*Original Research Article*

# Study of the Role of Protein S in Patients with Coronavirus Disease 2019


## Abstract:

**Background:** A novel coronavirus disease 2019 (COVID 19) was found for the first time in late 2019 in Wuhan, China, and swiftly transmitted throughout the globe, resulting in a pandemic. The virus was determined as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and the World Health Organization (WHO) recognized the condition as coronavirus disease 2019 (COVID-19)

**Aim of the Work:** to assess the role of protein S in COVID 19 cases.

**Patients and Methods:** Study area setting: The research was applied in Clinical Pathology Department, Faculty of Medicine, Tanta University. Group I: 15 normal subjects with matched age and sex to patient group as a control group and II: 40 patients diagnosed with PCR as COVID -19 positive. Which include 25% of patients had severe disease, 55% had moderate severity, and 20% had mild disease.

**Results:**COVID-19 infection has a significant influence on the blood coagulation cascade, which might result in the presentation of serious signs and elevated mortalities.



**Key word:**

severe acute respiratory syndrome coronavirus 2, coronavirus disease 2019and activated protein C


**Introduction**:

A novel coronavirus disease 2019 (COVID-19) was found for the first time in late 2019 in Wuhan, China, and swiftly spread throughout the globe, resulting in a pandemic. The virus was determined as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and the World Health Organization (WHO) designated the illness as COVID-19 (1, 2).

This illness's clinical course ranges from asymptomatic types and mild upper respiratory tract disease to severe pneumonia with associated acute respiratory distress syndrome. Progressive respiratory failure is considered as one of the primary causes of death (3-5).

The most common symptom is respiratory, but gastrointestinal, neurological, and other atypical symptoms can also be seen, although these symptoms are rare (6).

COVID-19, unlike the 2003 SARS-CoV pandemic, is not a simple upper respiratory illness. COVID-19 patients experience hypercoagulability and increased risk of venous thrombo-embolism. These thrombotic complications have been referred to as thrombo-inflammation or COVID-19-associated coagulopathy (7-10).

Protein S is a vitamin K –dependent anticoagulant protein that is synthesized in liver, endothelial cells and megakaryocytes (11).

Protein S facilitates the action of activated protein C (APC) on its substrates which is activated factor V (FVa) and activated factor VIII (FVIIIa). Protein S, also possesses another extremely important role, it activates the immunosuppressive Tyro3, Axl and MerTK receptors (TAM) receptors (12), their function is vital for avoiding a hyperinflammatory state, as observed in acute lung injury (13).

Studies done by *Carsana L et al.(2020)* and *Ackermann M et al.(2020)* have shown, besides a pattern of diffuse alveolar damage and hyaline membranes, signifier to acute lung injury, also platelet-fibrin thrombi in small arterial vessels (14).

**Aim of the Work**

This research objects to assess the role of protein S in COVID 19 cases.

**Patients and methods**

1 **Study area setting:** The research was applied in Clinical Pathology Department, Faculty of Medicine, Tanta University.

2 **Study subjects:** Patients with COVID -19 from CDCC (contagious disease control center), Tanta University in addition to healthy cases with matched age and sex as a control group.

3 **Study design:** Retrospective study.

**4 Sample size:**

**Individuals enrolled in this study are divided to:**

- **Group I:** 15 normal subjects with matched age and sex to patient group as a control group

- **Group II:** 40 patients diagnosed with PCR as COVID -19 positive. Which include 25% of patients had severe disease, 55% had moderate severity, and 20% had mild disease.

**Inclusion criteria:**

Patients classified as COVID-19 positive by polymerase chain reaction (PCR) for SARS-CoV-2 on nasopharyngeal swab specimen, regardless of the severity and manifestations.

Disease severity was assessed according to the study done by *Stoichitoiu LE et al.(2020),* We divided our subjects into three groups to include all ranges of clinical illness severity, We deemed a condition modest if the patient had minor signs (Smell and taste impairment, fatigue and mild fever) (15). Since studies have done by *XIE et al. (2020)* showed that a Peripheral oxygen saturation ($SpO_2$) < 90% was strongly related to mortality, independent of age and sex. Cases with a $SpO_2$ less than 90% and lung involvement on CT scan were classified as having severe form; cases who did not meet any of the above criteria were classified as having moderate one (16). Evaluation of illness severity was based on lung involvement on CT scan, cases were classified into four classifications: no lung involvement on CT scan, mild involvement if lower than 25% of the lung was affected, moderate disease if lung involvement was ≥25% but < 50%, severe disease if lung involvement was ≥50% but < 75%.

**Exclusion criteria:**

- Patients with coagulopathy.
- Patients treated with anticoagulants or antiplatelet therapy.
- Patients with liver diseases

**Statistical Analysis**

The statistical analysis was performed utilizing IBM SPSS software package version 20.0**.** (Armonk, NY: IBM Corp) Qualitative data were expressed as number and percent. The Shapiro-Wilk test was utilized to assess the normality of distribution. Quantitative data were expressed as range (minimum and maximum), mean and standard deviation. The average percentage range was compared with standard reference range of the respective parameter. P value ≤0.05 is deemed to be statistically significant.

## Results

- There was significant decline in platelet count among the two groups (P = 0.003). **Table (1)**

- There was no significant variance among the two groups regarding WBCs count, while neutrophil count was significantly elevated in group II than group I (P < 0.001). **Table (1)**

- Lymphocyte count was significantly decreased in group II than group I (P < 0.001). **Table (1)**

Ferritin and CRP showed significant elevation in group II than group I (P < 0.001). **Table (2)**

- 30 % of severely infected cases, 45.5% of moderately infected and 62.5% of mild infected had platelet count below reference range. **Table (3)**

- 60% of severely infected cases, 9.1% of moderately infected and 50% of mildly infected had PT and INR above reference range. **Table (3)**

- 30% of severely infected cases, 22.7% of moderately infected and 25% of mildly infected had aPTT above reference range. **Table (3)**

- 70% of severely infected cases, 59.1% of moderately infected and 25% of mildly infected had D-Dimer above reference range. **Table (3)**

- 90% of severely infected cases, 72.7% of moderately infected and 37.5% of mildly infected had protein S activity below reference range. **Table (3)**

- 90% of severely infected cases, 86.4% of moderately infected and 37.5% of mildly infected had CRP level above reference range. **Table (3)**

- 80% of severely infected cases, 81.8% of moderately infected and 62.5% of mildly infected had serum ferritin level above reference range. **Table (3)**

- There was significant decline in platelet count in non-survivors than survivors ($P < 0.001$). **Table (4)**

- There was a significant elevation in PT and PTT in non-survivors than survivors ($P = 0.022$ and 0.004 respectively). **Table (4)**

- There was a significant elevation in INR in non-survivors than survivors ($P = 0.049$). **Table (4)**

