Coagulopathy is Initiated with Endothelial Dysfunction and Disrupted Fibrinolysis in Patients with COVID-19

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Abstract

Background: A substantial group of patients suffer coagulopathy of Covid-19 (CAC) and are presented with thrombosis. The pathogenesis involved in CAC is not fully understood.

Objectives: We evaluated the hemostatic and inflammatory parameters of 51 hospitalized Covid-19 adult patients and 21 controls. The parameters analyzed were danger signal molecule (High molecular weight group box protein-1/HMGBP-1), platelet count, prothrombin time (PT), activated partial thromboplastin time (aPTT), D-dimer, fibrinogen, endothelial protein C receptor (EPCR), soluble E-selectin, soluble P-selectin, thrombomodulin, tissue plasminogen activator (TPA), plasminogen activator inhibitor-1 (PAI-1), soluble fibrin monomer complex (SFMC), platelet-derived microparticles (PDMP), β-thromboglobulin, antithrombin and protein C. The main objective of our study was to investigate which part of the hemostatic system was mostly affected at the admission of Covid-19 patients and whether these parameters could differentiate intensive care unit (ICU) and non-ICU patients.

Patients and Methods:

In this prospective case-control study, 51 patients ≥18 years who are hospitalized with the diagnosis of Covid-19 and 21 healthy control subjects were included. We divided the patients into two groups according to their medical progress, either into ICU and non-ICU group. Regarding the outcome, patients were again categorized as survivor and non-survivor groups. Blood samples were collected from patients at admission at the time of hospitalization before administration of any treatment for Covid-19. The analyzes of the study were made with the IBM SPSS V22 program. $p < 0.05$ was considered statistically significant.

Results:

A total of 51 adult patients (F/M: 24/27) (13 ICU and 38 non-ICU) were included in the study cohort. The mean age of the patients was 68.1 ± 14.4 years. The control group consisted of 21 age and sex-matched healthy individuals. All of the patients were hospitalized, in a group of 13 patients, Covid-19 progressed to severe form and were hospitalized at ICU. We found out that the levels of fibrinogen, prothrombin time (PT), endothelial protein-C receptor (EPCR), D-dimer, soluble E-selectin, soluble P-selectin, plasminogen activator inhibitor-1 (PAI-1), and tissue plasminogen activator (TPA) were increased; whereas, the levels of soluble fibrin monomer complex (SFMC), platelet-derived microparticles (PDMP), antithrombin and protein-C were decreased in Covid-19 patients compared to the control group at hospital admission. Tissue plasminogen activator was the only marker that had a significantly different median level between ICU and non-ICU groups ($p<0.001$).

Conclusions:

In accordance with the previous literature, we showed that Covid-19 associated coagulopathy is distinct from sepsis-induced DIC with prominent early endothelial involvement and fibrinolytic shut-down. Reconstruction of endothelial function at early stages of infection may protect patients to progress to ICU

hospitalization. We believe that after considering the patient's bleeding risk, early administration of LMWH therapy at Covid-19 even in at outpatient setting may be useful both for restoring endothelial function and anticoagulation. The intensity of anticoagulation in non-ICU and ICU Covid-19 patients should be clarified with further studies.

Introduction

As of June 2021, the COVID-19 pandemic has affected over 176 million worldwide confirmed cases and caused more than 3 million deaths [1]. In early studies from China, it has been shown that the non-survivors had significantly higher levels of D-dimer and fibrin degradation products, longer prothrombin time, and activated partial thromboplastin time when compared to survivors on admission [2]. Further studies have shown that the coagulopathy associated with COVID-19 (CAC) is quite different from disseminated intravascular coagulation (DIC) caused by sepsis [3, 4].

