Journal of Biosciences and Medicines, 2022, 10, 190-202
https://www.scirp.org/journal/jbm
ISSN Online: 2327-509X
ISSN Print: 2327-5081

Down Protein C and S Antigen Could Be Predictor of Mortality Outcome in Critical Egyptian COVID-19 Illness

Nadia El Menshawy[1*], Ghada Elsaid[2], Tarek Abo Zeid[3], Nora A. Farag[1,4]

[1]Clinical Pathology Department, Hematology Unit, Faculty of Medicine, Oncology Center, Mansoura University, Mansoura, Egypt
[2]Internal Medicine, Specialized Medicine Hospital, Mansoura University, Mansoura, Egypt
[3]Clinical Hematology, Faculty of Medicine, Oncology Center, Mansoura University, Mansoura, Egypt
[4]Clinical Pathology Department, Hematology Unit, Faculty of Medicine, Mansoura University, Delta University, Gamasa, Egypt
Email: *nadiamensh@yahoo.com

How to cite this paper: El Menshawy, N., Elsaid, G., Zeid, T.A. and Farag, N.A. (2022) Down Protein C and S Antigen Could Be Predictor of Mortality Outcome in Critical Egyptian COVID-19 Illness. *Journal of Biosciences and Medicines*, **10**, 190-202.
https://doi.org/10.4236/jbm.2022.103018

Received: January 10, 2022
Accepted: March 14, 2022
Published: March 17, 2022

Abstract

Background: One of the most serious morbidity and mortality features in COVID-19 is thrombo-embolic manifestation with disturbed hemostasis in natural anticoagulant. Protein S plays a critical role in cytokine storming and acts as cross talk between immune status and coagulation process. So, we aimed to address its role in addition to protein C in severity and outcome of disease, with its role in vaccinated people by AstraZeneca. **Methods:** The present study was conducted on 70 COVID-19 positive by real-time PCR. 40 of patients had moderate symptoms of respiratory, 30 cases were admitted into ICU of Mansoura University Hospital, some of them under mechanical ventilator. In addition to 40 healthy volunteers after first dose of vaccinated AstraZenka as reference, assessment of protein C&S antigen was done by ELIZA. **Results:** Significant increase in total leucocytic count in COVID-19 than vaccinated volunteer with significant reduction in absolute lymphocytosis, increased neutrophilia. First significant observation was higher MPV in COVID-19 than vaccinated, in addition to significantly higher C-reactive protein in severe cases than moderate and vaccinated. Prominent interesting finding in our study was significant reduction in Protein C&S in severe cases than moderate and vaccinated. Logistic regression analysis revealed that both could be predictor of mortality outcome as CRP in COVID-19 illness. **Conclusion:** There was significant reduction in protein C&S in severe cases than moderate and vaccinated. Regression analysis shows that could be predictor of mortality as well as CRP, D-dimer. Large extended study and follow up are needed to assess the incidence of thromboembolism and role of protein C&S in different variety protocols of vaccination programs.

Keywords

Protein C, S, COVID-19, AstraZeneca Vaccine

1. Introduction

COVID-19 is a global pandemic infectious disease affecting most of the world, spreads rapidly with high morbidity and mortality rate, and the spectrum of disease presentation was heterogeneous. It varies from asymptomatic into mild upper respiratory tract disease, to severe cases of pneumonia with associated acute respiratory distress syndrome, in 15% of the hospitalized patients with progressive respiratory failure that represents one of the primary causes of death [1].

Thromboembolic manifestations, ranging from venous thromboembolism to arterial events are one of major characteristic features of disease as detected in post mortem study of Carsana *et al.* [2] who analyzed lung tissue samples from acute lung injury and also platelet-fibrin thrombi in small arterial vessels. While Ackermann *et al.* showed that alveolar capillary microthrombi were 9 times more prevalent in patients with COVID-19 than in patients with influenza [3].

A lot of information and different theories have been postulated hemostasis disruption that happens in COVID-19 and may be major cause of morbidity and mortality. One of the interesting fields in thrombogenesis is the protein S and TAM family receptors network, which represents the very core of the balance between "the good inflammation" and "the bad inflammation". Recently, Lemke and Silverman proposed a theory suggesting that the intense process of blood clotting and the immune hyperreaction seen in patients with COVID-19 may be interlinked and may be the consequence of the dyshomeostasis of the same regulator, protein S [4].

Coagulopathy with the increase of D-dimer and clinical signs of organ damage indicating a significant hypercoagulable status were implicated in the formation of wide-spread microthrombi and multi-organ ischemia [5]. Although the pathological effect of SARS-CoV2 infection on coagulation system is unknown, release of various proinflammatory cytokines, vascular endothelial cell injuries, and platelet activation may play a role in the process. Indeed, data on coagulation activation in viral infection like coronaviruses are sparse [6] [7].