- The average D-Dimer level in non-survivors was elevated than the upper limit of reference range. **Table (4)**

- The average protein S activity was less than the lower limit of reference range in non-survivors when compared to survivors. **Table (4)**

- There was significant decrease in platelet count between cases with severe disease than with moderate one, also between cases with severe disease than with mild one ($P = 0.002$ and 0.028 respectively), while there was no significant variance between cases with mild disease than cases with moderate one regarding platelet count ($P = 0.746$). **Table (5)**
- There was significant increase in PT between cases with severe disease than with moderate one, also between cases with severe disease than with mild one ($P = 0.001$ and 0.002 respectively), while there was no significant variance between cases with mild disease than with moderate one regarding PT ($P = 0.852$). **Table (5)**
- There was significant increase in PTT between cases with severe disease than with moderate one, also between cases with severe disease than with mild one ($P = 0.045$ and 0.017 respectively), while there was no significant variance between cases with mild disease than with moderate one regarding PTT ($P = 0.562$). **Table (5)**
- There was significant increase in INR between cases with severe disease than with moderate one, also among cases with severe disease than with mild one ($P = 0.007$ and 0.012 respectively), while there was no significant variance between cases with mild disease than with moderate one regarding INR ($P = 0.868$). **Table (5)**
- There was significant increase in D-Dimer level between cases with severe disease than with moderate one, also between cases with severe disease than with mild one ($P = 0.044$ and 0.008 respectively), while there was no significant variance between cases with mild disease than with moderate one regarding D-Dimer level ($P = 0.243$). **Table (5)**
- There was significant decrease in Protein S activity between cases with severe disease than with moderate one, also between cases with severe disease than with mild one ($P = 0.002$ and 0.006 respectively), while there was no significant variance between cases with

mild disease than with moderate one regarding Protein S activity (P = 0.734). **Table (5)**

**Table (1): Comparison between Control Group (Group I) and Patient Group (Group II) according to Complete Blood Count (CBC)**

| | Group I (n = 15) | Group II (n = 40) | Test of Sig. | p |
|---|---|---|---|---|
| **Platelet Count (x10$^3$/mm)** | | | | |
| **Min. – Max.** | 100.0 – 320.0 | 91.0 – 285.0 | U= 144.0* | 0.003* |
| **Mean ± SD.** | 201.2 ± 66.36 | 150.7 ± 41.06 | | |
| **Median (IQR)** | 200.0(157.0 – 245.0) | 157.0(118.0 – 167.5) | | |
| **WBCs (x10$^3$/mm)** | | | | |
| **Min. – Max.** | 4.30 – 14.0 | 1.60 – 34.50 | U= 219.0 | 0.126 |
| **Mean ± SD.** | 7.24 ± 2.94 | 10.53 ± 7.45 | | |
| **Median (IQR)** | 6.40 (4.95 – 9.40) | 9.05 (5.50 – 12.90) | | |
| **Neutrophils (%)** | | | | |
| **Min. – Max.** | 55.0 – 71.0 | 78.0 – 96.0 | t= 11.158* | <0.001* |
| **Mean ± SD.** | 67.60 ± 4.08 | 83.52 ± 4.92 | | |
| **Median (IQR)** | 70.0 (66.50 – 70.0) | 82.50 (79.50 – 87.0) | | |
| **Lymphocytes (%)** | | | | |
| **Min. – Max.** | 18.0 – 30.0 | 2.0 – 16.0 | t= 10.071* | <0.001* |
| **Mean ± SD.** | 23.33 ± 3.85 | 11.61 ± 3.85 | | |
| **Median (IQR)** | 22.0 (20.0 – 25.0) | 12.50 (10.0 – 15.0) | | |

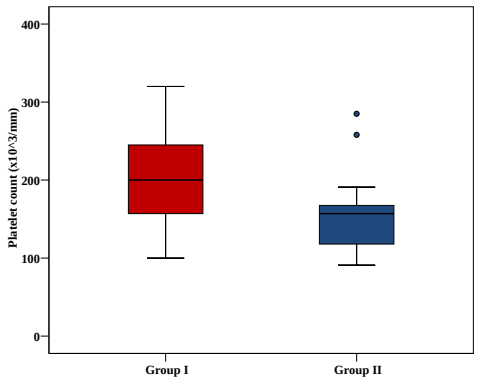


**Figure (1): Comparison between Group I and Group II according to PLT count**

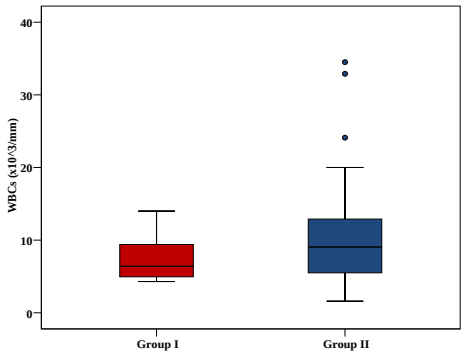


**Figure (2): Comparison between Group I and Group II according to WBCs count**

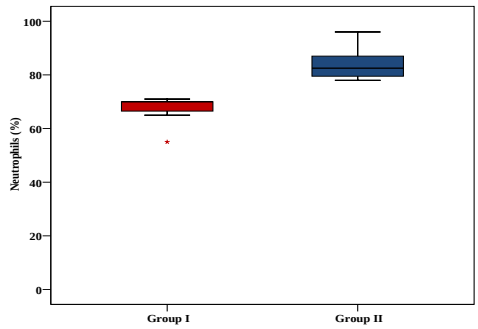


**Figure (3): Comparison between Group I and Group II according to Neutrophil count (%)**

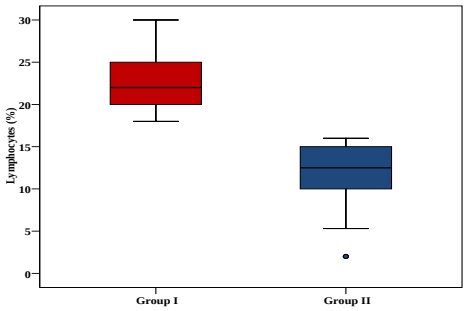


**Figure (4): Comparison between Group I and Group II according to Lymphocyte count (%)**

**Table (2): Comparison between Group I and Group II regarding inflammatory markers**

| | Group I (n = 15) | Group II (n = 40) | U | p |
|---|---|---|---|---|
| Ferritin (ng/ml) | | | | |
| **Min. – Max.** | 100.0 – 150.0 | 55.40 – 10000 | 63.0* | <0.001* |
| **Mean ± SD.** | 118.7 ± 17.93 | 1075 ± 1701 | | |
| **Median (IQR)** | 114.0(104.0 – 130.5) | 725.0(266.8 – 955.2) | | |
| CRP (mg/L) | | | | |
| **Min. – Max.** | 0.20 – 9.0 | 3.0 – 198.0 | 73.50* | <0.001* |
| **Mean ± SD.** | 5.39 ± 2.52 | 54.23 ± 48.74 | | |
| **Median (IQR)** | 5.10 (4.30 – 7.50) | 48.0 (12.0 – 96.0) | | |