We had hypothesized that pulmonary pathogenesis of COVID-19 should be caused not only by infecting pulmonary epithelium by binding to surface angiotensin-converting enzyme 2 (ACE2) receptors but also directly causing microvascular endothelial damage at the initial phase and we had called this process organ-specific 'Pulmonary Intravascular Coagulation-PIC' resembling a pulmonary counterpart of sinusoidal obstruction syndrome seen in hematopoietic stem cell transplantation patients [5]. Later, small autopsy and biopsy series in COVID-19 patients have shown that there were endotheliitis, endothelial viral inclusions, and fibrin deposits among intraalveolar spaces even at the early stages of infection [6, 7, 8, 9]. In addition to endotheliitis, increased local fibrinolysis in response to deposited fibrin is seen in CAC, in contrast to classical DIC. Hemostatic laboratory parameter changes are mainly characterized by high d-dimer and fibrinogen levels despite relatively normal prothrombin time and platelets [2, 3]. The role of neutrophil extracellular traps (NETs) in CAC is correlated with clinically evident thrombosis even in patients under anticoagulant therapy [10]. Neutrophils are assumed to release damage-associated molecular patterns (DAMPs), however, the pathophysiology of danger signals in COVID-19 and their association with coagulation is not fully understood. Platelets have shown to be mildly decreased in CAC in contrast to DIC, however circulating immature platelets and P-selectin were increased in COVID-19 [11, 12]. Considering the natural anticoagulant system, in previous studies; antithrombin and protein C levels were preserved in CAC supporting the hypothesis that CAC is different from DIC [13, 14]. To better understand the dynamic interactions of players at the pathophysiology of CAC, we performed a prospective study in hospitalized Covid-19 patients and evaluated the levels of a selected danger signal molecule; endothelial, fibrinolytic, thrombocyte activation markers, and natural anticoagulant proteins. The main objective of our study was to investigate which part of the hemostatic system was mostly affected at the admission of Covid-19 patients and whether these parameters could differentiate intensive care unit (ICU) and non-ICU patients. The secondary objective was to evaluate the relation of these hemostatic parameters with the clinical outcomes.

Methods

Study design and participants

We designed a prospective case-control study in Baskent University Ankara Hospital between November 2020 and April 2021. Patients, more than 18 years of age who were admitted to our Covid-19 outpatient clinics and were confirmed for the diagnosis of Covid-19 by PCR assays on nasopharyngeal swab samples were included in our study. The asymptomatic patients who were not hospitalized were excluded. Patient characteristics such as age, sex, comorbid diseases, admission complaints, history of thrombosis, previous anticoagulant usage were recorded. The data regarding hospitalization and therapy such as the extent of oxygen demand, treatment modalities, and thrombosis during hospitalization were collected. We divided the patients into two groups according to their medical progress, either into ICU and non-ICU group. Regarding the outcome, patients were again categorized as survivor and non-survivor groups. Clinical outcomes were assessed in June 2021. Samples from a control group of 21 age and sex-matched healthy volunteers were analyzed for the same biomarker measurements. Baskent University Institutional Review Board authorized the study. Permission for the usage of Covid-19 patient clinical data was obtained from the Turkish Ministry of Health. Informed consent was obtained from every patient and control subject at admission.

Procedures

Blood samples were collected from patients at admission at the time of hospitalization before administration of any treatment for Covid-19. For endothelial protein C receptor (EPCR), soluble E-selectin (sE-selectin), soluble fibrin monomer complex (SFMC), platelet-derived microparticles (PDMP), soluble P-selectin (sP-selectin), β-thromboglobulin, and High Mobility Group Box Protein 1 (HMGBP-1) measurements, blood samples were collected in a tube with a clot activator and gel separator. To obtain serum samples tubes were centrifuged at 1000 g for 10 minutes within 45 to 60 minutes after phlebotomy. For thrombomodulin, plasminogen activator inhibitor-1 (PAI-1), tissue plasminogen activator (TPA), antithrombin (AT), protein-C, prothrombin time (PT), activated partial thromboplastin time (aPTT), D-dimer, and fibrinogen level measurements blood samples were collected in 3.2% sodium citrate tubes and centrifuged at 1500 g for 15 minutes as the recommendation of Clinical and Laboratory Standards Institute [15]. After centrifugation, all samples were checked for hemolytic, lipemic, or icteric plasma that could affect the test results [16]. K2EDTA whole blood samples were used for complete blood count (CBC) analysis. CBC, PT, APTT, D-dimer, and fibrinogen levels were completed within 1 hour after venipuncture. For remaining tests, serum and plasma samples were aliquoted and immediately frozen at − 80°C until analysis. Batch analysis was performed after the collection of patient and control group samples. Measurements of PT (Siemens Innovin reagent), aPTT (Siemens Actin FSL reagent), Ddimer (Innovance D-dimer kit, Siemens), fibrinogen (Siemens Thrombin reagent), antithrombin (Siemens Innovance® antithrombin reagent), and Protein-C (Siemens Berichrom reagent) levels were performed using Sysmex CS2100 (Sysmex Corporation, Kobe, Japan) analyzer. Internal quality control studies were performed according to laboratory protocol for each test. CBC analyses were performed using Abbott CELL DYN Ruby hematology analyzer (Abbott Diagnostics, IL, USD). Endothelial protein C receptor, sE-selectin, thrombomodulin, PAI-1, Soluble fibrin monomer complex, TPA, PDMP, sP-selectin, β-

