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Circulating Naturally-Occurring Anticoagulants before Treatment and after Recovery from SARS-CoV-2 Infection in Ghana

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Abstract

Background: Disturbance in naturally-occurring anticoagulants may contribute to the hypercoagulable state in COVID-19. This study determined the plasma antigen levels of protein C (PC), protein S (PS), antithrombin-III (AT-III), and thrombomodulin (TM) before treatment and after recovery from COVID-19. *Materials and Methods*: This cross-sectional study, conducted from February to August 2022 at Kumasi South Hospital, recruited sixty-five RT-PCR-confirmed COVID-19 participants. A venous blood sample was taken for full blood count (FBC) analysis using a 3-part fully automated haematology analyzer, and PC, PS, AT-III, and TM antigen levels measured using ELISA. The data were analyzed using SPSS version 26.0. $P<0.05$ was considered statistically significant. *Results*: Severe COVID-19 participants had relatively lower haemoglobin ($p<0.001$), RBC ($p<0.001$), HCT% ($p<0.001$) and platelets ($p<0.001$), but higher RDW-CV% ($p=0.013$), WBC ($p<0.001$), and absolute lymphocyte counts ($p<0.001$) compared to those with the non-severe form

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of the disease. The overall prevalence of anaemia among the participants was 58.5%, and 32 (84.2%) and 6 (15.8%) of the anaemic participants had mild and moderate anaemia respectively. Protein C ($p<0.001$), PS ($p<0.001$) and ATIII ($p<0.001$) levels were lower among the severe COVID-19 participants than in the non-severe group. But severe COVID-19 group had higher TM levels ($p<0.001$) than the non-severe group. Again, participants had higher haemoglobin ($p<0.001$), RBC ($p<0.001$), HCT% ($p=0.049$), absolute neutrophil count ($p<0.001$) and platelets ($p<0.001$) after recovery from COVID-19 than the values on admission. Additionally, after recovery, participants had higher levels of PC ($p<0.001$), PS ($p<0.001$), and ATIII ($p<0.001$), but reduced TM ($p<0.001$). *Conclusion*: Severe COVID-19 patients had higher PC, PS, and AT-III, but lower TM levels. The changes in circulating anticoagulants may contribute to the hypercoagulable state of COVID-19. Blood cell indices are negatively affected during COVID-19. Complete recovery from the SARS-CoV-2 infection normalised the haematological indices. Assessment of naturally-occurring anticoagulants and the provision of anticoagulants are recommended in the management of COVID-19.

Keywords: Anaemia; Antithrombin-III; COVID-19; Protein C; Protein S; Thrombomodulin.

1. Introduction

The upsurge in morbidities and mortalities from the novel coronavirus disease 2019 (COVID-19) may be attributed to the associated fatal acute respiratory distress syndrome (ARDS) experienced during disease progression [1]. The COVID-19-causing pathogen, severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) requires angiotensin-converting enzyme-2 (ACE-2) for cell entry and adaptation, primarily in the respiratory tract. The widespread distribution of ACE-2 throughout the body enables SARS-CoV-2 entry into the general circulation and other parts of the body, inducing an inflammatory response and causing life-threatening complications [2, 3].

Severe COVID-19 patients suffer various haematological and biochemical derangements, and these have been linked to the associated cytokine storm and disruption of the endothelium [4–7], activation of procoagulants, hypofibrinolysis [8], altered synthesis and activation of naturally-occurring anticoagulants [9, 10], and stimulation of cell-death mechanisms [11]. Previous studies have suggested the occurrence of decreased haemoglobin (Hb) or anaemia [4-7], thrombocytopaenia [6, 12], leucocytosis [13, 14], and abnormal leucocyte differentials [12, 15] among severe COVID-19 patients. The deranged blood cell parameters have been linked to the cytokine storms with the subsequent increase and activation of circulating pro-inflammatory cytokines such as interleukin (IL)-6, IL-1, and tumour necrosis factor-alpha (TNF- α) during COVID-19 [16].

Efficient regulation of the synthesis of the naturally-occurring anticoagulants, protein C (PC), protein S (PS), anti-thrombin-III (AT-III), and thrombomodulin (TM), mostly by hepatocytes and endothelial cells, is very essential to controlling haemostatic activities; dysregulation of the process triggers thrombosis or haemorrhage [17]. Physiologically, thrombin forms a complex with TM, and the resulting thrombin-TM complex activates the most potent anticoagulant, PC. Activated PC (APC), in the presence of its cofactor PS, through proteolytic digestion, inactivates essential cofactors in the coagulation cascade, activated factor VIII (FVIIIa) and activated factor V (FVa), as well as inactivating the potent anti-fibrinolytic agent, plasminogen activator inhibitor-1 (PAI-1). This eventually controls the rate of fibrin formation to avoid excess thrombi generation [18]. Also, AT-III, enhanced by heparin, inhibits the activities of thrombin to ensure complete regulation of the haemostatic processes [19].

A previous histological study suggested the contributions of endothelial dysfunction (endotheliopathy) and widespread inflammation to the development of coagulopathy in severe COVID-19 patients [20]. Endotheliopathy was facilitated by the direct interactions of SARS-CoV-2 with endothelial cells through ACE-2 [21]. Earlier studies observed decreased plasma levels of PS [22, 23] and AT-III [22, 24] but increased TM levels [25] among hospitalized COVID-19 patients in the Intensive Care Unit (ICU). Variable PC levels have been reported in previous studies. While Kim and Kim [10], Corrêa et al. [22], and Al-Kuraishy et al. [26] studies identified decreased PC levels among mostly severe and critically ill COVID-19 patients at the ICU, the study by Esmaeel et al. [24] observed increased PC among the SARS-CoV-2-infected individuals. The disturbed endothelial function contributes significantly to the development of coagulopathy and thromboembolism in severe COVID-19 patients [27]. Approximately two-thirds of critically-ill COVID-19 patients in the ICU after receiving thromboprophylaxis still exhibited elevated levels of D-dimer and fibrinogen, as well as prolongation in prothrombin time (PT), signifying the occurrence of coagulopathy during the disease's progression [25, 28].

A recent case study in Ghana by Yasmine et al. [29] suggested the occurrence of thrombotic events among severe and critically ill COVID-19 patients after recording increased D-dimer levels. But the Yasmine et al. [29] case study, which involved three COVID-19 patients, only assessed D-dimer levels and could not give accounts on the effects of SARS-CoV-2 on naturally-occurring anticoagulants in Ghana. Hence, this study assessed the effects of COVID-19 on plasma PC, PS, AT-III, and TM, and determined the influence of the disease's management on the anticoagulants in Ghana.

2. Materials and Methods

2.1. Study Design/Setting

This cross-sectional study, from February to August 2022, was done at Kumasi South Hospital. This Public Health and Regional Hospital located in Kumasi, Ashanti Region, serves as one of the COVID-19 diagnostic centres in Ghana. The 140-bed capacity regional hospital located at longitude 1.300 West and latitudes 6.350 North and 6.400 South receives referrals from thirteen health facilities surrounding it [30].

2.2. Study population

Participants for the study were patients who had reverse transcriptase-polymerase chain reaction (RT-PCR)-confirmed COVID-19 and were receiving treatment at Kumasi South Hospital, Kumasi. A total of 65 COVID-19 patients were recruited for the study.

2.3. Inclusion Criteria

RT-PCR-confirmed COVID-19 patients who reported to Kumasi South Hospital and provided informed consent were included in the study.

2.4. Exclusion Criteria

COVID-19 patients with known chronic diseases, haematological disorders, on therapies and those who did not give their consent were excluded from the study.

2.5. Sample size determination

The sample size for the study was determined by the Cochran's formula [31]:

$$n = \frac{Z^2 P(1-p)}{d^2} \quad (1)$$

where: z is 1.96 at 95% confidence interval, p is prevalence of deep vein thrombosis in COVID-19, being 3% [31], d is margin of error (5%), n is minimum sample size required.

Forty-five (45) COVID-19 participants were required, but 65 participants were recruited for the study.