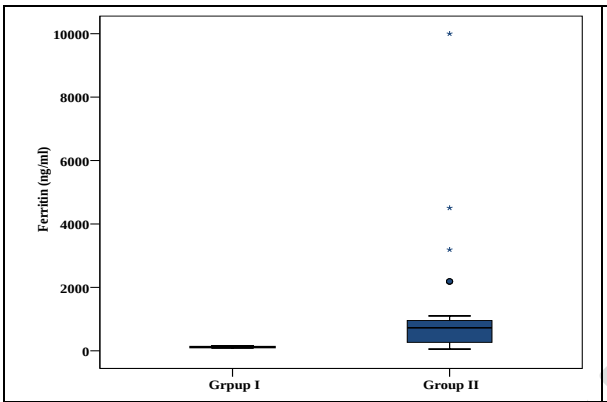


**Figure (5): Comparison between Group I and Group II according to serum ferritin level**

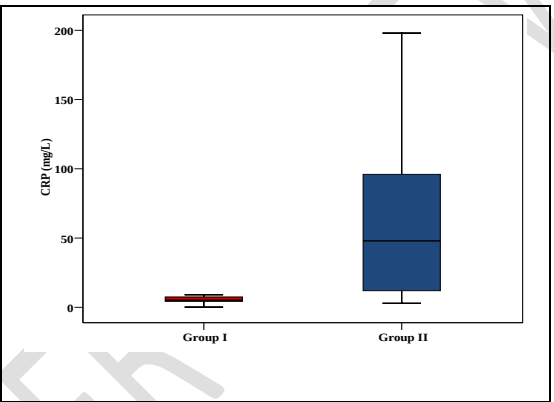


**Figure (6): Comparison between Group I and Group II according to CRP**

**Table (3): Percentage of patients (mild, moderate and severe) above, within and below reference range (compared to standard reference range) for each coagulation and inflammatory parameters**

| | Reference range | Disease severity | | | | | | $\chi^2$ | p |
|---|---|---|---|---|---|---|---|---|---|
| | | Mild (n = 8) | | Moderate (n = 22) | | Sever (n = 10) | | | |
| | | No. | % | No. | % | No. | % | | |
| Coagulation parameters | | | | | | | | | |
| Platelets | 150-450 | | | | | | | | |
| Above RR | x10$^3$/cmm | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1.856 | $^{MC}$p= |
| Within RR | | 3 | 37.5 | 12 | 54.5 | 7 | 70.0 | | 0.408 |
| Below RR | | 5 | 62.5 | 10 | 45.5 | 3 | 30.0 | | |
| PT | 12.5-14.5 | | | | | | | | |
| Above RR | sec. | 4 | 50.0 | 2 | 9.1 | 6 | 60.0 | 10.473* | $^{MC}$p= |
| Within RR | | 4 | 50.0 | 20 | 90.9 | 4 | 40.0 | | 0.004* |
| Below RR | | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | | |
| aPTT | 24.9-38.2 | | | | | | | | |
| Above RR | sec. | 2 | 25.0 | 5 | 22.7 | 3 | 30.0 | 0.407 | $^{MC}$p= |
| Within RR | | 6 | 75.0 | 17 | 77.3 | 7 | 70.0 | | 0.891 |
| Below RR | | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | | |
| INR | 0.9-1.2 | | | | | | | | |
| Above RR | | 4 | 50.0 | 2 | 9.1 | 6 | 60.0 | 10.473* | $^{MC}$p= |
| Within RR | | 4 | 50.0 | 20 | 90.9 | 4 | 40.0 | | 0.003* |
| Below RR | | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | | |
| D-Dimer | 0-0.5 | | | | | | | | |
| Above RR | ug/ml | 2 | 25.0 | 13 | 59.1 | 7 | 70.0 | 3.767 | $^{MC}$p= |
| Within RR | | 6 | 75.0 | 9 | 40.9 | 3 | 30.0 | | 0.180 |
| Below RR | | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | | |
| Protein S | 60-130% | | | | | | | | |
| Above RR | | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 5.453 | $^{MC}$p= |
| Within RR | | 5 | 62.5 | 6 | 27.3 | 1 | 10.0 | | 0.052 |
| Below RR | | 3 | 37.5 | 16 | 72.7 | 9 | 90.0 | | |
| Inflammatory parameters | | | | | | | | | |
| CRP | 0-3 mg/dl | | | | | | | | |
| Above RR | | 3 | 37.5 | 19 | 86.4 | 9 | 90.0 | 7.551* | $^{MC}$p= |
| Within RR | | 5 | 62.5 | 3 | 13.6 | 1 | 10.0 | | 0.015* |
| Below RR | | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | | |
| Ferritin | 100-150 | | | | | | | | |
| Above RR | ng/ml | 5 | 62.5 | 18 | 81.8 | 8 | 80.0 | 1.427 | $^{MC}$p= |
| Within RR | | 3 | 37.5 | 4 | 18.2 | 2 | 20.0 | | 0.521 |
| Below RR | | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | | |

$\chi^2$: **Chi square test** MC: **Monte Carlo**

p: p value for comparing between the different categories

*: Statistically significant at $p \leq 0.05$

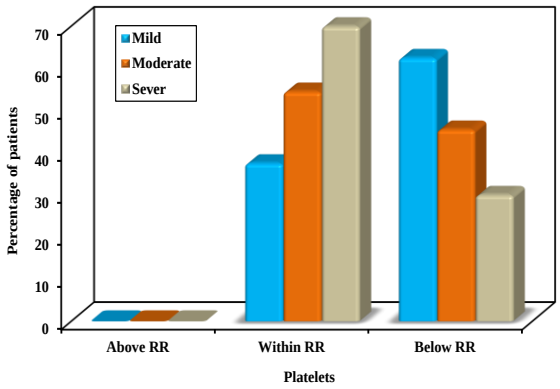


**Figure (5):** Relation between disease severity and platelets in patient group (Group II)

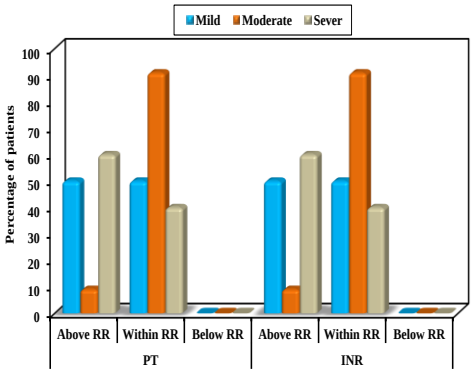


**Figure (6):** Relation between disease severity with PT and INR in patient group (Group II)

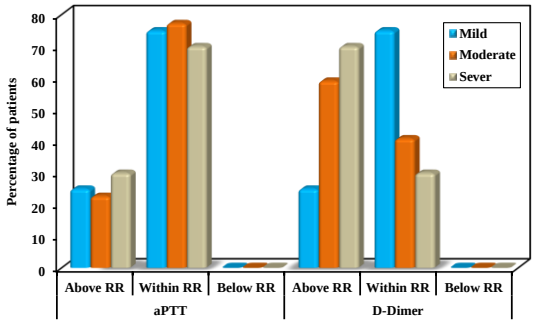


**Figure (7): Relation between disease severity with aPTT and D-Dimer in patient group (Group II)**

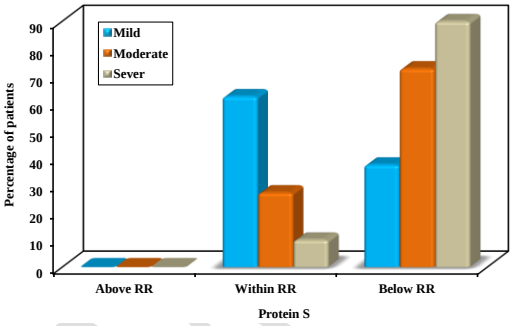


**Figure (8): Relation between disease severity and Protein S in patient group (Group II)**

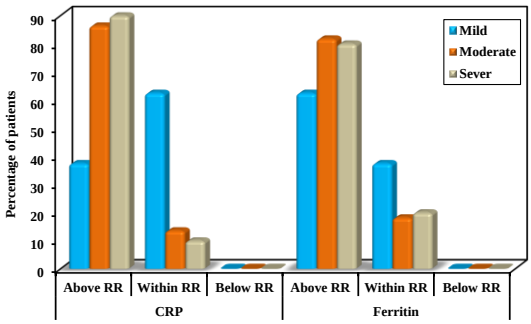


**Figure (9): Relation between disease severity with CRP and Ferritin in patient group (Group II)**

**Table (4): Average value of platelet count and coagulation parameters in survivors and non-survivor patients compared to standard reference range**

| | References Range | Outcome | | Test of Sig. | p |
|---|---|---|---|---|---|
| | | Survivors (n = 29) | Non survivor (n = 11) | | |
| **Platelet Count (x10$^3$/mm)**<br>Mean ± SD.<br>Median (Min. – Max.) | **150-450** | 164.0 ± 37.24<br>160.0 (92.0 – 285.0) | 115.8 ± 29.0<br>100.0 (91.0 – 181.0) | U= 45.50* | <0.001* |
| **PT (seconds)**<br>Mean ± SD.<br>Median (Min. – Max.) | **12.5-14.5 sec.** | 13.96 ± 0.87<br>13.80(13.0 – 15.90) | 18.68 ± 5.77<br>15.90 (15.30 – 35.10) | t= 2.700* | 0.022* |
| **aPTT (seconds)**<br>Mean ± SD.<br>Median (Min. – Max.) | **24.9-38.2 sec.** | 37.51 ± 12.28<br>30.80 (28.0 – 70.0) | 51.24 ± 14.04<br>49.0 (27.0 – 79.0) | t= 3.037* | 0.004* |
| **INR**<br>Mean ± SD.<br>Median (Min. – Max.) | **0.9-1.2** | 1.13 ± 0.11<br>1.15 (1.0 – 1.36) | 1.75 ± 0.93<br>1.36 (1.0 – 4.40) | t= 1.968 | 0.049* |
| **D-Dimer (Ug/ml)**<br>Mean ± SD.<br>Median (Min. – Max.) | **0-0.5 ug/ml** | 1.04 ± 1.03<br>0.66 (0.30 – 5.05) | 1.25 ± 0.60<br>1.07 (0.36 – 2.20) | U= 97.50 | 0.060 |
| **Protein S activity (%)**<br>Mean ± SD.<br>Median (Min. – Max.) | **60-130%** | 51.94 ± 16.41<br>48.10 (22.60 – 102.3) | 33.26 ± 10.89<br>30.40 (18.80 – 60.0) | U= 40.0* | <0.001* |