thromboglobulin, and HMGBP-1 measurements; serum was quantitatively assessed using commercially available ELISA kits according to manufacturer's instructions. All ELISA measurements have an intra-assay variability < 10% and an inter-assay variability < 12%.

Statistical Analysis

"As descriptive statistics in the study, depending on the assumptions for numerical variables, mean ± standard deviation or median (minimum-maximum); number (n) and percentage (%) are given for categorical data. Student's t-test or Analysis of Variance if parametric test assumptions are provided in determining whether there is a difference between groups in terms of numerical variables (which groups the difference originates from was determined by the Tukey test, one of the multiple comparison tests); If parametric test assumptions were not met, Mann-Whitney U test or Kruskal-Wallis test (which groups caused the difference was determined by Dunn-Bonferroni test from multiple comparison tests). Pearson Chi-Square test, Fisher's Exact test, or Fisher-Freeman-Halton Exact test were used in the evaluation of categorical data, depending on the assumptions. The analyzes of the study were made with the IBM SPSS V22 program. $p < 0.05$ was considered statistically significant."

Role of the funding source

The study was accomplished with the scientific research project support program of Turkish Blood Foundation. The funder did not have any role in study design, data collection, analysis, data interpretation and writing of the manuscript.

Results

A total of 51 adult patients (F/M: 24/27) (13 ICU and 38 non-ICU) were included in the study cohort. The mean age of the patients was 68.1 ± 14.4 years. The control group consisted of twenty-one age and sex-matched healthy individuals. All of the patients were hospitalized, in a group of 13 patients, Covid-19 progressed to severe form and were hospitalized at ICU. Accompanying comorbidities were present in 88.2% of the patients and the three most common comorbidities were hypertension in 58.8%, diabetes mellitus (DM) in 41.2%, and cardiac disease in 31.4%. The most common admission complaints were coughing (45.1%), fatigue (41.2%), fever (35.3%), and respiratory insufficiency (33%). History of thrombosis was present in 13.7% of the patients and 35.3% were using anticoagulants as 25.5% antiplatelet drugs, 3.9% low molecular weight heparin (LMWH), 3.9 % direct oral anticoagulant drugs (DOACs), and 2% warfarin. The patients were evaluated for hemostatic parameters at the time of admission before therapy for Covid-19 was initiated. There was positive thorax computed tomography findings in all of the patients among which 98% were ground-glass opacities. Twenty-four (47.1%) of the patients did not require oxygen therapy, whereas 43.1% were given oxygen therapy with mask and 3.9% with noninvasive mechanical ventilation, and 5.9% were intubated. Anticoagulant therapy was administered to all patients at admission to the ward either at prophylactic dose (1x4000 IU enoxaparin) or therapeutic dose (2x4000 IU enoxaparin), however, thrombosis was seen in 4 (7.8%) of the patients (2 deep vein thromboses, 1 pulmonary embolism, 1 peripheral arterial thrombosis). Other therapeutic