2.6. Specimen Collection and Processing

A nasopharyngeal specimen was collected from each participant in sterile tubes for the diagnosis of COVID-19. About 5 mL of whole blood were collected as soon as COVID-19 was diagnosed: 2 mL were dispensed into dipotassium ethylenediaminetetraacetic acid (K2EDTA) tubes for FBC, and 3 mL into sodium citrate tubes for analysis of plasma PC, PS, AT-III, and TM antigen levels. The blood in the respective tubes was adequately mixed to avoid coagulation. The plasma was aliquoted into Eppendorf tubes and stored at -20°C for the analysis of the anticoagulants using the Enzyme-Linked Immunosorbent Assay (ELISA) technique.

The aforementioned procedures were repeated for specimen collection, processing, storage, and batch analysis of CBC, PC, PS, AT-III, and TM immediately a participant attained complete recovery from COVID-19.

2.7. Diagnosis of COVID-19

The procedures for the diagnosis of COVID-19 from the nasopharyngeal swab through RT-PCR were adopted from the World Health Organization (WHO) guidelines [32]. The test was done at the COVID Laboratory at Kumasi South Regional Hospital. The W.H.O. provides protocols on the stratification of the severity of COVID-19 as described below. Mild COVID-19 was diagnosed when participants had SARS-CoV-2 present in the nasopharyngeal swab but no sign of severe or critical disease. Severe COVID-19 patients harboured SARS-CoV-2 and either oxygen saturation (SpO2) <90% at room temperature, signs of pneumonia, or severe respiratory distress. Patients were classified as having critical COVID-19 when there was an immediate requirement for life-threatening interventions, such as septic shock, sepsis, or acute respiratory distress syndrome (ARDS) [32].

2.8. Measurement of Full Blood Count

A full blood count was done on each day of blood sample collection at Kumasi South Regional Hospital, Kumasi, using a three-part automated haematology analyser (ABX Micros ES60 OT, Horiba Medical, France). The haematology analyser employed the coulter (impedance and flow cytometry) and the colorimetry principles. Red blood cells (RBCs)

and platelets were counted through the impedance principle. Flow cytometry enabled the counting of white blood cells (WBCs) and their differentials, while haemoglobin (Hb) estimation was achieved through the haemiglobin cyanide method at a wavelength of 540 nm. The procedure for the measurement of the blood cell indices was adopted from the study by Osei-Boakye et al. [33]. Diagnoses and stratification of anaemia among the study participants were based on the protocols described by the Chauhan et al. [34] study. Participants with Hb<11.5 g/dL were considered anaemic, and the severity of the anaemia was categorized into three based on the Hb concentration: mild (10.0–11.4 g/dL), moderate (7.0–9.9 g/dL), and severe (<7 g/dL) as recommended [34].

2.9. Plasma PC, PS, AT-III and TM Assay Using ELISA

The sandwich ELISA technique was used to measure the plasma levels of the anticoagulants PC, PS, AT-III, and TM. Commercially prepared ELISA reagents from Biobase, China, were used, and the tests were done according to the manufacturer's recommendations at Ankaase Methodist Hospital Laboratory, Ashanti Region, Ghana. The ELISA plates were splashed by manual technique, and the levels of the anticoagulants were measured with a microplate ELISA detector (Mindray MR-96A, China).

2.10. Statistical Analysis

IBM Statistical Package for the Social Sciences (SPSS) version 26.0 (IBM Corp., Armonk, NY, USA) was used to analyze the data. A one-sample Kolmogorov-Smirnov test and Shapiro-Wilk normality test were used to assess the distribution of the data. Descriptive data were presented as frequencies with corresponding percentages. Non-parametric data were presented in medians (25^{th}–75^{th} percentiles), while parametric data were in means ± standard deviation. Unpaired data were appropriately compared using the Student's T-test or Mann-Whitney U-test. Paired non-parametric data (on admission and after recovery) were compared with the Wilcoxon signed-rank test, and the paired parametric data were compared with the Paired Sample T-test. Statistical significance was set at $p<0.05$.

The flowchart of the research methodology that was used in this study has been shown in Figure 1.

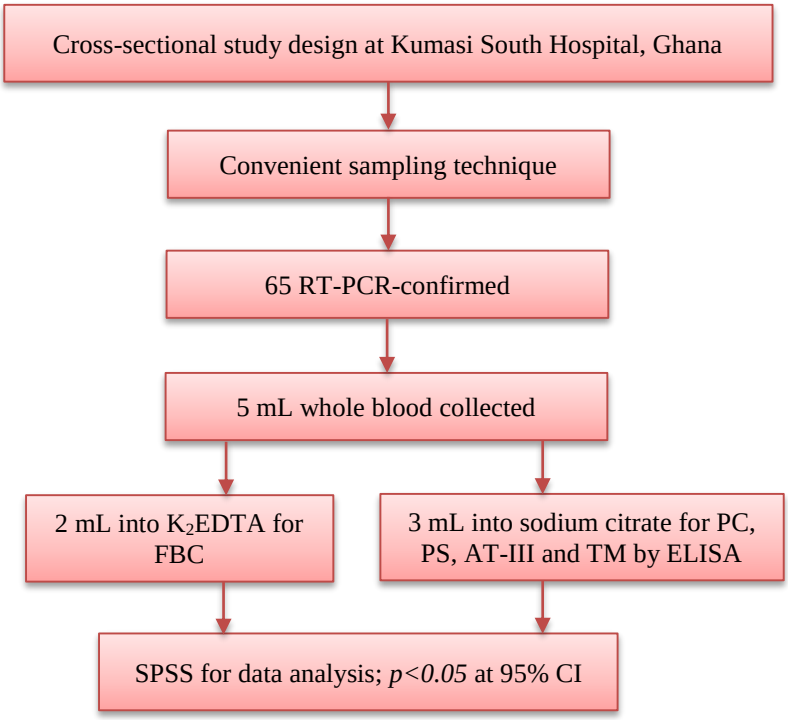

Figure 1. Flow chart of methodology

3. Results

3.1. Socio-demographic Characteristics of Study Participants Stratified by COVID-19 Severity

Table 1 shows the demographic and clinical characteristics of the study participants stratified by the severity of COVID-19. Of the 65 participants recruited into the study, majority (52/80%) had non-severe COVID-19, while 13 (20%) experienced a severe form of the disease. The median age of the study participants was 34 (29–44) years. The majority of the study participants were within 30-39 years (28/43.1%), females (43/66.2%), experienced cough

(41/63.1%), fever (40/61.5%), loss of taste (38/58.5%), and loss of smell (35/53.8%). Fever was present in all the severe COVID-19 participants, while only about half (27/51.9%) of the non-severe COVID-19 subjects had fever, and this was statistically significant (p<0.001) (Table 1).

Table 1. Participants' demographic and clinical characteristics stratified by COVID-19 severity

Variables	Total Participants (*n*=65)	COVID-19 Severity		*P-value*
		Non-severe (*n*=52, 80%)	Severe (*n*=13, 20%)	
Age (years)	**34 (29-44)**	**34 (28.3-45)**	**34 (29-37.5)**	**0.424**
Age group (years)				
20-29`	19 (29.2)	15 (28.5)	4 (30.8)	0.296
30-39	28 (43.1)	21 (40.4)	7 (53.8)	
40-49	8 (12.3)	7 (13.5)	1 (7.7)	
50-59	3 (4.6)	2 (3.8)	1 (7.7)	
>59	7 (10.5)	7 (13.5)	-	
Gender				
Male	22 (33.8)	17 (32.7)	5 (38.5)	0.694
Female	43 (66.2)	35 (67.3)	8 (61.5)	
Cough				
Yes	41 (63.1)	31 (59.6)	10 (76.9)	0.342
No	24 (36.9)	21 (40.4)	3 (23.1)	
Fever				
Yes	40 (61.5)	27 (51.9)	13 (100)	**0.001**
No	25 (38.5)	25 (48.1)	0	
Headache				
Yes	23 (35.4)	16 (30.8)	7 (53.8)	0.120
No	42 (64.6)	36 (69.2)	6 (46.2)	
Loss of taste				
Yes	38 (58.5)	30 (57.7)	8 (61.5)	0.801
No	27 (41.5)	22 (42.3)	5 (38.5)	
Loss of smell				
Yes	35 (53.8)	27 (51.9)	8 (61.5)	0.534
No	30 (46.2)	25 (48.1)	5 (38.5)	
Fatigue				
Yes	32 (49.2)	23 (44.2)	9 (69.2)	0.130
No	33 (50.8)	29 (55.8)	4 (30.8)	

n = Number of participants; COVID-19= Coronavirus disease-2019. Age was presented in median (25^{th}-75^{th} percentiles) and was compared using Mann Whitney U-test; Fisher's Exact test was used to compare Cough, Fever, and Fatigue; Chi-square test was used to compare Gender, Headache, Loss of taste and Loss of smell; ANOVA was used to compare the age categories., and all categorical data were presented in frequencies with percentages in parentheses. p<0.05 was considered statistically significant