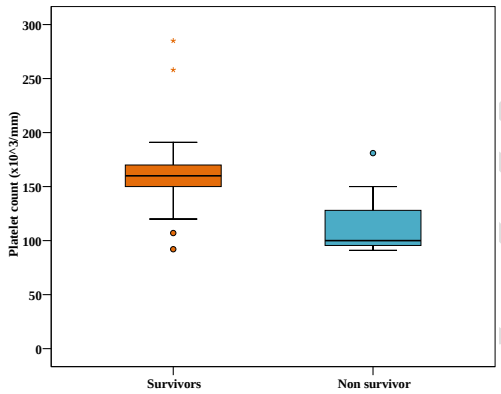


**Figure (10): Relation between outcome and Platelet Count in patient group (Group II)**

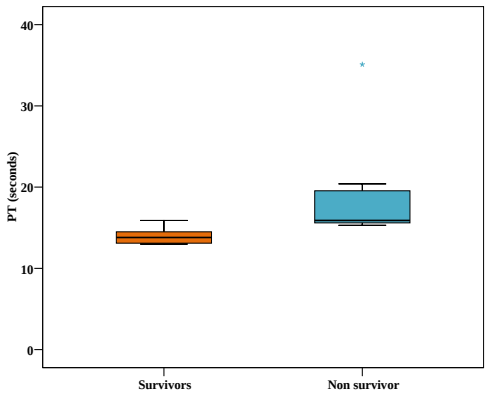


**Figure (11): Relation between outcome and PT in patient group (Group II)**

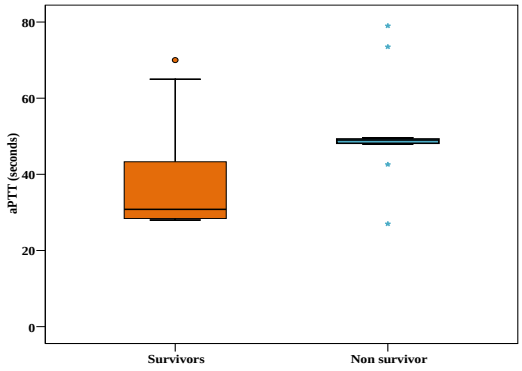


**Figure (12): Relation between outcome and aPTT in patient group (Group II)**

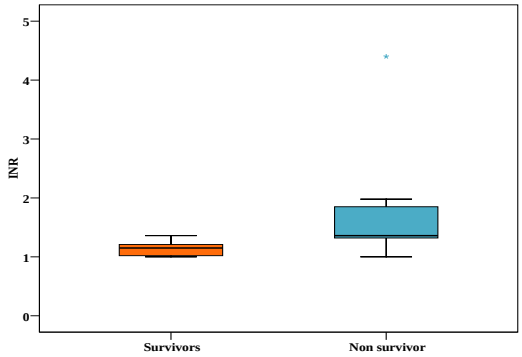


**Figure (13): Relation between outcome and INR in patient group (Group II)**

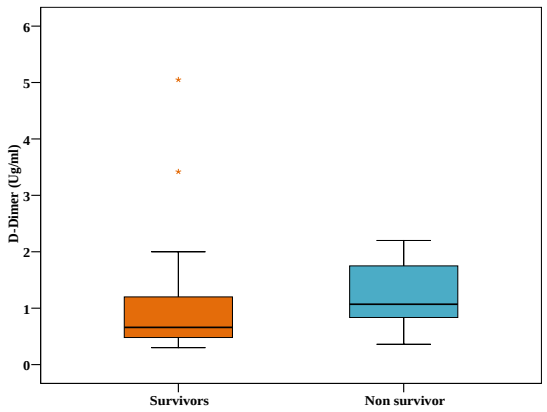


**Figure (14): Relation between outcome and D-Dimer in patient group (Group II)**

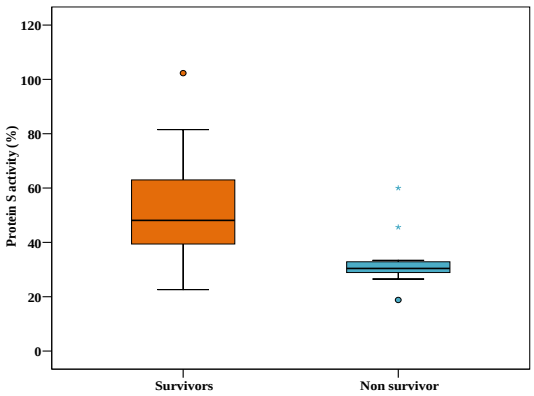


**Figure (15): Relation between outcome and Protein S activity in patient group (Group II)**

**Table (5): Characteristic details for coagulation and inflammatory parameters according to disease severity**

| | Disease severity | | | Mild vs. Moderate | Mild vs. Severe | Moderate vs. Severe |
|---|---|---|---|---|---|---|
| | Mild (n = 8) | Moderate (n = 22) | Sever (n = 10) | | | |
| **Platelet count (x10$^3$/mm)** | | | | | | |
| Mean ± SD. | 162.6 ± 57.52 | 161.6 ± 30.57 | 117.4 ± 30.07 | | | |
| Median | 162.5 | 160.0 | 105.0 | 0.746 | 0.028* | 0.002* |
| (Min. – Max.) | (92.0 – 285.0) | (100.0 – 258.0) | (91.0 – 181.0) | | | |
| **PT (seconds)** | | | | | | |
| Mean ± SD. | 13.53 ± 0.57 | 14.21 ± 0.97 | 18.96 ± 6.01 | | | |
| Median | 13.30 | 14.35 | 16.60 | 0.852 | 0.002* | 0.001* |
| (Min. – Max.) | (13.0 – 14.20) | (13.0 – 15.90) | (15.30 – 35.10) | | | |
| **aPTT (seconds)** | | | | | | |
| Mean ± SD. | 33.85 ± 6.31 | 39.33 ± 13.59 | 51.53 ± 14.77 | | | |
| Median | 31.90 | 30.45 | 49.0 | 0.562 | 0.017* | 0.045* |
| (Min. – Max.) | (28.0 – 43.30) | (28.0 – 70.0) | (27.0 – 79.0) | | | |
| **INR** | | | | | | |
| Mean ± SD. | 1.06 ± 0.07 | 1.17 ± 0.18 | 1.77 ± 0.96 | | | |
| Median | 1.03 | 1.17 | 1.36 | 0.868 | 0.012* | 0.007* |
| (Min. – Max.) | (1.0 – 1.15) | (1.0 – 1.81) | (1.28 – 4.40) | | | |
| **D-Dimer (Ug/ml)** | | | | | | |
| Mean ± SD. | 0.73 ± 0.59 | 1.13 ± 1.13 | 1.34 ± 0.55 | | | |
| Median | 0.46 | 0.68 | 1.17 | 0.243 | 0.008* | 0.044* |
| (Min. – Max.) | (0.30 – 2.0) | (0.30 – 5.05) | (0.74 – 2.20) | | | |
| **Protein S activity (%)** | | | | | | |
| Low | 4 (50.0%) | 15 (68.2%) | 9 (90.0%) | $^{FE}$p= | $^{FE}$p= | $^{FE}$p= |
| Normal | 4 (50.0%) | 7 (31.8%) | 1 (10.0%) | 0.431 | 0.118 | 0.380 |
| Mean ± SD. | 54.88 ± 24.61 | 49.86 ± 13.17 | 33.64 ± 11.41 | | | |
| Median | 52.50 | 47.90 | 31.0 | 0.734 | 0.006* | 0.002* |
| (Min. – Max.) | (22.60 – 102.3) | (29.50 – 81.50) | (18.80 – 60.0) | | | |