modalities used were favipiravir in 51 (100%), antibiotics in 45 (88.2%), steroids in 36 (70.6%), plasmapheresis in 5 (9.8%), colchicine in 1 (2%) and intravenous immunoglobulin (IVIG) in 1 (2%) patient (Table 1). The median days of hospitalization was 5 (1–17, minimum-maximum) in non-ICU and 18 (4–35, minimum-maximum) in the ICU group ($p < 0.05$). Forty-five (88.2%) of the patients survived and 6 (11.8%) were lost. (Supplementary file, Table A). Regarding patient characteristics and treatments; there were not any statistically significant differences among ICU and non-ICU groups despite oxygen demand and steroid usage ($p < 0.001$) (Table 2). When we compared survivor (n = 45) and non-survivor (n = 6) groups, again we did not observe any significant difference regarding patient characteristics but only oxygen demand (Supplementary file, Table B).

In terms of measurement of hemostatic parameters, we found that fibrinogen, EPCR, D-dimer, PT, sE-selectin, PAI-1, TPA, soluble fibrin monomer complex, sP-selectin, PDMP, AT, and protein C were significantly different in patient and control groups ($p < 0.05$). (Table 3). Endothelial activation markers EPCR, sE-selectin, and sP-selectin (a marker of both endothelial cell and platelet activation) were increased among all of the study cohort, with higher values in the ICU group even at admission ($p < 0.05$). Hemoglobin, platelet number, white blood cell (WBC) count, aPTT, thrombomodulin, and beta-thromboglobulin and high molecular weight group box protein (Danger signal molecule) levels did not differ significantly among patient vs control and ICU vs non-ICU groups ($p > 0.05$). When the fibrinolytic system was evaluated, it was found that PAI-1 levels were increased up to 5.6 times in the patient group when compared to the control group ($p < 0.05$). In accordance with PAI-1, TPA was increased and soluble fibrin monomer complex was decreased significantly in the patient group ($p < 0.001$). D-dimer as a representative marker of fibrin degradation was increased in ICU and non-ICU groups comparing to control levels ($p < 0.001$). However, the only marker that had a significantly different level between ICU and non-ICU groups was TPA ($p < 0.001$). Thrombomodulin showed an increasing trend in ICU patients compared to ICU and control groups, however, this was not statistically significant ($p > 0.05$). For endogenous anticoagulant system, we had shown that antithrombin (AT) and protein C were decreased when we pass from control to non-ICU then ICU groups, however, the levels were only statistically significant among ICU vs control and non-ICU vs control but not among ICU vs non-ICU groups (Fig. 1).

We had a small group of non-survivors (n = 6) among the patient group. Differences at levels of fibrinogen, EPCR, D-dimer, PT, PAI-1, TPA, Soluble fibrin monomer complex, sP-selectin, PDMP, AT, and protein C was found when we compared survivor, non-survivor, and control groups ($p < 0.05$), however, this difference was caused by survivor vs control or non-survivor vs control but not by survivor vs non-survivor groups (Fig. 2). None of the parameters at initial admission could differentiate between survivor and non-survivor groups.

Discussion

As the Covid-19 disease emerged, clinical observations have shown that the pathogenesis was frequently associated with coagulopathy and thrombotic complications. Initial studies have reported patients meeting the International Society of Hemostasis and Thrombosis (ISTH) criteria for DIC [17]. However,

soon it was shown that there were remarkable differences between Covid associated coagulopathy (CAC) and traditional sepsis-associated DIC. In contrast to DIC, the fibrin degradation products such as D-dimer are increased accompanied by a modest decrease in platelets and slight or no prolongation in PT and aPTT [2, 18, 19]. Another important key factor that is involved in CAC pathophysiology is the endothelium. We know that normal endothelial function refers to the control of the vascular tonus, permeability, cell adhesion, and anticoagulation [20]. Upon stimulation and attachment to ACE2 by SARS-CoV2; the endothelium turns into a state called 'endothelial dysfunction' that represents a proinflammatory and prothrombotic state [21]. We had postulated that Covid-19 pathogenesis was not only caused by respiratory epithelial but also endothelial involvement at the lungs and published the pathogenesis of pulmonary intravascular coagulation-PIC [5]. The autopsy series had also supported that endothelium is heavily causing small and firm microthrombi in sections of peripheral lung parenchyma even in the absence of gross inflammation [6, 7, 8, 9].