3.2. Comparison of Blood Cell Indices between Severe and Non-Severe COVID-19 Participants

Table 2 shows the blood cell indices of the study participants stratified by COVID-19 severity. Severe COVID-19 participants had relatively lower Hb (g/dL): 8.1 (7.6-8.4) vs 11.7 (11.0-12.4), p<0.001; red blood cell (RBC) × 10^{12}/L: 2.9 (2.6-3.1) vs 3.4 (3.1-4.1), p<0.001; HCT%: 25.5 (22.9-27.0) vs 34.8 (31.6-37.9), p<0.001; MCV (fL): 92.7 (80.2-98.7) vs 100.3 (89.3-107.7), p=0.036 and platelet × 10^{19}/L: 87.0 (62.6-94.3) vs 170.5 (125.8-210.3), p<0.001, compared to those with non-severe COVID-19. But, red cell distribution width-coefficient of variation (RDW-CV)%: 15.4 (14.6-20.4) vs 14.3 (13.4-15.1), p=0.013; white blood cell count (WBC) × 10^9/L: 11.6 (10.1-13.9) vs 5.5 (3.8-6.6), p<0.001; absolute lymphocyte count (Lymph#) × 10^9/L: 7.6 (6.5-10.0) vs 2.9 (1.9-3.7), p<0.001 and mixed population of monocytes, eosinophils and basophils (MID#) × 10^9/L: 0.6 (0.3-0.8) vs 0.4 (0.3-0.6), p<0.001, were higher among the severe COVID-19 participants than their counterparts with a non-severe form of the disease (Table 2).

Table 2. Comparison of blood cell indices between severe and non-severe COVID-19 Participants

Blood cell indices	Total Participants (*n*=65)	COVID-19 Severity		*P-value*
		Non-severe (*n*=52, 80%)	Severe (*n*=13, 20%)	
Hb (g/dL)	11.4 (8.7-12.3)	11.7 (11.0-12.4)	8.1 (7.6-8.4)	**<0.001**
RBC × 10^{12}/L	3.3 (2.9-3.8)	3.4 (3.1-4.1)	2.9 (2.6-3.1)	**<0.001**
HCT%	34.8 (31.4-38.7)	34.8 (31.6-37.9)	25.5 (22.9-27.0)	**<0.001**
MCV (fL)	98.6 (86.9-106.7)	100.3 (89.3-107.7)	92.7 (80.2-98.7)	**0.036**
MCH (pg)	34.4 (29.1-37.5)	35.6 (29.9-37.8)	32.4 (27.4-34.7)	0.051
MCHC (g/dL)	34.6 (33.8-35.8)	34.7 (33.6-35.9)	34.5 (34.1-35.7)	0.876
RDW-CV%	14.5 (13.6-15.5)	14.3 (13.4-15.1)	15.4 (14.6-20.4)	**0.013**
WBC × 10^9/L	5.8 (4.4-9.5)	5.5 (3.8-6.6)	11.6 (10.1-13.9)	**<0.001**
Lymph# × 10^9/L	3.2 (2.1-6.1)	2.9 (1.9-3.7)	7.6 (6.5-10.0)	**<0.001**
Neut# × 10^9/L	2.2 (1.7-3.0)	2.0 (1.5-2.5)	3.0 (2.9-4.0)	0.073
MID# × 10^9/L	0.4 (0.3-0.6)	0.4 (0.3-0.6)	0.6 (0.3-0.8)	**<0.001**
PLT × 10^9/L	153.0 (108.1-194.5)	170.5 (125.8-210.3)	87.0 (62.6-94.3)	**<0.001**
MPV (fL)	8.3 (7.7-8.8)	8.3 (7.5-9.0)	8.3 (8.0-8.4)	0.787
PDW%	15.4 (12.3-15.8)	15.4 (13.9-15.8)	15.3 (8.9-16.0)	0.961
PCT%	0.13 (0.1-0.2)	0.1 (0.1-0.2)	0.1 (0.1-0.2)	0.127

n= Number of participants, COVID-19= Coronavirus disease-2019, Hb=Haemoglobin, RBC=Red Blood Cell, HCT=Haematocrit, MCV=Mean Cell Volume, MCH=Mean Corpuscular Haemoglobin, MCHC=Mean Corpuscular Haemoglobin Concentration, RDW-CV=Red Cell Distribution Width-Coefficient of Variation, WBC= White Blood Cell, Lymph#=Absolute Lymphocyte count, Neut#= Absolute Neutrophil count, MID#= Mixed Populations of Monocytes, Eosinophils and Basophils, MPV=Mean Platelet Volume, PLT =Platelets, PDW= Platelet Distribution Width, PCT=Plateletcrit, g/dL=Grams per decilitre, fL=Femtolitre, pg=Picogram. Data were presented in medians (25^{th}-75^{th} percentiles) and were compared using Mann-Whitney U-Test. $p<0.05$ was considered statistically significant.

3.3. Prevalence and Severity of Anaemia among COVID-19 Patients

The overall prevalence of anaemia among the COVID-19 participants was 58.5% (38/65). Of the 38 anaemic COVID-19 participants, the majority (32/84.2%) had mild anaemia and 6 (15.8%) with moderate anaemia, but none of them had severe anaemia (Figure 2).

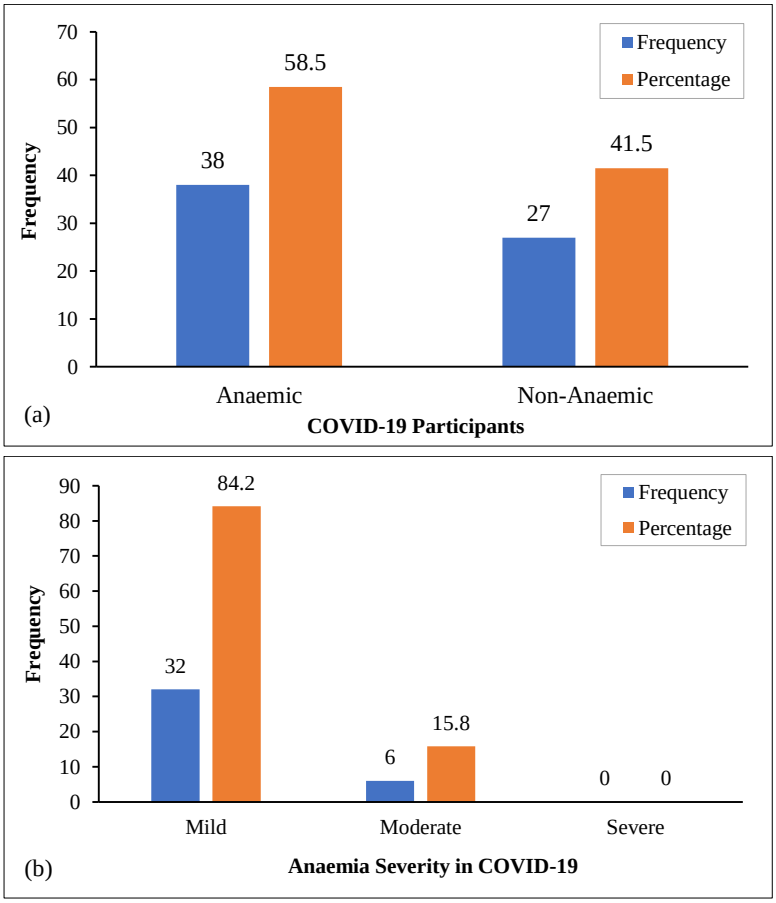

Figure 2. Prevalence (a) and Severity (b) of Anaemia among COVID-19 Participants. COVID-19=Coronavirus Disease-2019