Protein S, considered as well-known anticoagulant function as a cofactor for the activated protein C, possesses another extremely important role, which resides in the activation of the immunosuppressive TAM receptors, their function being essential in preventing a status of hyperinflammation, as the one seen in acute lung injury [8]. Amazingly, there are only sparse and indirect references to the role of the S-protein for the outcome of patients hospitalized with COVID-19. To the best of our knowledge and previous literature, PC&PS may be a player in thrombogenesis in COVID-19, may be a player of coagulopathy after vaccina-

tion. Our aim was to evaluate the role played by protein S and more with protein C in the severity of the COVID-19 induced lung lesions and, consequently, to test if protein S&C are involved in patients' outcome and predictor of death, in addition to evaluating them in vaccinated group.

2. Subject and Methods

This single-center cross-sectional study included all critically ill patients who were admitted to the intensive care unit (ICU). Our study was conducted on 70 patients admitted to special internal medicine hospital and ICU department from May 2020 to April 2021, all patients underwent nasopharyngeal swab or oropharyngeal swab and Real-time PCR was done for all with positive finding of COVID-19 in addition to 40 healthy vaccinated by AstraZeneca. We excluded from the study patients with known congenital or acquired thrombophilia, patients who were receiving anti-vitamin K anticoagulants at home, and patients with known liver disease or neoplasia.

All patients had screening firstly by chest standard radiographs, while the patients with pulmonary symptoms or signs had chest computer tomography's (CT scan). Besides, in all the patients with abnormalities on the chest radiography, we performed a chest CT scan; if the patient presented no abnormalities on the radiography, we considered that it was no involvement of the lung. If pulmonary symptoms or signs (including lowering of oxygen saturation) appeared during the hospitalization, chest CT scan was performed, and the worst pulmonary damage during hospitalization was considered as part of the outcome. Informed consent was taken from every participant.

In addition to routine laboratory investigation as CBC, complete liver and renal function, blood gases and electrolyte was done for sever and ICU patients. D. dimer and coagulation profiles protein S and protein-C activity for all participants.

3. Ethics Approval

All procedures performed in this study involving human participants were in accordance with the ethics standards of the institutional research committee of the investigating hospital (Institutional Review Board (IRB), Mansoura Faculty of Medicine, number) and the 1964 Helsinki Declaration and its later amendments or comparable ethics standards.

Protein S and protein C antigen assessment by enzyme-linked immunosorbent assay (ELISA)

Quantitative determination of Protein S Antigen from plasm patients using REAADS Protein S Antigen, with same procedure of Quantitative determination of Protein C Antigen from plasm patients using REAADS Protein C Antigen Test Kit (Corgnix, USA).

SPECIMEN COLLECTION AND PREPARATION

Plasma, collected with either 3.2% or 3.8% sodium citrate as an anticoagulant.

Predilute all plasma (1:2 dilution in sample diluent), reference plasma by adding 100 µl to 100 µl sample diluent, control and patient plasma by adding 20 µl to 20 µl sample diluent.

Prepare six reference dilutions as described in Table 1. Separate reference curves are used for Total Protein S assays, prepare one set of dilutions using pre-diluted reference plasma * Reference level value to be used for constructing reference curve only.

Prepare working dilutions of control and patient samples by adding 20 µL of prediluted plasma (1:2 dilution) to 500 µL Sample Diluent. Mix thoroughly, and add 100 µL of the working dilutions (reference plasmas, controls and patient samples) to the appropriate microwells. Add 100 µL of Sample Diluent to the reagent blank well. Leave the water blank well empty. Incubate 40 minutes at room temperature. Wash 4 times with working wash solution (PBS/Tween 20) using automatic washer PW40 system.

Add 100 µL Conjugate (blue) to each well (except the water blank well). Incubate for 10 minutes at room temperature. Wash 4 times with working wash solution (PBS/Tween 20) Add 100 µL Substrate to each well (except for the water blank well) and incubate for 10 minutes at room temperature. Add the substrate to the wells at a steady rate. Blue color will develop in wells with positive samples.

Add 100 µL Stopping Solution (0.36 N sulfuric acid) to each well (except for the water blank well). Do not add Stopping Solution to the water blank well. Instead, add 200 µL of reagent grade water to the water blank well. Blank or zero the plate reader against the water blank well. Read the O.D. of each well at 450 nm, against a 650 nm reference filter by Biorade ELIZA reader PR 400.

4. Statistics

The collected data was analyzed using Statistical package for Social Science (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.). Mann Whitney Test (U test) was used to assess the statistical significance of the difference of a non-parametric variable between two study groups. The Kruskal-Wallis test is was used to assess the statistical significance of the difference between more than two study group non-parametric variables. Chi-Square test was used to examine the relationship between two qualitative

Table 1. Different dilutional plasma level with reference level.

