suPAR LEVELS AND COVID-19: IS THERE A RELATIONSHIP BETWEEN suPAR LEVELS AND PROGNOSIS OF COVID-19 INFECTION?

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ABSTRACT

Objective*: suPAR is known as a marker for inflammation. In this study, we aimed to analyse suPAR levels and its correlation with disease prognosis in COVID-19 patients.*

Method*: Demographical, clinical and laboratory data of the 36 patients were recorded. Existence of suPAR levels and other parameters along with prognosis was studied.*

Result*: Of 36 patients included in this study, 15 were female (42%) and 21 were male (58%). The median age of the patients with mortality was 73 (min-max ,IR; 49-88, 25), and the median age of the patients with no mortality was 72 (min-max ,IR; 47-83, 21) revealing a statistically non-significant difference (p=0,596). Among lab tests, hemoglobin (p=0,044), urea (p=0,011), troponin(p=0,033), LDH (p=0,005), and procalcitonin (p=0,036) were significantly associated with mortality. Median suPAR level was 102 (min-max, IR; 29-540, 274) for the patients with no mortality whereas, median suPAR level was 61 (min-max, IR; 29-540, 355) for the patients with mortality, and the difference was statistically non-significant (p=0,607).*

Conclusion*: suPAR levels seem to be ineffective to predict disease severity and prognosis of COVID-19. More randomised controlled trials with larger groups are needed to clarify the association of suPAR levels and COVID-19.*

Keywords: *suPAR, COVID-19, Prognosis.*

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Introduction

COVID-19 has turned into a serious public health problem by causing a worldwide outbreak and as a result of this, was declared as a "pandemic" by World Health Organisation (WHO)[1,2]. Significant relationships were found between prognosis and parameters such as advanced age, comorbidities, trombocyte, neutrophil and lymphocyte counts, albumin, white blood cell (WBC), lactate dehydrogenase (LDH), C-reactive protein (CRP) in patients with COVID-19[3-7].

Some biomarkers have great importance in the diagnosis, prognosis, and treatment of some diseases[8]. Recently, suPAR was found as a biomarker for diagnosis, prognosis, and treatment of various diseases[9]. suPAR is a circulatory signaling protein which is coded on chromosome 19q13 by Plaur gene and reproduced by immature myeloid cells. It is also known to have significant physiological

and pathophysiological roles as an inflammatory biomarker[10]. Some studies show that suPAR is an inflammatory biomarker related to sepsis, disease activity, malignancy and mortality[11,12].

suPAR is a protein that reflects fibrinolysis and inflammation. Increased suPAR levels represent immune system activation in conditions such as inflammation and infection. Increased suPAR levels were reported in systemic inflammatory response syndrome, bacteraemia and sepsis. Increased levels were associated with poor prognosis in critically ill patients[13]. Urokinase-type plasminogen activator (uPA) is secreted from leukocytes, monocytes and activated T cells. It plays a role in cell adhesion, migration, differentiation, and proliferation. When uPA binds its receptor (uPAR), cell migration is activated for proteolysis of the extracellular matrix. uPAR interferes with integrin[14]. Tissue plasminogen is activated after this interaction. What is more important, suPAR is released. suPAR mobilizes neutrophils to inflammation sites with its direct chemotactic effect[14,15].

Autopsy studies show that endothelial injury and thrombotic micro embolism play a major role in the pathogenesis of COVID-19[16]. Although uPA and uPAR are parts of the fibrinolytic system, there is not enough evidence about the role of suPAR in thromboembolism. Some studies show a higher incidence of thromboembolism in patients with high suPAR levels[17].

In the light of all the data mentioned above, we aimed to analyse suPAR levels and its correlation with disease prognosis in COVID-19 patients.

Material and method

COVID diagnosis: In our hospital, patients who were hospitalized with suspicion of coronavirus infection and who had tomography findings and / or PCR + were diagnosed with COVID-19 infection. 36 patients who were followed up in the clinic of Sakarya University Research and Training Hospital with diagnosis of COVID-19 between the dates of 1 April 2020 and 31 May 2020 were included in the study as "patient group".

