cytokines and the production of free radicals [7].

SP-D provides interaction with pathogenic microorganisms (both gram-negative, such as Klebsiella pneumoniae, Haemophilus influenza, Pseudomonas aeruginosa and Escherichia coli, and gram-positive, mycobacteria, viruses, including influenza virus, fungi), acting as an attractant for immune cells, thereby performing the classic opsonizing functions [7, 16]. SP-D play crucial a role in the cascade and modeling of inflammatory responses. SP-D regulates the scavenging of apoptotic cells and bodies and inhibits the release of cytokines and other pro-inflammatory products [16, 17].

To date, there are many indications of how the structure of the surfactant and its components, in particular its proteins SP-D, changes during a respiratory infection [16, 18-20]. Pathogenic microorganisms, entering the respiratory tract, change the surface balance by several mechanisms, which leads to a direct decrease in the content of apoprotein and lipid components inside the alveoli, as well as to the degradation of collectins [20-22].

Most of the studies (more than 200 publications over the past 10 years) concern the levels of surfactant proteins SP-D in ARDS of various etiologies (it is important that up to 80% of ARDS associated with pneumonia), a significantly smaller number of publications - for chronic lung diseases and isolated ones for community-acquired pneumonia (CAP) [23, 24]. Serum levels of SP-D have been determined as biomarkers for other respiratory diseases, including COPD [25], systemic sclerosis [26] and interstitial lung diseases (including those associated with rheumatoid arthritis) [27], sarcoidosis [10], play an important role in differential diagnosis and have prognostic value.

Higher baseline concentrations of SP-D in blood plasma were associated with higher mortality and severe course of lower respiratory tract infection complicated by mechanical ventilation and the addition of organ failure [28]. In single studies of the SP-D protein level in patients with CAP compared with healthy patients, not only the presence of a higher indicator, but also a direct relationship of this

protein in the development of life-threatening complications and mortality in CAP has been shown [29, 30].

In a similar study, severe COVID-19 had higher SP-D levels than non-severe group according to the CT imaging results [30]. Saito et al. [31] also suggested that SP-D levels might be a distinctive biomarker in the diagnosis of COVID-19 infection, where CT image findings were not present [31]. In addition, in a study, it was observed that mortality decreased and the need for mechanical ventilation decreased after bronchoscopy was performed in ARDS cases caused by COVID-19 [32].

Kerget et al. [33] identified higher levels of SP-D in non-survivors compared to surviving patients from COVID-19. In another study conducted by Choreno-Parra and colleagues, SP-D levels were assessed in serious H1N1 and COVID-19 patients and declared SP-D levels as good prognostic markers in severe H1N1, but it is not a good biomarker for differentiation COVID-19 and H1N1 patients [34].

In the present study, we identified higher levels of SP-D in COVID-19 patients (both mild and severe/critical patients) compared to healthy controls; however, despite the mean values were higher, the difference between the mild patients and control were not statistically significant and thus, it is unlikely to indicate SP-D as a good diagnostic marker for COVID-19. And also we have determined increased SP-D levels in patients who have superinfection during the COVID-19 treatment in hospital. These results may show that SP-D levels might be a prognostic marker for predicting superinfected patients.

Furthermore, SP-D might be evaluated as a prognostic determinant after clinical diagnosis of severe/clinical patients with 86% of sensitivity and 51.6% specificity, which are parallel to previous studies in the literature. In addition, we identified a significant correlation between serum SP-D levels and CT severity score in COVID-19 patients. This finding contributes to the prognostic value of SP-D for COVID-19 patients, as well.

In conclusion, our findings clearly demonstrate the value of serum SP-D levels in predicting the clinical severity and prognosis of COVID-19 patients. SP-D is a valuable marker for predicting the clinical severity, prognosis and superinfections. Development of new methods for the optimization of these proteins will also improve differential diagnosis of COVID-19.

Conflict of interest: No conflict of interest was declared by the authors.

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Conception: R.S. and A.K.; Study design: R.S., A.K., E.K., P.B., and O.K.; Funding: A.K.; Materials: T.G., A.K., I.T. and O.K.; Data collection and processing: E.K., T.G., A.K., I.T. and R.S.; Literature review: E.K., R.S., A.K. and A.K.; Writers: E.K., R.S., A.K., P.B. and A.K.; Critical review: O.K.