3.4. Circulating Levels of PC, PS, AT-III and TM between Severe and Non-Severe COVID-19 Participants

Table 3 shows the plasma levels of the naturally-occurring anti-coagulants based on the COVID-19 severity of the study participants. The median circulating levels of the naturally-occurring anti-coagulants among the COVID-19 participants in the study were: PC (ng/mL): 21.0 ± 5.0; PS (ng/mL): 17.0 (15.3-19.8); AT-III (ng/mL): 40.0 (32.3-51.0) and TM (ng/mL): 16.0 (14.0-18.0). The plasma anticoagulants were significantly different between the severe and non-severe COVID-19 participants. Protein C (ng/mL): 9.2 ± 3.9 vs 21.0 ± 5.0, p <0.001: PS (ng/mL): 13.0 (12.0-14.0) vs 17.0 (15.3-19.8), p<0.001 and AT-III (ng/mL): 30.0 (29.0-32.0) vs 40.0 (32.3-51.0), p <0.001 were significantly lower among the severe COVID-19 participants compared to the non-severe group. But the severe COVID-19 group had relatively higher plasma levels of TM than the non-severe participants: TM (ng/dL): 25.0 (24.0-27.0) vs 16.0 (14.0-18.0), p <0.001, as shown in Table 3.

Table 3. Circulating levels of PC, PS, AT-III and TM between severe and non-severe COVID-19 Participants

Anticoagulants	Total Participants (n=65)	COVID-19 Participants		*P-value*
		Non-Severe (n=52, 80%)	Severe (n=13, 20%)	
PC (ng/mL)	21.0 ± 5.0	21.0 ± 5.0	9.2 ± 3.9	<0.001
PS (ng/mL)	17.0 (15.3-19.8)	17.0 (15.3-19.8)	13.0 (12.0-14.0)	<0.001
AT-III (ng/mL)	40.0 (32.3-51.0)	40.0 (32.3-51.0)	30.0 (29.0-32.0)	<0.001
TM (ng/mL)	16.0 (14.0-18.0)	16.0 (14.0-18.0)	25.0 (24.0-27.0)	<0.001

PC= Protein C, PS= Protein S, AT-III= Antithrombin-III, TM=Thrombomodulin, ng/mL=Nano gram per millilitre. Non-Parametric data were compared using Mann Whitney U test, and PC by Student's T-test. p< 0.05 was considered statistically significant.

3.5. Participants' Blood Cell Indices before Treatment and after Recovery from the SARS-Cov-2 Infection

Most blood cell indices significantly improved after a successful recovery from COVID-19. Hb (g/dL): 12.4 (11.6-13.6) vs 11.4 (8.7-12.3), p<0.001; RBC × 10^{12}/L: 4.2 (3.3-4.6) vs 3.3 (2.9-3.8), p<0.001; HCT%: 35.6 (31.5-41.3) vs 34.8 (31.4-38.7), p=0.049; absolute neutrophil count × 10^9/L: 4.7 (3.4-5.5) vs 2.2 (1.7-3.0), p<0.001 and platelet × 10^9/L: 225.0 (160.5-273.5) vs 153.0 (108.1-194.5), p<0.001 significantly increased after complete recovery from the SARS-CoV-2 infection compared to the treatment-naïve values. Conversely, absolute lymphocyte count was reduced after participants recovered from the infection (p<0.001). However, MCHC, RDW-CV, WBC, mixed cell population and MPV did not differ after recovery from COVID-19 when compared to the values before treatment commenced, as shown in Table 4.

Table 4.; Participants' blood cell indices before treatment and after recovery from the SARS-CoV-2 infection

Variables	COVID-19 Participants		*P-value*
	Before Treatment	At Recovery	
Hb (g/dL)	11.4 (8.7-12.3)	12.4 (11.6-13.6)	**<0.001**
RBC × 10^{12}/L	3.3 (2.9-3.8)	4.2 (3.3-4.6)	**<0.001**
HCT%	34.8 (31.4-38.7)	35.6 (31.5-41.3)	**0.049**
MCV (fL)	98.6 (86.9-106.7)	91.3 (84.3-98.2)	**0.006**
MCH (pg)	34.4 (29.1-37.5)	30.5 (26.9-35.2)	**0.003**
MCHC (g/dL)	34.6 (33.8-35.8)	34.3 (33.3-35.9)	0.397
RDW-CV%	14.5 (13.6-15.5)	14.2 (13.7-15.5)	0.563
WBC × (10^9/L)	5.8 (4.4-9.5)	6.7 (5.8-7.9)	0.834
Lymph# × 10^9/L	3.2 (2.1-6.1)	1.7 (1.2-2.1)	**<0.001**
Neut# × 10^9/L	2.2 (1.7-3.0)	4.7 (3.4-5.5)	**<0.001**
MID# × 10^9/L	0.4 (0.3-0.6)	0.5 (0.4-0.7)	0.443
PLT × 10^9/L	153.0 (108.1-194.5)	225.0 (160.5-273.5)	**<0.001**
MPV (fL)	8.3 (7.7-8.8)	8.2 (7.6-8.9)	0.615
PDW%	15.4 (12.3-15.8)	15.6 (15.5-15.8)	**0.004**
PCT%	0.1 (0.1-0.2)	0.2 (0.1-0.2)	**0.012**

COVID-19= Coronavirus disease-2019, Hb=Haemoglobin, RBC=Red Blood Cell, HCT=Haematocrit, MCV=Mean Cell Volume, MCH=Mean Corpuscular Haemoglobin, MCHC=Mean Corpuscular Haemoglobin Concentration, RDW-CV=Red Cell Distribution Width-Coefficient of Variation, WBC= White Blood Cell, Lymph#=Absolute Lymphocyte count, Neut#= Absolute Neutrophil count MPV=Mean Platelet Volume, PLT =Platelets, PDW= Platelet Distribution Width, PCT= Plateletcrit. g/dL= Grams per decilitre, fL=Femtolitre, pg=Picogram. Wilcoxon signed-rank test was used to determine the significant difference in blood cell indices during COVID-19 management. p<0.05 was considered statistically significant.

3.6. Comparison of Participants' Plasma Naturally-Occurring Anticoagulants before Treatment and after Recovery from COVID-19

Table 5 shows the plasma antigen levels of the naturally-occurring anticoagulants before treatment and after recovery from COVID-19. Following successful recovery from SARS-CoV-2 infection, plasma levels of PC, PS, and AT-III significantly increased compared to the values before therapy commenced: PC (ng/mL): 32.0 ± 8.4 vs. 21.0 ± 5.0, p <0.001; PS (ng/mL): 21.0 (18.0-24.5) vs. 17.0 (15.3-19.8), p <0.001 and AT III (ng/mL): 64.0 (56.0-75.0) vs. 40.0 (32.3-51.0), p <0.001. On the other hand, circulating TM was relatively higher before treatment [16.0 (14.0–18.0) ng/mL] than after recovery from COVID-19 [13.0 (12.0–15.0) ng/mL], and this was significant (p <0.001) as seen in Table 5.

Table 5. Comparison of participants' circulating naturally-occurring anticoagulants before treatment and after recovery from COVID-19

Anticoagulants	Before Treatment (n=65)	At Recovery (n=65)	P-value
PC (ng/mL)	21.0 ± 5.0	32.0 ± 8.4	<0.001
PS (ng/mL)	17.0 (15.3-19.8)	21.0 (18.0-24.5)	<0.001
AT-III (ng/mL)	40.0 (32.3-51.0)	64.0 (56.0-75.0)	<0.001
TM (ng/mL)	16.0 (14.0-18.0)	13.0 (12.0-15.0)	<0.001

PC= Protein C, PS= Protein S, AT-III= Antithrombin-III, TM= Thrombomodulin, ng/mL=Nano gram per millilitre. Wilcoxon signed-rank test was used to compare PS, AT-III and TM Ag levels before and after COVID-19 management, and PC compared using Paired T-test. p< 0.05 was considered statistically significant.