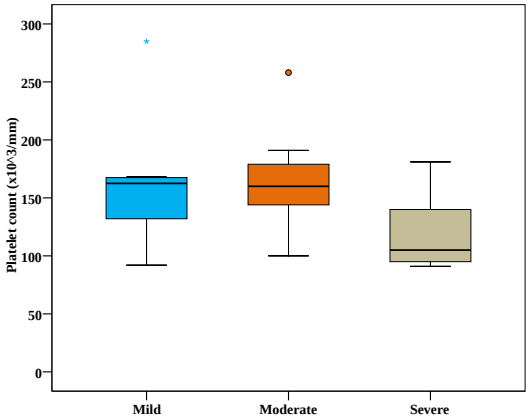


**Figure (16): Relation between disease severity and Platelet Count in patient group (Group II)**

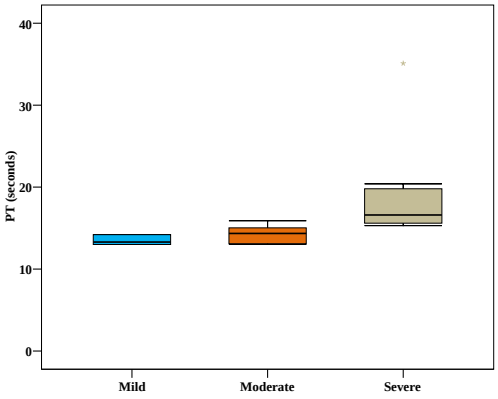


**Figure (17): Relation between disease severity and PT in patient group (Group II)**

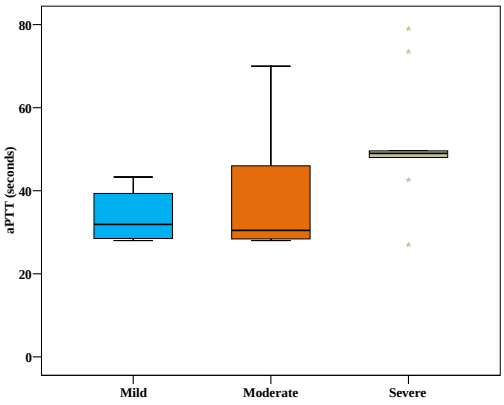


**Figure (18): Relation between disease severity and aPTT in patient group (Group II)**

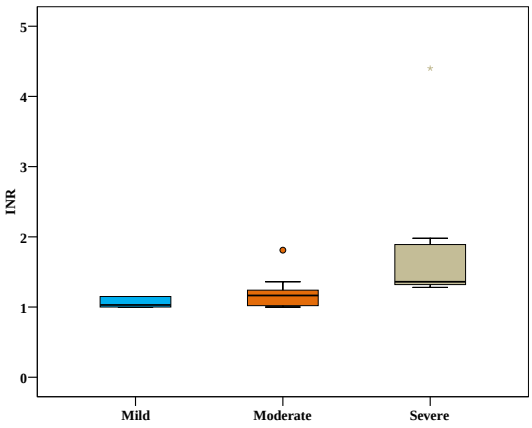


**Figure (19): Relation between disease severity and INR in patient group (Group II)**

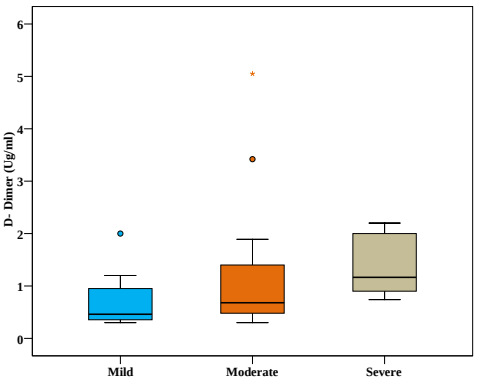


**Figure (20): Relation between disease severity and D-Dimer in patient group (Group II)**

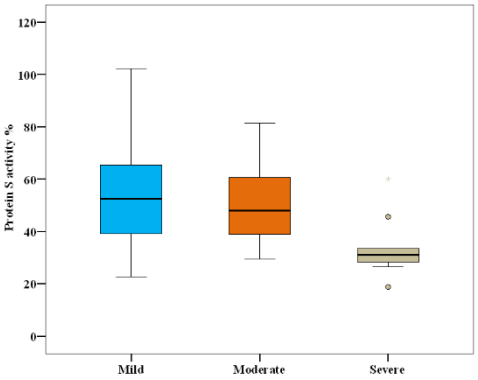


**Figure (21): Relation between disease severity and Protein S activity (%) in patient group (Group II)**

## DISCUSSION

The SARS-CoV-2 virus does not seem to have inherent procoagulant properties; somewhat, the coagulopathy is likely due to the intense COVID-19 inflammatory reaction and endothelial activation/damage (17). Current COVID-19 autopsy research reveal pulmonary endothelial viral inclusions and apoptosis, as well as enhanced angiogenesis and capillary microthrombi (18, 19).

This research objects to assess the role of protein S in COVID-19 cases. We studied 2 groups, Group I (control group) included 15 normal persons with age and sex matched to patient group, and Group II included 40 patients diagnosed as COVID-19 positive using PCR. Patient group included 25 males and 15 Females with age from 26-75 years. All patients were evaluated for disease severity following *Stoichitoiu LE et al.(2020)* and divided to mild ( 20%), moderate ( 55%) and severe ( 25%) (15).

Our results revealed there was significant elevation in neutrophil count in group II than group I, while there was significant decline in lymphocyte count in group II than group I.

These findings are in line with *Kong et al.(2020)* who revealed higher number of neutrophils is related to the negative outcome of COVID-19 cases (20), *Stoichitoiu LE et al.(2020)* also reported death is also related to advanced age, more concentrations of inflammatory markers and neutrophils. Neutrophils were also linked to illness severity, lung involvement, and prognosis (15).

In our patients, platelet count was significantly reduced in group II than group I.

There was significant decrease in platelet count when comparing patients with severe disease versus mild and moderate disease (P = 0.028 and 0.002 respectively) as shown in table 5.

We found there was significant reduction in platelet count in non-survivors than survivors.

Our findings are in line with *Liu Y et al. (2019)* who revealed thrombocytopenia upon admission in COVID-19 cases was related with an elevated risk of inpatient death (21)**.** *Li et al.( 2020)* demonstrated COVID-19 cases with severe illness had a lower platelet count (22).

Our results were not in line with *Rampotas et al.(2021); Hottz et al(.2020)* who reported platelet concentrations were significantly elevated in severe cases than in mild ones (23, 24).

Younger platelets have greater amounts of activation to agonists and hence induce platelet aggregation more easily (25). As it is recognized that immature platelets are more active, this may be an additional explanation for elevated clotting events in COVID-19 (26).

In our results there is significant increase in inflammatory markers (Ferritin and CRP) in group II than group I.

There was a significant elevation in CRP above reference range (90%, 86.4% and 37.5%) with increasing disease severity.

Our results was aggrement with *Lippi G et al.(2020)* who reported CRP is an acute phase reactant whose

concentration rises dramatically during the acute phase response (27) and *Bao C et al.(2020)* reported that CRP is associated with elevated IL-6 (17).

Our results revealed that 62.5%, 81.8% and 80% of mild, moderate and severely infected patients had ferritin level above reference range.

*Stoichitoiu LE et al.(2020)* reported that death is related to older age, more concentrations of inflammatory markers (CRP and ferritin) (15).

In our results, there was non-significant elevation in PT, aPTT and INR in group II than group I.

This was in line with *Panigada M et al.(2020)* who found normal or slightly lengthened PT and aPTT in COVID cases than controls (28).

PT was significantly elevated in cases with severe versus mild and moderate illness.

aPTT was significantly elevated in cases with severe versus mild and moderate illness.

INR was significantly elevated in cases with severe versus mild and moderate illness.

We found a significant elevation in PT, aPTT and INR in non-survivors than survivors.

This was in concurrence with the findings of *Long H et al.(2020)* who demonstrated D-Dimer and PT values might be utilised as possible predictors of death owing to COVID-19 infections (29). *Tan et al.(2020)* reported three ICU patients with mildly prolonged aPTT and clotting times (30).