Nasopharyngeal swab real time Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) test was used as gold standard for definitive diagnosis. Patients who were RT-PCR negative and had definitive diagnosis of malignancy, bacterial and/or fungal co-infection were excluded. Our study was approved by Sakarya University Medical Faculty Ethical Committee. Demographical, clinical and laboratory data of the patients were recorded.

Sample collection, nucleic acid isolation and SARS CoV-2 reverse transcriptase PCR reaction

Combined nasopharyngeal and oropharynx swab samples were taken into the viral transport medium immediately after being taken with dacron swab, kept in 2-8 °C, and delivered to the laboratory. The samples were sent to the laboratory in accordance with the cold chain rules with the triple transport system, following the infection prevention and control procedures. After acceptance of the samples in the microbiology laboratory, the samples were taken to a level 3 biosafe negative pressure chamber. Bio-Speedy® Viral Nucleic Acid Isolation Kit for the isolation of total nucleic acid from samples (bioeks, Turkey) were used. The isolation procedure was carried out in line with the manufacturer's recommendations. Bio-Speedy® COVID-19 RT-qPCR Detection Kit (bioeksen, Turkey) was used for RT-PCR analysis. PCR amplification and evaluation of the results were carried out in line with the manufacturer's recommendations.

suPAR

SuPAR has been analysed in a fully automated micro-ELISA device (Grifols, Triturus, Spain) by using the kit of SinoGeneClon Biotech Human Soluble urokinase-type plasminogen activator receptor (suPAR) ELISA Kit (Republic of China). Micro ELISA test procedure (piping, incubation, washing and reading operations) was applied in accordance with the manufacturer's instructions, and the serum levels of suPAR were measured quantitatively by removing the cut-off and calibration curve. Results were measured as pg/mL and recorded. Reference range for suPAR was taken into consideration as between 12 and 360 pg/mL. Analytic sensitivity of the kit is 3 pg/mL.

Statistical Analyses

Descriptive analyses were performed to provide information on the general characteristics of the study population. Visual (probability plots, histograms) and analytical methods (Kolmogorov-Smirnov/Shapiro-Wilk's test) were used to determine whether or not they are normally distributed. Descriptive analyses were presented using medians with minimum-maximum values, interquartile range (IR) for the non-normally distributed variables; using means with standard deviation (±SD). The

Mann-Whitney U test was used for nonparametric tests, Student's t-test was used for parametric tests to compare these parameters. Chi-square test was used to compare the categorical variables between two groups. The categorical variables were presented as the frequency (% percentage). The significance level for all of the statistical tests was set at p<0.05. Analyses were performed using SPSS statistical software (IBM SPSS Statistics, Version 23.0. Armonk, NY: IBM Corp.)

Results

36 patients were included in the study. 15 patients were female (42%), and 21 patients were male (58%). 18 patients were intubated (50%) and 15 patients died (42%) throughout the course of the disease. Of 15 patients with mortality, 7 were female (46,7%) and 8 were male (53,3%) (p=0,607). The median age of the patients with mortality was 73 (min-max ,IR; 49-88, 25), and the median age of the patients with no mortality was 72 (min-max, IR; 47-83, 21) revealing a statistically non-significant difference (p=0,596). There was no association between mortality and symptoms at the time of diagnosis such as fever, cough, sore throat, shortness of breath (p>0,05).

	survivor n=21	nonsurvivor n=15	total=36	p
AGE, median (min-max) [IR]	72 (47-83) [21]	73 (49-88) [25]	72 (47-88) [22]	0,596**
SEX female, n (%)	8 (38,10%)	7 (46,70%)	15 (41,70%)	0,607***
male, n (%)	13 (61,90%)	8 (53,30%)	21 (58,30%)	
Non-intubated, n (%)	18 (85,7%)	0 (0,00%)	18 (50,00%)	**0,000*** **
Intubated, n (%)	3 (14,30%)	15 (100,00%)	18 (50,00%)	
Renal replasman tedavisi, n (%)	1 (4,80%)	9 (60,00%)	10 (27,80%)	**0,000*** **

Table 1: Demographical features of the patients with COVID-19.