Volume Reference Plasma	Volume Sample Diluent	*Reference Level
30 µL	500 µL	150%
20 µL	500 µL	100%
15 µL	500 µL	75%
10 µL	500 µL	50%
10 µL	1000 µL	25%
10 µL	2000 µL	12.5%

variables. Logistic regression analysis was used for prediction of risk factors, using generalized linear models. A p value < 0.05 was considered to be significant.

5. Result

The present study was conducted on 70 COVID-19 positive by real-time PCR. 40 of patients had moderate symptoms of respiratory tract infection as documented by X-ray and CT SCAN with evidence of hilar consolidation and chest pneumonia. 30 cases were admitted into ICU of Mansoura university hospital, some of them under mechanical ventilator. In addition to 40 healthy volunteers after first dose of vaccinated Astrazenka as reference.

Total leucocytic count is higher in COVID-19 than vaccinated with more significant increase in severe cases than moderate p < 0.001 while Hb is mildly reduced in all patients than normal healthy vaccinated despite RBCs and platelet show no significant changes.

Absolute lymphocytosis is reduced in COVID-19 than control with more significant reduction in sever than moderate cases (P1 0.008, P2 0.002). While absolute neutrophilia is reduced in all COVID-19 compared to control with high significant difference p < 0.001, more interesting finding is prominently increased in severe cases compared to moderate cases p < 0.002.

Neutrophil/lymphocyte ratio as marker of systemic inflammatory condition is reduced in COVID-19 cases than vaccinated, however is increased in severe cases than moderate with highly significant difference p < 0.001. As regarding platelet/neutrophil ratio is higher in COVID-19 than vaccinated with marked reduction in severe cases than moderate.

MPV is significant higher in COVID-19 than vaccinated with more significantly higher in sever compared to moderate cases.as presented in Table 2.

As regarding CRP (C-Reactive Protein) in COVID-19 cases, marked increased in severe cases (median 140 mg/dl) (range 68 - 292) than moderate cases (median 27 mg/dl) (range 8 - 40 mg/dl) with highly significant difference p < 0.001 as show in Table 3.

Prothrombin time is higher in all cases of COVID-19 with significant increase in sever than moderate with reduction in prothrombin activity in COVID-19 than vaccinated with no significant reduction among moderate and sever p 0.348. While INR is higher in all cases either sever or moderate than vaccinated p < 0.001.

Median D-Dimer is significant increase in COVID-19 than vaccinated (median 1.5 vs 0.47) with marked increase in severe cases (median 5.7 vs 0.86) with highly significant p value < 0.001.

As regarding Protein-C activity, significant reduction in all COVID-19 than healthy vaccinated with marked reduction in sever than moderate p 0.004 as illustrated in Table 4, Figure 1 with more reduction in non-survived than survive patients (Figure 2).

As regarding Protein-S activity, significant reduction in all COVID-19 than

Table 2. Comparison of hematological parameters among studied groups and subgroups.

	Vaccinated N = 40	COVID N = 70	Moderate N = 40	Sever N = 30	P1	P2
WBCs Median Range	5.42 4.8 - 10.5	6.45 3.8 - 19.8	5.75 3.8 - 7.5	11.85 6.5 - 19.8	<0.001	<0.001
RBCs Median Range	5.4 5.8 - 6.9	5.1 3.7 - 4.8	5.2 3.8 - 5.1	4.71 3.4 - 4.9	0.162	0.124
Hb Median Range	14.5 13.4 - 15.3	12.2 8.5 - 14.5	9.6 8.5 - 14.5	8.8 9.2 - 13.9	<0.001	0.011
PLT Median Range	185.5 158 - 325	145.5 95 - 198	189 139 - 249	146 95 - 190	0.145	0.122
LYMPH Median Range	2.7 2.9 - .3.2	3.4 1.6 - 3.8	1.65 1 - 3.1	1.23 1.0 - 1.6	0.008	0.002
NEUT Median Range	8.2 6.9 - 8.8	4.94 2.3 - 7.1	3.65 2.5 - 5.8	10.4 5.7 - 11.5	**<0.001**	0.002
Neut/l ratio Median Range	21.8 16 - 27.2	3.6 0.79 - 13.8	2.1 0.79 - 4.92	7.7 3.47 - 13.75	<0.001	<0.001
PltN ratio Median Range	0.43 0.36 - 0.51	3.87 1.01 - 11.4	4.42 2.13 - 11.4	1.6 1.01 - 9.8	**<0.001**	**0.001**
MPV Median Range	7.7 7.4 - 10.4	9.4 6.9 - 12.6	9.95 7.4 - 12.4	10 - 4 9.1 - 12.4	**0.013**	**<0.001**

P1, comparison between control and total cases; P2, comparison between moderate and severe cases.

Table 3. Comparison of CRP among studied groups and subgroups.

		COVID N = 70	Moderate N = 40	Severe N = 30	P
CRP	Median	33.8	21	134	**<0.001**
	Minimum	8	8	86	
	Maximum	292	40	292	

CRP: C-Reactive Protein.