4. Discussion

Deranged circulating levels of naturally-occurring anticoagulants may contribute to the noticeable thrombosis and deaths occurring during COVID-19 progression. This study determined plasma PC, PS, AT-III, and TM before treatment and after recovery from SARS-CoV-2 infection.

The COVID-19 participants in this study experienced cough, fever, and loss of taste and smell, and this is consistent with similar studies elsewhere [35–37]. The clinical manifestations exhibited by the SARS-CoV-2-infected participants could be related to the virus' vigorous interactions with ACE-2 in the host's epithelial cells, especially in the pulmonary region. The interaction consequently triggers a cytokine-mediated physiological response, activating both innate and adaptive immunity involving adrenergic stimulation pathways and enhancing the occurrence of the clinical features [38]. The damaged endothelial cells activate dendritic cells, which then trigger the release of cytokines and subsequent activation of T-cells, causing inflammation and its associated manifestations [39].

The present study agrees with the negative effects of severe COVID-19 on red cell parameters, as this has been described by earlier studies where RBC, HCT, and Hb were reduced in Ghana [40, 41] and other parts of the world [4–7]. The 58.6% prevalence of anaemia among COVID-19 patients recorded in this study is higher than the 19.23% identified in Liaocheng, China [42], 35.6% [43], and 38.2% [44] of hospitalized COVID-19 patients in Wuhan, China, and 42.7% among COVID-19 survivors in a 30-day prospective cohort study during the initial wave of the outbreak in Italy [5]. The prevalence is, however, lower compared to the 62.8% found in a retrospective study in Riyadh, Kingdom of Saudi Arabia [6], 66.7% among COVID-19 non-survivors in Italy [5], and 74.3% from the CURE cohort (from Kaiser Permanente Georgia's (KPGA) electronic medical record (EMR), which used data generated from members of a southeastern integrated healthcare system testing positive for COVID-19 [7]. The anaemia may be due to the COVID-19-associated cytokine storm that influences iron absorption and re-uptake, and inhibits erythropoietin. IL-6 is a crucial controller of inflammation-induced iron dysregulation as it triggers the release of hepcidin, the chief regulator of iron homeostasis. The hormone hepcidin regulates iron efflux by interfering with the only cellular iron exporter, ferroportin 1 (FPN1), leading to cellular iron retention in iron-storage tissues such as macrophages and limiting iron absorption in the duodenum and jejunum [4]. Again, TNF-α and IL-1 inhibit EPO release and stimulation by the peritubular interstitial cells of the renals, and hepatocytes, leading to the downregulation of erythropoiesis [45–47].

The studies by Yan et al. [48] and Lippi & Plebani [13] observed an increase in total leucocytes with a corresponding elevation in lymphocytes in severe COVID-19 patients. This observation is linked to the probable overwhelming stimulation of the immune response by inflammatory cytokines during COVID-19 progression, triggering the release of immune cells to keep the virus in check [49]. Findings from the present study agree with the earlier observations [13, 48]. Conversely, reduced lymphocyte counts have been identified among severe COVID-19 patients [6, 50]. The disparity in the findings may be related to the variations in participants' selection in the studies.

The severe COVID-19 patients in this study had a reduced platelet count, which is similar to an earlier study in Northwest Ethiopia [51]. A similar study by Elderdery et al. [6] observed that 82.5% of individuals infected with SARS-CoV-2 developed thrombocytopaenia. This finding may be due to the suppression of medullary haemopoiesis, and direct viral interactions with megakaryocytes, affecting the primary synthesis of thrombocytes [52, 53], SARS-CoV-2 disruption of the endothelium, stimulating platelet activation and aggregation [54], as well as the probable generation of anti-platelet proteins [55].

Plasma antigen levels of naturally-occurring anticoagulants: PC, PS, and AT-III were decreased in severe COVID-19 participants in this study, and this is comparable with similar studies elsewhere [9, 10, 22, 24]. These findings could be due to the enhanced coagulopathy associated with severe COVID-19. To ensure effective haemostasis, activated PC in the presence of its cofactor PS deactivates FVa and FVIIIa to limit the rate of thrombi generation [10, 24], and AT-III regulates the activities of the coagulation factor thrombin, which helps to control excessive coagulation [28, 56]. Continuous excessive consumption of endogenous anticoagulants depletes their plasma levels, and minimizes the expected inhibition of the coagulation process to regulate the rate of clot formation. Again, the presence of ACE-2 on hepatocytes permits SARS-CoV-2 interaction with the liver cells and eventually suppresses the liver's ability to synthesize the anticoagulants, leading to their low concentrations in the plasma in severe COVID-19 patients [57, 58]. Additionally, the Kim and Kim [10] study suggests that activated plasma anticoagulants, such as PC, have a short half-life and rapidly get depleted from circulation during the disease's progression. However, the present study recorded increased circulating levels of TM among the severe COVID-19 participants, and this agrees with the findings from an earlier study in the United States [25]. This could be due to the increased loss of TM from the endothelial cell surface resulting from the excessive disruption of the endothelium by the explosive cytokine release during severe COVID-19 progression [25].

Blood cell indices and the anticoagulants were significantly changed after recovery from COVID-19. The improved erythrocyte parameters after severe COVID-19 participants recovered from the infection identified in this study agree with findings from earlier studies [4, 45, 46, 59]. Effective regulation of iron homeostasis due to the suppression of the inflammatory response and the sufficient release of EPO by the peritubular interstitial cells of the kidneys after recovery from COVID-19 could eventually promote erythropoiesis and increase peripheral numbers of the RBC parameters [4, 46]. Conversely, another study in China noticed reduced haemoglobin concentrations among COVID-19 patients post-treatment and associated the anaemia with the probable effect of the drugs [60].

Following successful recovery from the SARS-CoV-2 infection, platelet counts in peripheral blood were restored to normal in the current study. This could be related to a probable drop in the SARS-CoV-2 viral load with a subsequent limited inflammatory response resulting in well-regulated thrombocyte consumption and sufficient hepatic thrombopoietin release [60].

Comparatively, the plasma levels of PC, PS, and AT-III were higher after recovery from COVID-19 than the values at admission. This could be due to the restoration of endogenous synthesis by the liver following the suppression of the inflammatory response. Also, the reduced consumption of anticoagulants after recovery from the SARS-CoV-2 infection, when the rate of coagulation may be physiologically regulated, could account for the findings [24, 49]. Additionally, effective endothelial repairs following limited inflammation and suppressed endotheliopathy with a subsequent reduction in the release of TM from the endothelial cells could account for the lower TM levels found after recovery from COVID-19 [25].

This study could not assess the entire haemostatic system of the study participants.

5. Conclusion

Severely infected COVID-19 patients had higher PC, PS, and AT-III, but lower TM plasma levels. The significant changes in circulating anticoagulants may contribute to the hypercoagulable state of COVID-19. Blood cell indices were negatively affected during COVID-19 disease progression. Complete recovery from the SARS-CoV-2 infection normalised the haematological indices. Assessment of naturally-occurring anticoagulants and the provision of appropriate anticoagulants are recommended in the management of COVID-19 to prevent thrombotic complications. A future study to assess the entire haemostatic system of COVID-19 patients is recommended.