There was no association between mortality and comorbidities such as diabetes, hypertension, coronary artery disease, COPD, asthma, chronic kidney disease, cerebrovascular disease, atrial fibrillation (p>0,05). There were 10 patients receiving renal replacement therapy (hemodialysis, hemodiafiltration) (27,8%) and 1 patient not receiving renal replacement therapy (4,8%) in the group with mortality (p=0,000). Among lab tests, hemoglobin (p=0,044), urea (p=0,011), troponin(p=0,033), LDH (p=0,005), and procalcitonin (p=0,036) were significantly associated with mortality. Median suPAR level was 102 (min-max, IR; 29-540, 274) for the patients with no mortality whereas, median suPAR level was 61 (min-max, IR; 29-540, 355) for the patients with mortality, and the difference was statistically non-significant (p=0,607).

	survivor n=21	nonsurvivor n=15	total=36	p
WBC, median (min-max) [IR]	5280 (3100-21700) [3710]	7200 (3590-26000) [10050]	5990 (3100-26000) [4760]	0,205**
HB, mean (±SD)	13,4 (2,1)	11,9 (2,2)	12,8 (2,3)	**0,044***
LEU, median (min-max) [IR]	1110 (209-2550) [1000]	806 (250-2030) [574]	913,5 (209-2550) [1018,5]	0,098**
NEU, median (min-max) [IR]	3830 (1640-19200) [3510]	6290 (3010-23100) [8840]	4970 (1640-23100) [4635]	0,065**
PLT, median (min-max) [IR]	181 (98-421) [80]	163 (49,2-717) [153]	177 (49,2-717) [107]	1**
GLUCOSE, median (min-max) [IR]	119 (91-280) [38]	110 (46-246) [64]	116 (46-280) [45]	0,360**
UREA, median (min-max) [IR]	41 (14-136) [28]	67 (29-188) [83]	57,5 (14-188) [44,5]	**0,011****
CRE, median (min-max) [IR]	0,9 (0,5-5,4) [0,3]	1,2 (0,7-7,3) [1,1]	1 (0,5-7,3) [0,5]	0,092**
PT, median (min-max) [IR]	13,4 (10,6-22,3) [1,9]	13,3 (11,1-17,5) [4,1]	13,3 (10,6-22,3) [2,4]	0,701**
APTT, median (min-max) [IR]	29,3 (21,5-69,4) [9]	29,3 (24,1-56,3) [6,9]	29,3 (21,5-69,4) [7,4]	0,925**
INR, median (min-max) [IR]	1,23 (1,05-2,05) [0,18]	1,22 (1,02-1,61) [0,37]	1,22 (1,02-2,05) [0,22]	0,920**
DDIMER, median (min-max) [IR]	759 (25-24800) [2182]	1070 (135-25000) [2047]	1040 (25-25000) [2145,5]	0,217**
TROP, median (min-max) [IR]	11,7 (0,3-524) [28,1]	36,1 (6,2-173,7) [40,2]	18,5 (0,3-524) [30,3]	**0,033****
FERRITIN, median (min-max) [IR]	292 (12-34323) [442]	734 (119-40000) [1462]	457 (12-40000) [943]	0,178**
LDH, median (min-max) [IR]	280,5 (127-876) [97]	551 (235-7171) [306]	311 (127-7171) [338]	**0,005****
CRP, median (min-max) [IR]	49 (10-236) [134]	141 (3-194) [81]	130 (3-236) [146]	0,156**
Procalcitonin, median (min-max) [IR]	0,13 (0,02-100) [0,23]	0,31 (0,07-9,87) [1,1]	0,25 (0,02-100) [0,42]	**0,036****
CK, median (min-max) [IR]	71,5 (19-304) [70,5]	77 (33-1964) [458]	73,5 (19-1964) [80]	0,248**
FIBRINOGEN, mean (±SD)	362,5 (69,7)	421,1 (85,7)	395,3 (83)	0,08*
suPAR, median (min-max) [IR]	102 (29-540) [274]	61 (29-540) [355]	88 (29-540) [299]	0,908**
*Student's t-test, **Mann–Whitney U test, ***chi-squared test				