On the other hand, these results are against those of *Spyropoulos et al (2020)* who found short PT and aPTT. The shortened aPTT is often linked with higher Factor VIII (FVIII) as an acute phase reaction (31).

In our results, D-Dimer level showed significant elevation in group II than group I.

Our findings are in line with *Helms et al. (2020)* who revealed D-Dimer is significantly elevated in COVID-19 cases, likely reflecting pulmonary vascular bed thrombosis and fibrinolysis (32).

*Wright FL et al. (2020)* explained PAI-1 production from endothelial cells is increased during SARS-CoV-2 infection, which inhibits urokinase plasminogen activator and tissue plasminogen activator. Consequently, by inhibiting the conversion of plasminogen to plasmin, fibrin destruction is diminished. In venous thrombosis, incomplete destruction of intravascular thrombus leads in elevated blood levels of intermediate degradation products such as D-Dimer (33).

We found significant increase in D-Dimer when comparing severely ill patients with mild and moderately ill patients (P = 0.008 and 0.044 respectively).

70% of severely infected cases, 59.1% of moderately infected and 25% of mildly infected had D-Dimer above reference range

Our findings are in line with *Tng J et al.(2020)* who demonstrated there was a correlation among high D-Dimer concentrations and illness severity and 28-day mortality (34), they also reported that

D-Dimer concentrations have been demonstrated to be directly related to illness severity. Multivariate analysis indicates that a high D-Dimer level, normal or moderately extended PT and aPTT, and mild thrombocytopenia related with 28-day mortality (34).

*Helms J et al. (2020), Han H et al.(2020)* reported that D-Dimers are representative of fibrin clot formation, clot crosslinking by FXIIIa, and fibrinolysis. The substantial rise of D-Dimers in COVID-19 seems to be attributable to coagulation activation caused by viremia and cytokine storm (32, 35).

Regarding protein S activity, our results showed that 28 out of 40 cases had low protein S activity (70%) while 12 cases had normal protein S activity (30%). There was significant reduction in protein S activity in group II than group I.

This findings is in line with *Stoichitoiu LE et al.(2020)* who found 64.8% of patients had protein S activity lower than normal (15), while *Bouadma et al.(2020)* found that only 20% of patients had protein S deficiency (36).

*Pilli VS et al. (2018)* observed that Protein S supplementation in the plasma of thrombotic mice was able to reduce the risk of thrombosis. Notably, the addition of Protein S to normal mouse plasma also decreased thrombin production. These findings suggest Protein S supplementation may be beneficial in the treatment of thrombotic problems (37).

It seems that all three components of Virchow's triad are present in COVID-19 cases. These are endothelial injury, stasis, and hypercoagulable state (38).

We found 90% of severely infected cases, 72.7% of moderately infected and 37.5% of mildly infected had protein S activity below reference range.

Also, we found significant decrease in Protein S activity in comparing severely infected with mild and moderately infected.

There was significant decrease in Protein S activity in non-survivors than survivors.

This was in line with *Stoichitoiu LE et al.(2020)* who found decrease protein S activity was correlated with illness severity according to lung involvement and CT scan (15) . The correlation was revealed by *Srivastava et al.(2022)* who stated that 91% of cases with severe infection had lower protein S concentrations than reference range (39), while *Bouadma et al.(2020)* informed that deficiency of protein S was not different between severe and non-severe patients (36).

*Amouri et al. (2020)* reveled a new concept linking the severity of the pathology to the antibody response to covid-19 infection, as this infection stimulates the development of anti-spike protein antibodies against the viral spike protein. Due to this protein's similarity to protein S, the antibodies target protein S, resulting in its degradation or malfunction (40).

## References

.1 Guan W-j, Ni Z-y, Hu Y, Liang W-h, Ou C-q, He J-x, et al. Clinical Characteristics of Coronavirus Disease 2019 in China. New England Journal of Medicine. 2020;382(18):1708-20.

.2 Singhania N, Bansal S, Nimmatoori DP, Ejaz AA, McCullough PA, Singhania G .Current Overview on Hypercoagulability in COVID-19.

Am J Cardiovasc Drugs. 2020;20(5):393-403. Epub 2020/08/05.

.3 Cai A, McClafferty B, Benson J, Ramgobin D, Kalayanamitra R, Shahid Z, et al. COVID-19: Catastrophic Cause of Acute Lung Injury. S D Med .60-252:(6)73;2020 .Epub 2020/06/25.

.4 Connors JM, Levy JH. Thromboinflammation and the hypercoagulability of COVID-19. J Thromb Haemost. 2020;18(7):1559-61. Epub 2020/04/18.

.5 Carsana L, Sonzogni A, Nasr A, Rossi RS, Pellegrinelli A, Zerbi P, et al. Pulmonary post-mortem findings in a series of COVID-19 cases from northern Italy: a two-centre descriptive study. Lancet Infect Dis. 2020;20(10):1135-40. Epub 2020/06/12.

.6 Singhania N, Bansal S, Singhania G. An atypical presentation of novel coronavirus disease 2019 (COVID-19). Am J Med. 2020;133(7):e365.

.7 Bikdeli B, Madhavan MV, Jimenez D, Chuich T, Dreyfus I, Driggin E, et al. COVID-19 and Thrombotic or Thromboembolic Disease: Implications for Prevention, Antithrombotic Therapy, and Follow-Up: JACC State-of-the-Art Review. J Am Coll Cardiol. 2020;75(23):2950-73. Epub 2020/04/21.

.8 Becker RC. COVID-19 update: Covid-19-associated coagulopathy. J Thromb Thrombolysis. 2020;50(1):54-67. Epub 2020/05/18.

.9 Fogarty H, Townsend L, Ni Cheallaigh C, Bergin C ,Martin-Loeches I, Browne P, et al. COVID19 coagulopathy in Caucasian patients. Br J Haematol. 2020;189(6):1044-9. Epub 2020/04/25.

.10 Levi M, Thachil J, Iba T, Levy JH. Coagulation abnormalities and thrombosis in patients with COVID-19. Lancet Haematol . :(6)7;2020e438-e40. Epub 2020/05/15.

.11 Lundwall A, Dackowski W, Cohen E, Shaffer M, Mahr A, Dahlback B, et al. Isolation and sequence of the cDNA for human protein S, a regulator of blood coagulation. Proc Natl Acad Sci U S A. 1986;83(18):6716-20.

.12 Mulangu S, Dodd LE, Davey RT, Jr., Tshiani Mbaya O, Proschan M, Mukadi D, et al. A Randomized, Controlled Trial of Ebola Virus Disease Therapeutics. N Engl J Med. 2019;381(24):2293-303. Epub 2019/11/28.