Table 4. Comparison of hemostatic parameters among studied groups and subgroups.

		vaccinated	COVID	Moderate	Severe	P1	P2
		N = 40	N = 70	N = 40	N = 30		
PT	**Median**	12.15	16.05	15.55	17.2	**<0.001**	**0.011**
	Minimum	11.4	13.4	13.4	14.5		
	maximum	12.5	22.1	21.5	22.1		
%	**Median**	103	83.5	82	84	**<0.001**	0.348
	Minimum	98	64	64	75		
	maximum	110	98	98	98		
INR	**Median**	0.94	1.23	1.15	1.32	**<0.001**	**<0.001**
	Minimum	0.9	0.8	0.8	1.1		
	maximum	10	2.3	1.5	2.3		

Continued

	Median	0.47	1.5	0.86	5.7		
DDIMER	**Minimum**	0.3	0.2	0.2	2.8	**<0.001**	**<0.001**
	maximum	0.9	6.7	2.9	6.7		
	Median	95	77	83.5	67.5		
Protein C	**Minimum**	89	55	65	55	**<0.001**	**0.004**
	maximum	110	97	97	79		
	Median	94	81	84	55		
Protein S	**Minimum**	81	35	48	35	**<0.001**	**<0.001**
	maximum	150	90	95	95		

P1, comparison between vaccinated volunteer and total cases of COVID-19; P2, comparison between moderate and severe cases of COVID-19.

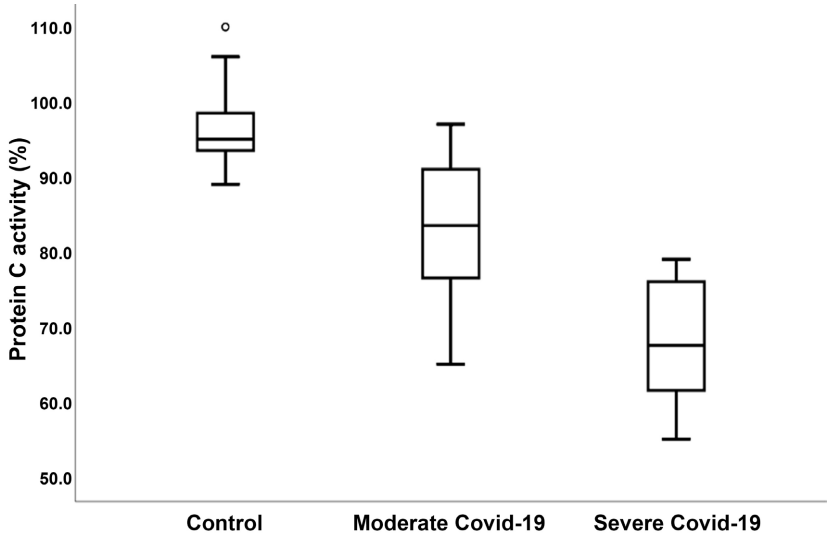

Figure 1. Protein C activity in studied groups.

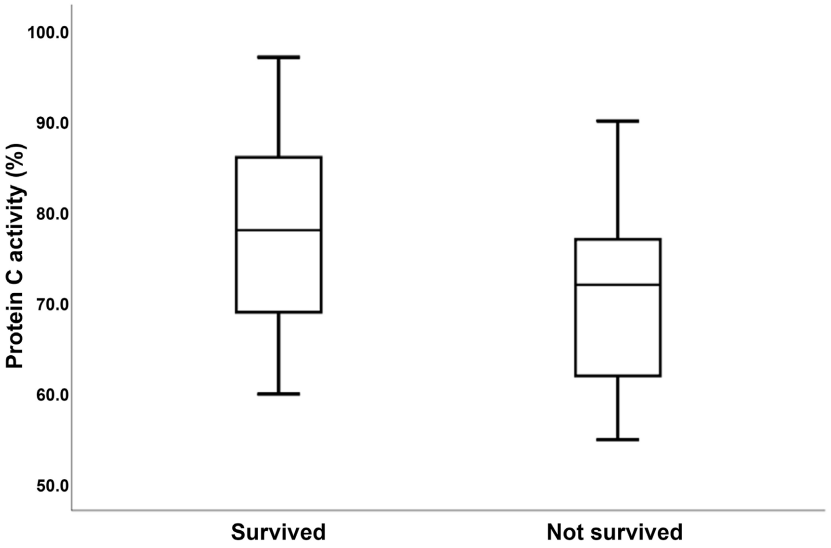

Figure 2. Protein C activity in survived and not survived COVID-19 cases.

healthy vaccinated with marked reduction in sever than moderate p 0.004 as illustrated in Table 4, Figure 3 with more reduction in non-survived than survive patients Figure 4. Follow up of survival of our study group show (20/70) 28.6% of cased died, 16 of them (53.5%) from severe cases while 4 (10%) from moderate. Most of severe cases are complicated by acute respiratory failure, cerebral stroke, few cases are ended by renal impairment. The mortality rate among studied groups and subgroups is shown in Table 5.