Table 2: Analysis of the survivor and non-survivor patients with COVID-19.

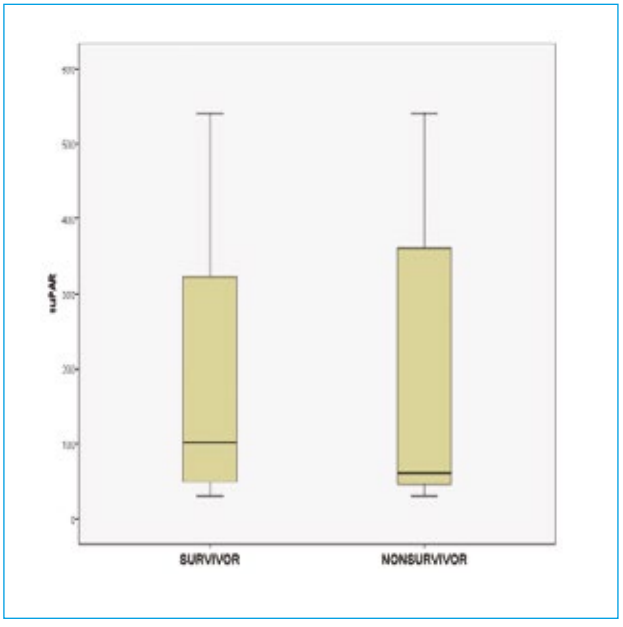

Fig. 1: Demonstration of suPAR levels in survivor and non-survivor patients with Box-plot graphic.

Discussion

High levels of suPAR was found to be associated with poor prognosis in patients with cancer, HIV, tuberculosis, pneumonia, diabetes, various bacterial and parasitic infections, and sepsis[18]. We tried to figure out whether suPAR levels are associated with prognosis in COVID-19 patients in this study. Median suPAR level was 88 (29-540) (299) for all patients, 102 for survivor group (29-540) (274) and 61 (29-540) (355) for nonsurvivor group. No significant relationship was found between suPAR levels and mortality (p>0,05).

The significant role of thromboembolism was shown in pathogenesis of COVID-19. The significance of coagulopathy in COVID-19

pathogenesis was shown by high levels of D-dimer and fibrin products in the patients. The increase of PT and PTT and decrease of antithrombin levels were found less in COVID-19 patients when compared to sepsis related coagulopathy[16,19-21]. The cause of thrombocytopenia in COVID-19 patients is not certain yet. Since the relationship of suPAR with prognosis of COVID-19 patients was analysed in this study, we consider that this study can make a contribution to the literature.

High levels of suPAR were found to be associated with poor prognosis in sepsis in a meta-analysis consisting of 30 study/6906 cases. However, even in this meta-analysis, it is emphasized that more studies are needed to clarify the role of suPAR[12]. In another study, suPAR levels <4 ng/ml was found to be associated with safe discharge while suPAR levels >6 ng/ml was found as a risk factor for poor outcomes. In addition to this, suPAR levels must be interpreted according to the history of the patient. Levels >12 ng/ml in critically ill patients are related with 17-50% of 28 days mortality rate[22]. suPAR showed a promising diagnostic effect in a study which suPAR and procalcitonin levels of 59 patients with sepsis and 29 patients with systemic inflammatory response syndrome (SIRS) were analysed[23].