.13 Lemke G, Silverman GJ. Blood clots and TAM receptor signalling in COVID-19 pathogenesis. Nat Rev Immunol. 2020;20(7):395-6.

.14 Ackermann M, Verleden SE, Kuehnel M, Haverich A, Welte T, Laenger F, et al. Pulmonary Vascular Endothelialitis, Thrombosis, and Angiogenesis in Covid-19. N Engl J Med. 2020;383 .8-120:(2)Epub 2020/05/22.

.15 Stoichitoiu LE, Pinte L, Balea MI, Nedelcu V, Badea C, Baicus C. Anticoagulant protein S in COVID-19: low activity, and associated with outcome. Rom J Intern Med. 2020;58(4):251-8.

.16 XIE J, COVASSIN N, FAN Z, SINGH P, GAO W, LI G, et al. Association Between Hypoxemia and Mortality in Patients With COVID-19. Mayo Clin Proc. 2020;95(6):1138-1147. doi:10.1016/j.mayocp.2020.04.006

.17 Bao C, Liu X, Zhang H, Li Y, Liu J. Coronavirus disease 2019 (COVID-19) CT findings: a systematic review and meta-analysis. Journal of the American college of radiology. 2020;17(6):701-9.

.18 Ackermann M, Verleden SE, Kuehnel M, Haverich A, Welte T, Laenger F, et al. Pulmonary vascular endothelialitis, thrombosis, and angiogenesis in Covid-19 .New England Journal of Medicine. 2020;383(2):120-8.

.19 Varga Z, Flammer AJ, Steiger P, Haberecker M, Andermatt R, Zinkernagel AS, et al. Endothelial cell infection and endotheliitis in COVID-19. The Lancet. 2020;395(10234):1417-8.

.20 Kong M, Zhang H, Cao X, Mao X, Lu Z. Higher level of neutrophil-to-lymphocyte is associated with severe COVID-19. Epidemiology & Infection. 2020;148.

.21 Liu Y, Sun W, Guo Y, Chen L, Zhang L, Zhao S, et al. Association between platelet parameters and mortality in coronavirus disease 2019: retrospective cohort study. Platelets. 2020 May; 31(4): 490–6.

.22 Li Q, Cao Y, Chen L, Wu D, Yu J, Wang H, et al. Hematological features of persons with COVID-19. Leukemia. 2020 Aug; 34(8): 2163–72

.23 Rampotas A, Pavord S. Platelet aggregates, a marker of severe COVID-19 disease. Journal of Clinical Pathology. 2021;74(11):750-1.

.24 Hottz ED, Azevedo-Quintanilha IG, Palhinha L, Teixeira L, Barreto EA, Pão CR, et al. Platelet activation and platelet-monocyte aggregate formation trigger tissue factor expression in patients with severe COVID-19. Blood. 2020;136(11):1330-41.

.25 Hille L, Lenz M, Vlachos A, Gruning B, Hein L, Neumann FJ, et al. Ultrastructural, transcriptional, and functional differences between human reticulated and non-reticulated platelets. J Thromb Haemost. 2020 Aug; 18(8): 2034–46.

.26 Lador A, Leshem-Lev D, Spectre G, Abelow A, Kornowski R, Lev EI. Characterization of surface antigens of reticulated immature platelets. J Thromb Thrombolysis. 2017 Oct; 44(3): 291–7.

.27 Lippi G, Plebani M. Laboratory abnormalities in patients with COVID-2019 infection. Clin Chem Lab Med. 2020 Jun;58(7):1131–4

.28 Panigada M, Bottino N, Tagliabue P, Grasselli G, Novembrino C, Chantarangkul V, et al. Hypercoagulability of COVID-19 patients in Intensive Care Unit. A report of thromboelastography fndings and other parameters of hemostasis. J Thromb Haemost. 2020;18(7):1738–42. https://doi.org/10.1111/jth.14850 .

.29 Long H, Nie L, Xiang X, Li H, Zhang X, Fu X, et al. D-dimer and prothrombin time are the significant indicators of severe COVID-19 and poor prognosis. Biomed Res Int. 2020 Oct;2020:6159720

.30 Tan W, Hao F, McIntyre RS, Jiang L, Jiang X, Zhang L, et al. Is returning to work during the COVID-19 pandemic stressful? A study on immediate mental health status and psychoneuroimmunity prevention measures of Chinese workforce. Brain, behavior, and immunity. 2020;87:84-92.

.31 Spyropoulos AC, Levy JH, Ageno W, Connors JM, Hunt BJ, Iba T, et al. Scientific and standardization committee communication: Clinical guidance on the diagnosis, prevention and treatment of venous thromboembolism in hospitalized patients with COVID-19. J Thromb Haemost. 2020.

.32 Helms J, Tacquard C, Severac F, Leonard-Lorant I, Ohana M, Delabranche X, et al. High risk of thrombosis in patients with severe SARS-CoV-2 infection: a multicenter prospective cohort study. Intensive care medicine. 2020;46(6):1089-98.

.33 Wright FL, Vogler TO, Moore EE, Moore HB, Wohlauer MV, Urban S, et al. Fibrinolysis shutdown correlation with thromboembolic events in severe COVID-19 infection. Journal of the American College of Surgeons. 2020;231(2):193-203. e1.

.34 Tang N, Bai H, Chen X, Gong J, Li D, Sun Z. Anticoagulant treatment is associated with decreased mortality in severe coronavirus disease 2019 patients with coagulopathy. Journal of thrombosis and haemostasis. 2020;18(5):1094-9.

.35 Han H, Yang L, Liu R, Liu F, Wu K-l, Li J, et al. Prominent changes in blood coagulation of patients with SARS-CoV-2 infection. Clinical

Chemistry and Laboratory Medicine (CCLM). 2020;58(7):1116-20.

.36 Bouadma L, Lescure F-X, Lucet J-C, Yazdanpanah Y, Timsit J-F. Severe SARS-CoV-2 infections: practical considerations and management strategy for intensivists. Intensive care medicine. 2020;46(4.82-579:(

.37 Pilli VS, Datta A, Afreen S, Catalano D, Szabo G, Majumder R. Hypoxia downregulates protein S expression. Blood, The Journal of the American Society of Hematology. 2018;132(4):452-5.

.38 CONNORS, Jean M.; LEVY, Jerrold H. COVID-19 and its implications for thrombosis and anticoagulation. Blood, 2020, 135.23: 2033-2040.

.39 Srivastava S, Garg I, Dogra V, Bargotya M, Bhattar S, Gupta U, et al. Implications of COVID-19 on Thrombotic Profile of Severely Affected Patients. Pathobiology. 2022:1-1.1

.40 Amouri A, Alaparthy VT, Morgera SD. A machine learning based intrusion detection system for mobile Internet of Things. Sensors. 2020;20(2):461.