Endothelial activation-dysfunction:

In our study, we postulated that the CAC starts with endothelial dysfunction and is accompanied by increased fibrinolysis followed by fibrinolytic shut-down and loss of natural anticoagulants making the clinical picture more serious. We analyzed the hemostatic parameters at admission. Fibrinogen levels (presumably due to increase as an acute phase reactant) and D-dimer levels were found to be increased in the patient group in accordance with the previous studies [22, 23]. Prothrombin time was slightly prolonged as well. When we evaluated the endothelial dysfunction; we found that EPCR, sE-selectin, sP-selectin, PAI-1, TPA were significantly increased in Covid-19 patients when compared to normal controls. These biomarkers also showed an increasing trend between non-ICU versus ICU and survivor versus non-survivor groups, however, only a statistically significant difference was seen in median TPA levels between ICU vs non-ICU groups ($p < 0.001$). These findings suggested that early endotheliopathy at admission plays an important role in CAC.

Fibrinolytic system:

The Fibrinolytic system is controlled by plasminogen activators and inhibitors with a result of plasminogen converted to plasmin. Tissue plasminogen activator, urea plasminogen activator (uPA), and their inhibitor PAI-1 are the key players of this system. Plasma TPA and PAI-1 levels were shown to be increased in patients with Covid-19 and worse outcomes [24]. In our cohort, despite the levels of the mentioned biomarkers were different among ICU, non-ICU, and control groups, the only statistically different marker was TPA between ICU and non-ICU groups. When we compared survivor and non-survivor groups, median levels of none of the biomarkers including TPA were found to be different among groups. This finding may be caused by the small number of patients in the non-survivor group. The major source of TPA in Covid-19 patients is likely to be the endothelium, PAI-1 is secreted from the endothelium and to a lesser extent from the platelets that make us think the endothelial activation-dysfunction is accompanied by platelet activation at early stages. Neutrophil extracellular traps present at inflammatory infiltration of lungs may also contribute to the secretion of PAI-1 as can be speculated from a study by

Zuo et al [10]. They have shown a correlation between absolute neutrophil counts and TPA/PAI-1 levels. Another fibrinolytic system element that we analyzed was soluble fibrin monomer complex (SFMC) that is increased in hypercoagulable states and at the early stages of DIC [25, 26]. It was also shown to be a useful marker in hypercoagulable states with increased fibrinolysis [27]. In our cohort, median SFMC levels were significantly lower in Covid-19 patients, especially patients in the ICU group, when comparing to controls showing the fibrinolytic shut-down is present even at admission.

Platelet activation and danger signal HMGBP-1:

When we analyzed the platelet activation markers, we evaluated the levels of sP-selectin (which is an activation marker of both endothelium and platelets), PDMP, and β-thromboglobulin. Soluble P-selectin was increased in Covid-19 patients especially in the ICU group when compared to controls in our cohort ($p < 0.001$). The molecular interaction between sP-selectin on platelets and endothelial cells is known to rapidly cause the expression of tissue factor (TF) on monocytes. The increase in sP-selectin may show an early activation of platelets causing localized intravascular micro-thrombosis. Our findings were consistent with the study by Agrati et al who had also shown increased levels of sP-selectin in Covid-19 patients [28]. These findings make us think that in Covid-19 pathogenesis, the endothelium is activated initially causing an interaction between endothelium and platelets leading to further activation of the inflammatory system and resulting in fibrin formation.

An interesting finding of our study was that the median PDMP levels were found to be lower in Covid-19 patients when compared to the control group. Circulating microparticles are increased in malignant and non-malignant Covid-19 patients recently [29]. Another study by Rausch et al showed that peripheral blood mononuclear cell binding to microparticles correlated with disease severity in Covid-19 patients [30]. Our results were in contrast with the previous literature as we had expected an increase in PDMPs in Covid-19 patients. It was recently shown that platelets can form aggregates with activated leukocytes and monocytes in Covid-19 patients [31]. The decrease in PDMP levels may be explained by the corporation of PDMPs to these aggregates, therefore, causing a decrease in plasma levels, however, this hypothesis needs to be tested with further studies.