Pneumonia, acute lung injury and ARDS can occur during the course of COVID-19. Urokinase plasminogen activator system can cause the inflammatory process. It has been speculated that serum suPAR levels could be used as a biomarker for disease progression in COVID-19 and medications targeting uPA/uPAR system could be used as a treatment in COVID-19[24,25]. In another study in which 15 patients with COVID-19 were analysed, suPAR levels ≥ 6 ng/ml were significantly associated with a higher need for mechanical ventilation[26]. In a study in which 352 patients with COVID-19 were analyzed, it was reported that acute renal failure was found in 91 patients (25,9%) and 25 patients had to be treated with dialysis (7%). As a result of this study, it was stated that high suPAR levels can be predictive for acute renal failure and need for dialysis in patients with COVID-19. None of the patients with suPAR levels <4,60 ng/ml required dialysis therapy, however mortality rates were not reported in this study[27]. In our study it was found that there was an association between dialysis requirement and mortality in patients with COVID-19. However, in our study, there was not any significant relationship between suPAR levels and prognosis.

Limitation

Before the last comment, we must report the limitations of our study. The number of cases are limited in both arms of this study (survivor-nonsurvivor). For this reason, we consider that more studies with a higher number of cases are needed to clarify the relationships between suPAR levels and diagnosis, follow up and treatment processes.

Conclusion

There was not any relationship between suPAR levels and prognosis and severity of COVID-19. More randomised controlled trials with larger groups are needed to clarify the association of suPAR levels and COVID-19.

References

1) Zarocostas J. What next for the coronavirus response? The Lancet. 2020; 395(10222):401. doi:10.1016/s0140-6736(20)30292-0
2) Sohrabi C, Alsafi Z, O'Neill N, et al. World Health Organization declares global emergency: A review of the 2019 novel coronavirus (COVID-19). Int J Surg. 2020; 76: 71-76.
3) Lauc G, Sinclair D. Biomarkers of biological age as predictors of COVID-19 disease severity. Aging . 2020; 12(8): 6490-6491.
4) Kang SJ, Jung SI. Age-Related Morbidity and Mortality among Patients with COVID-19 . Infect Chemother. June 2020. https://www.ncbi.nlm.nih.gov/pubmed/32537961.
5) Lippi G, Plebani M, Henry BM. Thrombocytopenia is associated with severe coronavirus disease 2019 (COVID-19) infections: A meta-analysis. Clin Chim Acta. 2020; 506: 145-148.
6) Henry BM, de Oliveira MHS, Benoit S, Plebani M, Lippi G. Hematologic, biochemical and immune biomarker abnormalities associated with severe illness and mortality in coronavirus disease 2019 (COVID-19): a meta-analysis. Clin Chem Lab Med. 2020; 58(7): 1021-1028.
7) Violi F, Cangemi R, Romiti GF, et al. Is Albumin Predictor of Mortality in COVID-19 ? Antioxid Redox Signal. June 2020. doi:10.1089/ars.2020.8142
8) Strimbu K, Tavel JA. What are biomarkers? Curr Opin HIV AIDS. 2010; 5(6): 463-466.
9) Law RHP, Abu-Ssaydeh D, Whisstock JC. New insights into the structure and function of the plasminogen/plasmin system. Curr Opin Struct Biol. 2013; 23(6): 836-841.
10) Castellino FJ, Ploplis VA. Structure and function of the plasminogen/plasmin system. Thromb Haemost. 2005; 93(4): 647-654.
11) Chen D, Wu X, Yang J, Yu L. Serum plasminogen activator urokinase receptor predicts elevated risk of acute respiratory distress syndrome in patients with

sepsis and is positively associated with disease severity, inflammation, and mortality. Exp Ther Med. 2019; 18(4): 2984-2992.

12) Huang Q, Xiong H, Yan P, et al. The Diagnostic and Prognostic Value of suPAR in Patients with Sepsis: A Systematic Review and Meta-Analysis. Shock. 2020; 53(4): 416-425.