Table 5. Comparison of mortality rate among studied groups and subgroups.

	COVID N = 70		Moderate N = 40		Severe N = 30		p
	N	%	N	%	N	%	
Death	20	28.6%	4	10%	16	53.5%	**<0.001**

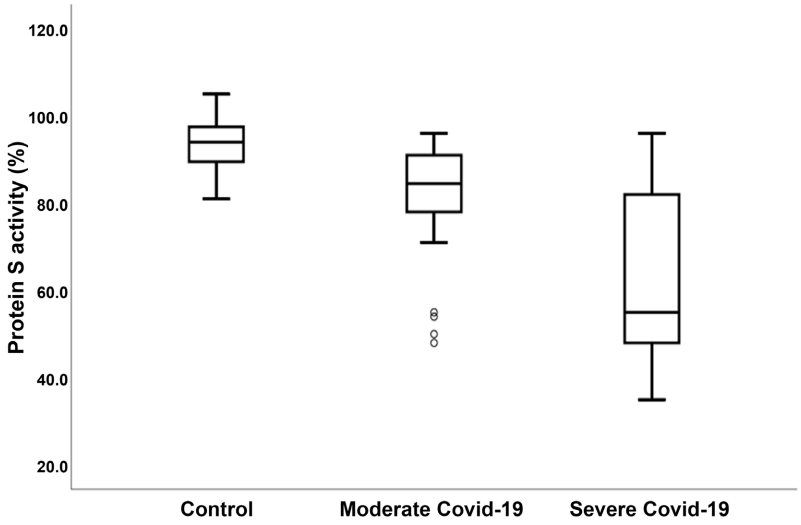

Figure 3. Protein S activity in studied groups.

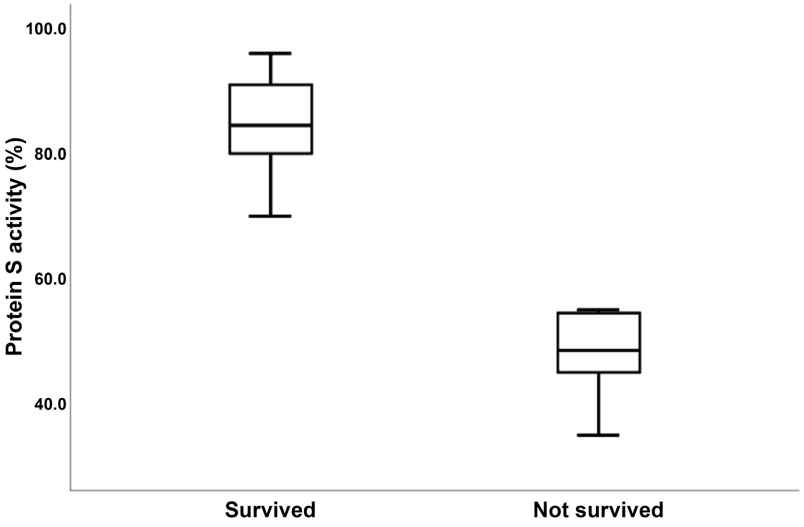

Figure 4. Protein S activity among survived and not survived COVID-19 cases.

6. Discussion

Coagulopathy with disturbed hemostasis is major evidence of thromboembolic, manifestation and serious causes of morbidity and mortality in COVID-19 patients. Most of sever hospitalized at ICU under prophylactic and standard doses of therapeutic anticoagulant however it could not prevent the exuberant thrombotic events in those patients. llitjos *et al.* 2020 [9] concluded that hemostasis disturbance in COVID-19 patients is quite unique.

Extensive cross talk occurs between the immune and coagulation system, previous study has clarified that interleukin-6, IL-17 and tumor necrosis factor α and other cytokines activate coagulation [10]. COVID-19 is associated with inflammatory response and increased IL-6 fibrinogen and ferritin in more increased D-dimer suggesting extensive thrombosis and fibrinolysis [11] [12]. Hypercoagubility is an important hallmark of inflammation, pro-inflammatory cytokines are critically involved in abnormal clot formation and platelet hyperactivation in more downregulation of important physiological anticoagulation pathway [13].

Pathophysiology of thrombosis is of high interest of a lot of researchers because defining it will make us understand this resistance to standard anticoagulant and will certainly change the way in which we will manage the COVID-19 patients. Regarding the mechanisms and factors that enhance this abnormal process of coagulation, several hypotheses have been postulated until now, varying from a local state of pulmonary inflammation and endothelial disfunction with or without associated antiphospolipid antibodies, to neutrophil extracellular traps, dysregulated complement activation, and dysregulated renin angiotensin system [14] [15].