6. Declarations

6.1. Author Contributions

Conceptualization, C.N., M.O., L.D.A., M.O.T., N.A-K., F.P.A., Y.I., R.A.A.W., F.P.A-D., E.B.A., C.A.E.W. and K.O.A.; methodology, C.N., M.O., L.D.A., M.O.T., N.A-K., F.P.A., Y.I., R.A.A.W., F.P.A-D., E.B.A., C.A.E.W. and K.O.A.; validation, C.N., K.M., S.K.A., M.O., K.O.A., N.A-K., F.P.A., M.O.T., Y.I., R.A.A.W., F.P.A-D., C.A.E.W., C.B.D. and F.O-B.; formal analysis, L.D.A., D.A.A., C.N., E.B.A., F. O-B., K.O.A., N.A-K., F.P.A., M.O.T., Y.I., R.A.A.W., F.P.A-D., and C.A.E.W.; resources, C.N., F.E.C., H.A.O., K.O.A., N.A-K., F.P.A., M.O.T., Y.I., R.A.A.W., F.P.A-D., C.A.E.W., L.D.A., S.B.B., Y.Q., A-W.I., P.E.A., K.M., D.A.A., and S.K.A.; data curation, L.D.A., E.B.A., K.O.A., N.A-K., F.P.A., M.O.T., Y.I., R.A.A.W., F.PA-D, and C.A.E.W.; writing—original draft preparation, C.N., K.M., S.K.A., Y.Q., F.O-B., G.A., S.D., C.A.D., F.A.A., H.A.O., M.O., D.S., S.B.B., and E.B.A.; writing—review and editing, C.N., M.O., S.K.A., G.A., S.D., K.M., L.D.A., F.O-B., Y.Q., C.A.D., F.A.A., S.B.B., E.B.A., C.B.D., H.A.O., F.E.C., D.A.A., D.S., K.O.A., N.A-K., F.P.A., M.O.T., Y.I., R.A.A.W., F.P.A-D., P.E.A., and C.A.E.W. All authors have read and agreed to the published version of the manuscript.

6.2. Data Availability Statement

Data presented in the study are available on request from the corresponding author.

6.3. Funding

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6.5. Institutional Review Board Statement and Ethical Approval

The Committee on Human Research, Publications and Ethics (CHRPE) of Kwame Nkrumah University of Science and Technology (KNUST), Kumasi (Ref: CHRPE/AP/012/22) provided approval for the study. Permission was obtained from the Management of Kumasi South Regional Hospital, and informed consent was given by the participants.

6.6. Informed Consent Statement

Not Applicable.

6.7. Declaration of Competing Interest

The authors declare that there is no conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancies have been completely observed by the authors.

7. References

[1] Cabrera-Garcia, D., Miltiades, A., Parsons, S., Elisman, K., Mansouri, M. T., Wagener, G., & Harrison, N. L. (2021). High levels of plasminogen activator inhibitor-1, tissue plasminogen activator and fibrinogen in patients with severe COVID-19. MedRxiv, 2020.12.29.20248869. doi:10.1101/2020.12.29.20248869.

[2] Vabret, N., Britton, G. J., Gruber, C., Hegde, S., Kim, J., Kuksin, M., Levantovsky, R., Malle, L., Moreira, A., Park, M. D., Pia, L., Risson, E., Saffern, M., Salomé, B., Esai Selvan, M., Spindler, M. P., Tan, J., van der Heide, V., Gregory, J. K., … Laserson, U. (2020). Immunology of COVID-19: Current State of the Science. Immunity, 52(6), 910–941. doi:10.1016/j.immuni.2020.05.002.

[3] Akin, I. M., Kanburoglu, M. K., Tayman, C., Oncel, M. Y., Imdadoglu, T., Dilek, M., ... & Koc, E. (2022). Epidemiologic and clinical characteristics of neonates with late-onset COVID-19: 1-year data of Turkish Neonatal Society. European journal of pediatrics, 181(5), 1933-1942. doi:10.1007/s00431-021-04358-8.

[4] Sonnweber, T., Boehm, A., Sahanic, S., Pizzini, A., Aichner, M., Sonnweber, B., Kurz, K., Koppelstätter, S., Haschka, D., Petzer, V., Hilbe, R., Theurl, M., Lehner, D., Nairz, M., Puchner, B., Luger, A., Schwabl, C., Bellmann-Weiler, R., Wöll, E., … Weiss, G. (2020). Persisting alterations of iron homeostasis in COVID-19 are associated with non-resolving lung pathologies and poor patients' performance: a prospective observational cohort study. Respiratory Research, 21(1), 1–9. doi:10.1186/s12931-020-01546-2.

[5] Giacomelli, A., Ridolfo, A. L., Milazzo, L., Oreni, L., Bernacchia, D., Siano, M., Bonazzetti, C., Covizzi, A., Schiuma, M., Passerini, M., Piscaglia, M., Coen, M., Gubertini, G., Rizzardini, G., Cogliati, C., Brambilla, A. M., Colombo, R., Castelli, A., Rech, R., … Galli, M. (2020). 30-day mortality in patients hospitalized with COVID-19 during the first wave of the Italian epidemic: A prospective cohort study. Pharmacological Research, 158, 104931. doi:10.1016/j.phrs.2020.104931.

[6] Elderdery, A. Y., Elkhalifa, A. M. E., Alsrhani, A., Zawbaee, K. I., Alsurayea, S. M., Escandarani, F. K., Alhamidi, A. H., Idris, H. M. E., Abbas, A. M., Shalabi, M. G., & Mills, J. (2022). Complete Blood Count Alterations of COVID-19 Patients in Riyadh, Kingdom of Saudi Arabia. Journal of Nanomaterials, 2022. doi:10.1155/2022/6529641.

[7] Hudgins IV, A. F. (2022). Assessing the Prevalence of Anemia Post COVID-19 Infection in Adult Members of a Southeastern Integrated Healthcare System. Master Thesis, Georgia State University, Atlanta, United States. doi:10.57709/29959212.

[8] Zuo, Y., Warnock, M., Harbaugh, A., Yalavarthi, S., Gockman, K., Zuo, M., Madison, J. A., Knight, J. S., Kanthi, Y., & Lawrence, D. A. (2021). Plasma tissue plasminogen activator and plasminogen activator inhibitor-1 in hospitalized COVID-19 patients. Scientific Reports, 11(1). doi:10.1038/s41598-020-80010-z.

[9] Wójcik, K., Bazan-Socha, S., CelejewS.K.A.-Wójcik, N., Górka, K., Lichołai, S., Polok, K., Stachura, T., Zaręba, L., Dziedzic, R., Gradzikiewicz, A., Sanak, M., Musiał, J., Sładek, K., & Iwaniec, T. (2023). Decreased protein C activity, lower ADAMTS13 antigen and free protein S levels accompanied by unchanged thrombin generation potential in hospitalized COVID-19 patients. Thrombosis Research, 223, 80–86. doi:10.1016/j.thromres.2023.01.016.

[10] Kim, H. A., & Kim, J. E. (2022). Development of Nafamostat Mesylate Immediate-Release Tablet by Drug Repositioning Using Quality-by-Design Approach. Pharmaceutics, 14(6), 1219. doi:10.3390/pharmaceutics14061219.

[11] Iba, T., Levy, J. H., Connors, J. M., Warkentin, T. E., Thachil, J., & Levi, M. (2020). The unique characteristics of COVID-19 coagulopathy. Critical Care, 24(1), 1–8. doi:10.1186/s13054-020-03077-0.

[12] Palladino, M. (2021). Complete blood count alterations in covid-19 patients: A narrative review. Biochemia Medica, 31(3), 1–13. doi:10.11613/BM.2021.030501.

[13] Lippi, G., & Plebani, M. (2020). The critical role of laboratory medicine during coronavirus disease 2019 (COVID-19) and other viral outbreaks. Clinical Chemistry and Laboratory Medicine, 58(7), 1063–1069. doi:10.1515/cclm-2020-0240.

[14] Henry, B. M. (2020). COVID-19, ECMO, and lymphopenia: a word of caution. The Lancet Respiratory Medicine, 8(4), e24. doi:10.1016/S2213-2600(20)30119-3.

[15] Mu, T., Yi, Z., Wang, M., Wang, J., Zhang, C., Chen, H., Bai, M., Jiang, L., & Zhang, Y. (2021). Expression of eosinophil in peripheral blood of patients with COVID-19 and its clinical significance. Journal of Clinical Laboratory Analysis, 35(1), 23620. doi:10.1002/jcla.23620.

[16] Conti, P., Ronconi, G., Caraffa, A., Gallenga, C. E., Ross, R., Frydas, I., & Kritas, S. K. (2020). Induction of pro-inflammatory cytokines (IL-1 and IL-6) and lung inflammation by Coronavirus-19 (COVI-19 or SARS-CoV-2): anti-inflammatory strategies. Journal of Biological Regulators and Homeostatic Agents, 34(2), 327–331. doi:10.23812/CONTI-E.