13) Bilgili B, Cinel İ. Yoğun Bakım Hastalarında "Soluble Urokinase Plasminogen Activator Receptor (suPAR)"ın Önemi. Türk Yoğun Bakım Derneği Dergisi. 2013; 11(1): 33-39. doi:10.4274/tybdd.33042

14) Mustjoki S, Sidenius N, Vaheri A. Enhanced release of soluble urokinase receptor by endothelial cells in contact with peripheral blood cells. FEBS Lett. 2000; 486(3): 237-242.

15) Jackson MR, Moore NR, Ritchie HM, Booth NA. Enhanced cell-associated u-PA activity of endothelial cells in response to soluble factors released by human peripheral blood monocytes. Blood Coagulation & Fibrinolysis. 1996; 7(7): 728. doi:10.1097/00001721-199610000-00026

16v Wichmann D, Sperhake J-P, Lütgehetmann M, et al. Autopsy Findings and Venous Thromboembolism in Patients With COVID-19 . Ann Intern Med. May 2020. doi:10.7326/M20-2003

17) Engström G, Zöller B, Svensson PJ, Melander O, Persson M. Soluble urokinase plasminogen activator receptor and incidence of venous thromboembolism. Thromb Haemost. 2016; 115(3): 657-662.

18) Eugen-Olsen J, Giamarellos-Bourboulis EJ. suPAR: The unspecific marker for disease presence, severity and prognosis. Int J Antimicrob Agents. 2015; 46 Suppl 1: S33-S34.

19) Hippensteel JA, Burnham EL, Jolley SE. Prevalence of venous thromboembolism in critically ill patients with COVID-19. British Journal of Haematology. 2020. doi:10.1111/bjh.16908

20) Porfidia A, Pola R. Venous thromboembolism in COVID-19 patients. Journal of Thrombosis and Haemostasis. 2020; 18(6): 1516-1517. doi:10.1111/jth.14842

21) Iba T, Levy JH, Levi M, Thachil J. Coagulopathy in COVID-19 . J Thromb Haemost. June 2020. doi:10.1111/jth.14975

22) Velissaris D, Dimopoulos G, Parissis J, et al. Prognostic Role of Soluble Urokinase Plasminogen Activator Receptor at the Emergency Department: A Position Paper by the Hellenic Sepsis Study Group. Infect Dis Ther. May 2020. doi:10.1007/s40121-020-00301-w

23) Sharma A, Ray S, Mamidipalli R, et al. A Comparative Study of the Diagnostic and Prognostic Utility of Soluble Urokinase-type Plasminogen Activator Receptor and Procalcitonin in Patients with Sepsis and Systemic Inflammation Response Syndrome. Indian J Crit Care Med. 2020; 24(4): 245-251.

24) D'Alonzo D, De Fenza M, Pavone V. COVID-19 and pneumonia: a role for the uPA/uPAR system. Drug Discov Today. June 2020. doi:10.1016/j.drudis.2020.06.013.

25) Chalkias A, Mouzarou A, Samara E, Xanthos T, Ischaki E, Pantazopoulos I. Soluble Urokinase Plasminogen Activator Receptor: A Biomarker for Predicting Complications and Critical Care Admission of COVID-19 Patients. Mol Diagn Ther. 2020 1: 1-5. DOI: 10.1007/s40291-020-00481-8

26) Rovina N, Akinosoglou K, Eugen-Olsen J, Hayek S, Reiser J, Giamarellos-Bourboulis EJ. Soluble urokinase plasminogen activator receptor (suPAR) as an early predictor of severe respiratory failure in patients with COVID-19 pneumonia. Crit Care. 2020; 24(1): 187.

27) Tariq U Azam, Husam R Shadid , Pennelope Blakely, Patrick O'Hayer, Hanna Berlin , Michael Pan , et al. Soluble Urokinase Receptor (SuPAR) in COVID-19-Related AKI. J Am Soc Nephrol. 2020 Nov; 31(11): 2725-2735. DOI: 10.1681/ASN.2020060829.

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