Recently, a new theory was proposed, that protein S is an activating ligand for MER and AXL receptors comprised by the TAM family of receptor tyrosin kinases [4]. When activated, MER receptors, who are found on the surface of macrophages and other immune cells, are broadly immunosuppressive, and so they temper the production of cytokines, (type 1 interferon, IL-6, TNF), the same cytokines that are linked to cytokine storm and consequently to acute lung injury [16]

Few studies revealed relation of protein S only to outcome and severity of disease so we aimed to address its role in combination with protein C as predictor of mortality and assess its role in vaccinated healthy volunteer.

Total leucocytic count is higher in COVID-19 than vaccinated with reduction of absolute lymphocytosis and increased absolute neutrophilia. This finding matched with [17]. This could be explained by a significant role of neutrophil trapping theory in thrombogenesis and may play a trigger role in lung tissue damage and severity of disease and outcome.

In current study one interesting finding and first observation that Mean platelet volume is significantly higher in COVID-19 than vaccinated with significant increase in severe than moderate. This could be explained that large platelet may contribute in pathophysiology of thrombus formation [18].

Our result revealed significant increase in C-reactive protein (CRP) in severe cases than moderate. This finding could be related to association of CRP with severity and worsening condition of disease. In addition, Logistic regression analysis shows that CRP could be independent predictor of mortality. This finding matched with Sharifpour [19]. That concluded in his study CRP rise is indicative of disease worsening and measure of underlying systemic inflammatory response. Emerging evidence about causal link between systemic inflammation and clinical outcome in COVID-19 by dexamethasone therapy with inhibition of systemic inflammation and reduce COVID-19 related mortality [20] [21], in more it could be used to risk-stratify patients early and guide intensive management respiratory support and or immune suppression with corticosteroid along with monitoring response to therapy [22].

Prominent and interesting finding in our study that both significant reduction in Protein C& Protein S in severe cases than moderate and even vaccinated. Logistic regression analysis revealed that both could be predictor of mortality in COVID-19 patients (**Table 6**). To the best of our knowledge that first study of both prothrombotic markers and its assessment in vaccinated people. This deficiency in Protein S in accordance with conclusion of Stoichitoiu [23], until this time no studies on vaccinated people. This deficiency of PC&PS could be explained that increased consumption of both natural anticoagulant in coagulation cascade in early event of disease, may be reduced in synthesis from endothelium due to endothelititis as consequences of inflammatory process induced by COVID-19 [24]. Other explanation due to increased C4BP that binds to PS however previous study showed that C4BP levels are not correlated with free protein S levels [25]. In more addition and another explanation due to antiphospholipid syndrome with hyperactivation and autoimmune diseases through MER-protein S complex [26].

Our interesting and new data reported about clinical value of PC&PS in vaccinated healthy volunteers with Asrtazenca show no significant changes while

Table 6. Prediction of death.

	Univariable				Multivariable			
	p	OR	95% CI		p	OR	95% CI	
Neutrophils	0.335	1.015	0.985	1.046				
C reactive protein	**0.002**	1.006	1.002	1.010	0.484	1.003	0.995	1.010
D. Dimer	**0.008**	1.016	1.004	1.028	0.800	0.997	0.974	1.021
Protein S	**<0.001**	0.919	0.888	0.950	**0.003**	0.930	0.888	0.975
Protein C	**0.015**	0.962	0.933	0.992	**0.012**	0.989	0.939	0.998

OR, odds ratio; CI, confidence interval. Logistic regression analysis was conducted for prediction of mortality in COVID-19 cases, using neutrophils, CRP, D. Dimer, Protein C and protein S as covariates. Higher CRP, D. Dimer, lower PC and PS were associated with higher mortality rates in univariable analysis. While in multivariable analysis, lower PC & PS were suggested to be independent predictors of death among COVID-19 patients.

more reduction was observed in severe COVID-19 than moderate and even vaccinated. No more study about role of natural anticoagulant in rare documented thromboembolic disorder in vaccinated people however some suggested explanation by [27].

Role of antibodies against platelet factor 4 (PF4), direct interaction between adenovirus vector and platelet., m RNA Vaccines leading to spike protein synthesis and its presentation in the context of MHC with triggering cytotoxic response to CD8+ cells [28] Gene encoding SARS-Covid-2 can occur in vitro experimented [29].

7. Conclusion

Significant reduction was observed in protein S&C at severe cases than moderate and vaccinated. Logistic regression analysis revealed that could be predictor of mortality as CRP, D-dimer. Large extended study is needed to assess incidence of thromboembolism and role of PS&C in different vaccinated regimens with different protocols.

Acknowledgements

We would like to thank all patients and healthy vaccinated people, who participated in our study and help to release our study.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Cai, A., Mcclafferty, B., Benson, J., Ramgobin, D., Kalayanamitra, R., Shahid, Z., *et al.* (2020) COVID-19: Catastrophic Cause of Acute Lung Injury. *South Dakota Medicine*, **73**, 252-260.