[17] Nkansah, C., Addai-Mensah, O., Mensah, K., Owusu, M., Ephraim, R. K. D., Adu, P., Osei-Boakye, F., Appiah, S. K., Serwaa, D., Derigubah, C. A., & Debrah, A. Y. (2021). Plasminogen Activator Inhibitor-1 in poorly controlled vs well controlled Type-2 Diabetes Mellitus patients: A case-control study in a district hospital in Ghana. PLoS ONE, 16(4 April). doi:10.1371/journal.pone.0250090.

[18] Griffin, J. H., & Lyden, P. (2020). COVID-19 hypothesis: Activated protein C for therapy of virus-induced pathologic thromboinflammation. Research and Practice in Thrombosis and Haemostasis, 4(4), 506–509. doi:10.1002/rth2.12362.

[19] Faggian, G., Bernabei, A., Tropea, I., Francica, A., & Onorati, F. (2023). Hemostasis during cardiopulmonary bypass. In Cardiopulmonary Bypass (pp. 295–309). Academic Press. doi:10.1016/b978-0-443-18918-0.00018-8.

[20] Marchetti, M., Gomez-Rosas, P., Sanga, E., Gamba, S., Verzeroli, C., Russo, L., Restuccia, F., Schieppati, F., Bonanomi, E., Rizzi, M., Fagiuoli, S., D'Alessio, A., Lorini, L., & Falanga, A. (2021). Endothelium Activation Markers in Severe Hospitalized COVID-19 Patients: Role in Mortality Risk Prediction. TH Open, 05(03), e253–e263. doi:10.1055/s-0041-1731711.

[21] Flaumenhaft, R., Enjyoji, K., & Schmaier, A. A. (2022). Vasculopathy in COVID-19. Blood, 140(3), 222–235. doi:10.1182/blood.2021012250.

[22] Corrêa, T. D., Cordioli, R. L., Campos Guerra, J. C., Caldin da Silva, B., dos Reis Rodrigues, R., de Souza, G. M., Midega, T. D., Campos, N. S., Carneiro, B. V., Campos, F. N. D., Guimarães, H. P., de Matos, G. F. J., de Aranda, V. F., & Rolim Ferraz, L. J. (2020). Coagulation profile of COVID-19 patients admitted to the ICU: An exploratory study. PLOS ONE, 15(12), e0243604. doi:10.1371/journal.pone.0243604.

[23] Stoichitoiu, L. E., Pinte, L., Balea, M. I., Nedelcu, V., Badea, C., & Baicus, C. (2020). Anticoagulant protein S in COVID-19: low activity, and associated with outcome. Romanian Journal of Internal Medicine, 58(4), 251–258. doi:10.2478/rjim-2020-0024.

[24] Esmaeel, H. M., Ahmed, H. A., Elbadry, M. I., Khalaf, A. R., Mohammed, N. A., Mahmoud, H. A., & Taha, E. M. (2022). Coagulation parameters abnormalities and their relation to clinical outcomes in hospitalized and severe COVID-19 patients: prospective study. Scientific Reports, 12(1), 13155. doi:10.1038/s41598-022-16915-8.

[25] Goshua, G., Pine, A. B., Meizlish, M. L., Chang, C.-H., Zhang, H., Bahel, P., Baluha, A., Bar, N., Bona, R. D., Burns, A. J., Dela Cruz, C. S., Dumont, A., Halene, S., Hwa, J., Koff, J., Menninger, H., Neparidze, N., Price, C., Siner, J. M., … Lee, A. I. (2020). Endotheliopathy in COVID-19-associated coagulopathy: evidence from a single-centre, cross-sectional study. The Lancet Haematology, 7(8), e575–e582. doi:10.1016/s2352-3026(20)30216-7.

[26] Al-Kuraishy, H. M., Al-Gareeb, A. I., Al-Harcan, N. A. H., Alexiou, A., & Batiha, G. E.-S. (2022). Tranexamic Acid and Plasminogen/Plasmin Glaring Paradox in COVID-19. Endocrine, Metabolic & Immune Disorders - Drug Targets, 23(1), 35–45. doi:10.2174/1871530322666220801102402.

[27] McConnell, M. J., Kondo, R., Kawaguchi, N., & Iwakiri, Y. (2022). Covid-19 and Liver Injury: Role of Inflammatory Endotheliopathy, Platelet Dysfunction, and Thrombosis. Hepatology Communications, 6(2), 255–269. doi:10.1002/hep4.1843.

[28] Panigada, M., Bottino, N., Tagliabue, P., Grasselli, G., Novembrino, C., Chantarangkul, V., Pesenti, A., Peyvandi, F., & Tripodi, A. (2020). Hypercoagulability of COVID-19 patients in intensive care unit: A report of thromboelastography findings and other parameters of hemostasis. Journal of Thrombosis and Haemostasis, 18(7), 1738–1742. doi:10.1111/jth.14850.

[29] Yasmine, H. O., Emmanuel, O., Kwaku, D. G., James, A.-D., & Rita, L.-R. (2022). COVID-19 Associated Pulmonary Thromboembolic Events in Anti-Coagulated Patients: Case Series from a Tertiary Facility in Ghana. Medical and Clinical Case Reports, 2(1). doi:10.33425/2768-6647.1017.

[30] Adu, C., Mensah, K. A., Ahinkorah, B. O., Osei, D., Tetteh, A. W., & Seidu, A. A. (2022). Socio-demographic factors associated with medication adherence among People Living with HIV in the Kumasi Metropolis, Ghana. AIDS Research and Therapy, 19(1), 50. doi:10.1186/s12981-022-00474-z.

[31] Voicu, S., Bonnin, P., Stépanian, A., Chousterman, B. G., Le Gall, A., Malissin, I., Deye, N., Siguret, V., Mebazaa, A., & Mégarbane, B. (2020). High Prevalence of Deep Vein Thrombosis in Mechanically Ventilated COVID-19 Patients. Journal of the American College of Cardiology, 76(4), 480–482. doi:10.1016/j.jacc.2020.05.053.

[32] World Health Organization (WHO). (2022). Clinical management of COVID-19: Living guideline. World Health Organization (WHO), Geneva, Switzerland.

[33] Osei-Boakye, F., Addai-Mensah, O., Owusu, M., Saasi, A. R., Appiah, S. K., Nkansah, C., Wiafe, Y. A., & Debrah, A. Y. (2022). Effect of pulmonary tuberculosis on natural anticoagulant activity in therapy-naïve Ghanaian adults; a case-control study. Journal of Immunoassay and Immunochemistry, 43(3), 271–287. doi:10.1080/15321819.2021.2001002.

[34] Chauhan, S., Kumar, P., Marbaniang, S. P., Srivastava, S., & Patel, R. (2022). Prevalence and predictors of anaemia among adolescents in Bihar and Uttar Pradesh, India. Scientific Reports, 12(1), 1–9 8197. doi:10.1038/s41598-022-12258-6.

[35] Struyf, T., Deeks, J. J., Dinnes, J., Takwoingi, Y., Davenport, C., Leeflang, M. M., Spijker, R., Hooft, L., Emperador, D., Dittrich, S., Domen, J., Horn, S. R. A., & Van den Bruel, A. (2020). Signs and symptoms to determine if a patient presenting in primary care or hospital outpatient settings has COVID-19 disease. Cochrane Database of Systematic Reviews. doi:10.1002/14651858.cd013665.

[36] Sadoff, J., Gray, G., Vandebosch, A., Cárdenas, V., Shukarev, G., Grinsztejn, B., Goepfert, P. A., Truyers, C., Van Dromme, I., Spiessens, B., Vingerhoets, J., Custers, J., Scheper, G., … Douoguih, M. (2022). Final Analysis of Efficacy and Safety of Single-Dose Ad26.COV2.S. New England Journal of Medicine, 386(9), 847–860. doi:10.1056/nejmoa2117608.