[2] Carsana, L., Sonzogni, A., Naser, A., Rossi, R.S., Pellegrinelli, A., Zerbi, P., *et al.* (2020) Pulmonary Post-Mortem Findings in a Series of COVID-19 Cases from Northern Italy: A Two-Center Descriptive Study. *The Lancet Infectious Diseases*, **20**, 1135-1140. https://doi.org/10.1016/S1473-3099(20)30434-5

[3] Ackermann, M., Verleden, S.E., Kuehnel, M., Haverich, A., Welte, T., Laenger, F., *et al.* (2020) Pulmonary Vascular Endothelialitis, Thrombosis, and Angiogenesis in Covid-19. *The New England Journal of Medicine*, **383**, 120-128. https://doi.org/10.1056/NEJMoa2015432

[4] Lemke, G. and Silverman, G.J. (2020) Blood Clots and TAM Receptor Signalling in COVID-19 Pathogenesis. *Nature Reviews Immunology*, **20**, 395-396. https://doi.org/10.1038/s41577-020-0354-x

[5] Connors, J.M. and Levy, J.H. (2020) Thromboinflammation and the Hypercoagulability of COVID-19. *Journal of Thrombosis and Haemostasis*, **18**, 1559-1561. https://doi.org/10.1111/jth.14849

[6] Zhang, Y., Cao, W., Xiao, M., Li, Y.J., Yang, Y., Zhao, J., Zhou, X., Jiang, W., Zhao, Y.Q., Zhang, S.Y. and Li, T.S. (2020) Clinical and Coagulation Characteristics of 7

Patients with Critical COVID-2019 Pneumonia and Acro-Ischemia. *Chinese Journal of Hematology*, **41**, E006.

[7] Zhou, F., Yu, T., Du, R., Fan, G., Liu, Y., Liu, Z., Xiang, J., Wang, Y., Song, B., Gu, X., Guan, L., Wei, Y., Li, H., Wu, X., Xu, J., Tu, S., Zhang, Y., Chen, H. and Cao, B. (2020) Clinical Course and Risk Factors for Mortality of Adult Inpatients with COVID-19 in Wuhan, China: A Retrospective Cohort Study. *The Lancet*, **395**, 1054-1062. https://doi.org/10.1016/S0140-6736(20)30566-3

[8] Klok, F.A., Mjha, K., Njm, V.M., Arbous, M.S., Dampj, G., Kant, K.M., *et al.* (2020) Incidence of Thrombotic Complications in Critically Ill ICU Patients with COVID-19. *Thrombosis Research*, **191**, 145-147. https://doi.org/10.1016/j.thromres.2020.04.013

[9] Llitjogs, J.F., Leclerc, M., Chochois, C., Monsallier, J.M., Ramakas, M., Auvray, M., *et al.* (2020) High Incidence of Venous Thromboembolic Events in Anticoagulated Severe COVID-19 Patients. *Journal of Thrombosis and Haemostasis*, **18**, 1743-1746. https://doi.org/10.1111/jth.14869

[10] Ulhaq, Z.S. and Soraya, G.V. (2020) Interleukin-6 as a Potential Biomarker of COVID-19 Progression. *Médecine et Maladies Infectieuses*, **50**, 382-383. https://doi.org/10.1016/j.medmal.2020.04.002

[11] Stouthard, J.M., Levi, M., Hack, C.E., *et al.* (1996) Interleukin-6 Stimulates Coagulation, Not Fibrinolysis, in Humans. *Thrombosis and Haemostasis*, **76**, 738-742. https://doi.org/10.1055/s-0038-1650653

[12] Gabay, C. and Kushner, I. (1999) Acute-Phase Proteins and Other Systemic Responses to Inflammation. *New England Journal of Medicine*, **340**, 448-454. https://doi.org/10.1056/NEJM199902113400607

[13] Tang, N., Li, D., Wang, X. and Sun, Z. (2020) Abnormal Coagulation Parameters Are Associated with Poor Prognosis in Patients with Novel Coronavirus Pneumonia. *Journal of Thrombosis and Haemostasis*, **18**, 844-847. https://doi.org/10.1111/jth.14768

[14] Ranucci, M., Ballotta, A., Di Dedda, U., Bayshnikova, E., Dei Poli, M., Resta, M., Falco, M., Albano, G. and Menicanti, L. (2020) The Procoagulant Pattern of Patients with COVID-19 Acute Respiratory Distress Syndrome. *Journal of Thrombosis and Haemostasis*, **18**, 1747-1751. https://doi.org/10.1111/jth.14854

[15] Lodigiani, C., Iapichino, G., Carenzo, L., Cecconi, M., Ferrazzi, P., Sebastian, T., *et al.* (2020) Venous and Arterial Thromboembolic Complications in COVID-19 Patients Admitted to an Academic Hospital in Milan, Italy. *Thrombosis Research*, **191**, 9-14. https://doi.org/10.1016/j.thromres.2020.04.024

[16] Lemke, G. (2013) Biology of the TAM Receptors. *Cold Spring Harbor Perspectives in Biology*, **5**, a009076. https://doi.org/10.1101/cshperspect.a009076

[17] Li, X., Wang, L., Yan, S., Yang, F., Xiang, L., Zhu, J., Shen, B. and Gong, Z. (2020) Clinical Characteristics of 25 Death Cases with COVID-19: A Retrospective Review of Medical Records in a Single Medical Center, Wuhan, China. *International Journal of Infectious Diseases*, **94**, 128-132. https://doi.org/10.1016/j.ijid.2020.03.053

[18] Lippi, G., Sanchis-Gomar, F. and Favaloro, E.J. (2020) Mean Platelet Volume in Arterial and Venous Thrombotic Disorders. *Journal of Laboratory Medicine*, **44**, 1-8. https://doi.org/10.1515/labmed-2019-0201

[19] Sharifpour, M., Rangaraju, S., Liu, M., Fadi, D.A., Nahab, B., Christina, M. and Jabaley, B.C. (2020) C-Reactive Protein as a Prognostic Indicator in Hospitalized Patients with COVID-19. *PLoS ONE*, **15**, e0242400. https://doi.org/10.1371/journal.pone.0242400

[20] Chen, W., Zheng, K.I., Liu, S., Yan, Z., Xu, C. and Qiao, Z. (2020) Plasma CRP Level Is Positively Associated with the Severity of COVID-19. *Annals of Clinical Microbiology and Antimicrobials*, **19**, 18. https://doi.org/10.1186/s12941-020-00362-2

[21] Horby, P., Lim, W.S., *et al.* (2020) Dexamethasone in Hospitalized Patients with Covid-19—Preliminary Report. *The New England Journal of Medicine*, **384**, 693-704.

[22] Stallmach, A., Kortgen, A., Gonnert, F., Coldewey, S.M., Reuken, P. and Bauer, M. (2020) Infliximab against Severe COVID-19-Induced Cytokine Storm Syndrome with Organ Failure—A Cautionary Case Series. *Critical Care*, **24**, 444. https://doi.org/10.1186/s13054-020-03158-0

[23] Stoichitoiu, L.E., Pinte, L., Balea, M.I., Nedel, V., Badea, C. and Baicus, C. (2020) Anticoagulant Protein S in COVID-19: Low Activity, and Associated with Outcome ROM. *Journal of Internal Medicine*, **58**, 251-258. https://doi.org/10.2478/rjim-2020-0024

[24] Varga, Z., Flammer, A.J., Steiger, P., Haberecker, M., Andermatt, R., Zinkernagel, A.S., *et al.* (2020) Endothelial Cell Infection and Endothelitis in COVID-19. *The Lancet*, **395**, 1417-1418. https://doi.org/10.1016/S0140-6736(20)30937-5

[25] Boulianger, N., Elkouri, D., Trossaert, M., Truchaud, F., Trewick, D. and Planchon, B. (1998) Coagulation Activation in Patients with an Inflammatory Syndrome: Is There a Link with Acquired Protein S Deficiency? *Blood Coagulation & Fibrinolysis*, **9**, 167-171. https://doi.org/10.1097/00001721-199803000-00007

[26] Erbe, M., Rickerts, V., Bauersachs, R.M. and Lindhoff-last, E. (2003) Acquired Protein C and Protein S Deficiency in HIV Infected Patients. *Clinical and Applied Thrombosis/Hemostasis*, **9**, 325-331. https://doi.org/10.1177/107602960300900408

[27] Rzymski, P., Perek, B. and Flisiak, R. (2021) Thrombotic Thrombocytopenia after COVID-19 Vaccination: In Search of the Underlying Mechanism. *Vaccines*, **9**, 559. https://doi.org/10.3390/vaccines9060559

[28] Chapman, L.M., Aggrey, A.A., Field, D.J., Srivastava, K., Ture, S., Yui, K., Topham, D.J., Baldwin, W.M. and Morrell, C.N. (2012) Platelets Present Antigen in the Context of MHC Class I. *The Journal of Immunology*, **189**, 916-923. https://doi.org/10.4049/jimmunol.1200580

[29] Almuqrin, A., Davidson, A.D., Williamson, M.K., Lewis, P.A., Heesom, K.J., Morris, S., Gilbert, S.C. and Matthews, D.A. (2021) SARS-CoV-2 Vaccine ChAdOx1 nCoV-19 Infection of Human Cell Lines Reveals Low Levels of Viral Backbone Gene Transcription Alongside Very High Levels of SARS-CoV-2 S Glycoprotein Gene Transcription. *Genome Medicine*, **13**, 43. https://doi.org/10.1186/s13073-021-00859-1