[37] Xu, P., Zhou, Q., & Xu, J. (2020). Mechanism of thrombocytopenia in COVID-19 patients. Annals of Hematology, 99(6), 1205–1208. doi:10.1007/s00277-020-04019-0.

[38] Gul, M. H., Htun, Z. M., & Inayat, A. (2021). Role of fever and ambient temperature in COVID-19. Expert Review of Respiratory Medicine, 15(2), 171–173. doi:10.1080/17476348.2020.1816172.

[39] Caruso, P. F., Angelotti, G., Greco, M., Albini, M., Savevski, V., Azzolini, E., Briani, M., Ciccarelli, M., Aghemo, A., Kurihara, H., Voza, A., Badalamenti, S., Lagioia, M., & Cecconi, M. (2022). The effect of COVID-19 epidemic on vital signs in hospitalized patients: a pre-post heat-map study from a large teaching hospital. Journal of Clinical Monitoring and Computing, 36(3), 829–837. doi:10.1007/s10877-021-00715-y.

[40] Konlaan, Y., Asamoah Sakyi, S., Kumi Asare, K., Amoah Barnie, P., Opoku, S., Nakotey, G. K., Victor Nuvor, S., & Amoani, B. (2022). Evaluating immunohaematological profile among COVID-19 active infection and recovered patients in Ghana. PLOS ONE, 17(9), e0273969. doi:10.1371/journal.pone.0273969.

[41] Attoh, S. A., Sarkodie, E., Fatchu, R., Kuma, A. B. A., Asumanu, E., McAddy, M., Toppar, A., Hobenu, F., Agyeman-Bediako, K., Edusei, L., & Akakpo, P. K. (2022). COVID-19 and sickle cell disease: autopsy findings of three deaths at the 37 Military Hospital, Accra, Ghana. Pan African Medical Journal, 41. doi:10.11604/pamj.2022.41.332.30035.

[42] Wang, L., Duan, Y., Zhang, W., Liang, J., Xu, J., Zhang, Y., Wu, C., Xu, Y., & Li, H. (2020). Epidemiologic and clinical characteristics of 26 cases of covid-19 arising from patient-to-patient transmission in liaocheng, china. Clinical Epidemiology, 12, 387–391. doi:10.2147/CLEP.S249903.

[43] Tao, Z., Xu, J., Chen, W., Yang, Z., Xu, X., Liu, L., Chen, R., Xie, J., Liu, M., Wu, J., Wang, H., & Liu, J. (2021). Anemia is associated with severe illness in COVID-19: A retrospective cohort study. Journal of Medical Virology, 93(3), 1478–1488. doi:10.1002/jmv.26444.

[44] Huang, Y., Tu, M., Wang, S., Chen, S., Zhou, W., Chen, D., Zhou, L., Wang, M., Zhao, Y., Zeng, W., Huang, Q., Xu, H., Liu, Z., & Guo, L. (2020). Clinical characteristics of laboratory confirmed positive cases of SARS-CoV-2 infection in Wuhan, China: A retrospective single center analysis. Travel Medicine and Infectious Disease, 36. doi:10.1016/j.tmaid.2020.101606.

[45] Lanser, L., Burkert, F. R., Bellmann-Weiler, R., Schroll, A., Wildner, S., Fritsche, G., & Weiss, G. (2021). Dynamics in anemia development and dysregulation of iron homeostasis in hospitalized patients with covid-19. Metabolites, 11(10). doi:10.3390/metabo11100653.

[46] Lanser, L., Fuchs, D., Kurz, K., & Weiss, G. (2021). Physiology and inflammation driven pathophysiology of iron homeostasis—mechanistic insights into anemia of inflammation and its treatment. Nutrients, 13(11). doi:10.3390/nu13113732.

[47] Weiss, G., Ganz, T., & Goodnough, L. T. (2019). Anemia of inflammation. Blood, 133(1), 40–50. doi:10.1182/blood-2018-06-856500.

[48] Yan, W., Chen, D., Bigambo, F. M., Wei, H., Wang, X., & Xia, Y. (2021). Differences of blood cells, lymphocyte subsets and cytokines in COVID-19 patients with different clinical stages: a network meta-analysis. BMC Infectious Diseases, 21(1). doi:10.1186/s12879-021-05847-9.

[49] Chatterjee, S., Sengupta, T., Majumder, S., & Majumder, R. (2020). COVID-19: A probable role of the anticoagulant Protein S in managing COVID-19-associated coagulopathy. Aging, 12(16), 15954–15961. doi:10.18632/aging.103869.

[50] Afriyie-Mensah, J., Aboagye, E. T., Ganu, V. J., Bondzi, S., Tetteh, D., Kwarteng, E., Akamah, J., Doku, A., & Adjei, P. (2021). Clinical and therapeutic outcomes of covid-19 intensive care units (Icu) patients: A retrospective study in ghana. Pan African Medical Journal, 38, 38. doi:10.11604/pamj.2021.38.107.27131.

[51] Asrie, F., Tekle, E., Gelaw, Y., Dagnew, M., Gelaw, A., Negash, M., Kassa, E., Bizuneh, S., & Wudineh, D. (2022). Baseline Thrombocytopenia and Disease Severity Among COVID-19 Patients, Tibebe Ghion Specialized Hospital COVID-19 Treatment Center, Northwest Ethiopia. Journal of Blood Medicine, 13, 315–325. doi:10.2147/JBM.S366478.

[52] Ropa, J., Cooper, S., Capitano, M. L., Van't Hof, W., & Broxmeyer, H. E. (2021). Human Hematopoietic Stem, Progenitor, and Immune Cells Respond Ex Vivo to SARS-CoV-2 Spike Protein. Stem Cell Reviews and Reports, 17(1), 253–265. doi:10.1007/s12015-020-10056-z.

[53] Ropa, J., Trinh, T., Aljoufi, A., & Broxmeyer, H. E. (2021). Consequences of coronavirus infections for primitive and mature hematopoietic cells: New insights and why it matters. Current Opinion in Hematology, 28(4), 231–242. doi:10.1097/MOH.0000000000000645.

[54] Iba, T., Levy, J. H., Levi, M., Connors, J. M., & Thachil, J. (2020). Coagulopathy of Coronavirus Disease 2019. Critical Care Medicine, 48(9), 1358–1364. doi:10.1097/CCM.0000000000004458.

[55] Wool, G. D., & Miller, J. L. (2021). The Impact of COVID-19 Disease on Platelets and Coagulation. Pathobiology, 88(1), 15–27. doi:10.1159/000512007.

[56] Ranucci, M., Ballotta, A., Di Dedda, U., Bayshnikova, E., Dei Poli, M., Resta, M., Falco, M., Albano, G., & Menicanti, L. (2020). The procoagulant pattern of patients with COVID-19 acute respiratory distress syndrome. Journal of Thrombosis and Haemostasis, 18(7), 1747–1751. doi:10.1111/jth.14854.

[57] Boettler, T., Newsome, P. N., Mondelli, M. U., Maticic, M., Cordero, E., Cornberg, M., & Berg, T. (2020). Care of patients with liver disease during the COVID-19 pandemic: EASL-ESCMID position paper. JHEP Reports, 2(3), 100113. doi:10.1016/j.jhepr.2020.100113.

[58] Sun, J., Aghemo, A., Forner, A., & Valenti, L. (2020). COVID-19 and liver disease. Liver International, 40(6), 1278–1281. doi:10.1111/liv.14470.

[59] Bellmann-Weiler, R., Lanser, L., Barket, R., Rangger, L., Schapfl, A., Schaber, M., Fritsche, G., Wöll, E., & Weiss, G. (2020). Prevalence and predictive value of anemia and dysregulated iron homeostasis in patients with COVID-19 infection. Journal of Clinical Medicine, 9(8), 1–11. doi:10.3390/jcm9082429.

[60] Liang, J., Nong, S., Jiang, L., Chi, X., Bi, D., Cao, J., Mo, L., Luo, X., & Huang, H. (2021). Correlations of disease severity and age with hematology parameter variations in patients with COVID-19 pre- and post-treatment. Journal of Clinical Laboratory Analysis, 35(1), 23609. doi:10.1002/jcla.23609.