The other biomarker that we did not find any difference among patient and control groups was β-thromboglobulin. High molecular weight group box protein-1 (HMGBP-1) and β-thromboglobulin were not studied previously in Covid-19 patients, our findings showed no difference of both markers in patient and control groups.

Natural anticoagulant proteins:

We studied soluble thrombomodulin (both as an endothelial marker and an anticoagulant protein), AT, and protein C as natural anticoagulants. Despite the median levels of s-thrombomudulin was not different between ICU, non-ICU, and control groups, in a study by Goshua et al, it was shown to be correlated with the hospital discharge status and mortality in Covid-19 patients [13]. One of our study limitations is that we could not evaluate the correlation of levels of biomarkers with mortality as our

cohort only contained 6 patients in the non-survivor group. Median AT and protein C levels were found to be significantly decreased in patient group compared to the control group and this decrease was more profound in the ICU group showing an initial decrease of the anticoagulant system.

Limitations of our study:

Our study had several limitations. The number of patients in the cohort was small, even smaller in subgroups. The budget of our grant limited the number of patients studied, therefore we could only include the minimum number of patient and control subjects that can enable statistical analysis. Despite we tried to evaluate different parts of coagulation activation, we could not correlate these biomarkers with a viscoelastic evaluation such as TEG or ROTEM as we lacked this system. For PDMP levels it would have been more specific to evaluate them via flow cytometry, however, this method required immediate evaluation of cells and microparticles with a fresh sample, we could only collect and freeze samples and study all in once therefore we preferred the ELISA method in every marker to have the opportunity to study them together. Another limitation was that the number of patients in ICU, non-ICU, survivor, and non-survivor subgroups were low, disabling us to evaluate the effect of the markers over survival and ICU stay. Because of a limited budget of the grant, we could not evaluate tissue factor, von Willebrand factor, and other coagulation factor levels. The evaluation of all factors will also have a reflection on how coagulation is activated in Covid-19.

Conclusion

Despite all our limitations, our study provided a global sight on Covid-19 coagulopathy. In accordance with previous literature, we showed that Covid-19 associated coagulopathy is distinct from sepsis-induced DIC with prominent early endothelial involvement and fibrinolytic shut-down. Reconstruction of endothelial function at early stages of infection may protect patients from progressing to ICU hospitalization. The occurrence of thrombosis despite therapeutic anticoagulation in our cohort revealed the need for evaluation of anticoagulant intensity with further studies. We believe that after considering the patient's bleeding risk, early administration of LMWH therapy at Covid-19 even in at outpatient setting may be useful both for restoring endothelial function and anticoagulation.

Declarations

Contributions:

FBBA designed the study and conributed in writing of the manuscript, GY and YEF obtained patient data and blood samples, AG provided the ethical board admission and made the data collection, DIT analyzed the blood samples and contributed to wiritng of the manuscipt. TS analyzed the data. All authors revised the manuscipt.

Conflict of interest:

None of the authors report any conflict of interest.

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Tables

Table 1

Patient characteristics

Patient characteristics	n (%)
Sex (F/M)	24/27 (52.9%/47.1%)
Comorbid disease	45 (88.2 %)
Hypertension	30 (58.8%)
Diabetes mellitus	21 (41.2%)
Cardiac disease	16 (31.4%)
Chronic lung disease	11 (21.6%)
Cancer	9 (17.6%)
Chronic renal disease	4 (7.8%)
Chronic liver disease	2 (3.9%)
İmmune deficiency	1 (2%)
Admission complaint	23 (45.1%)
Cough	21 (41.2%)
Fatigue	18 (35.3%)
Fever	17 (33%)
Respiratory insufficiency	10 (19.6%)
Myalgia	5 (9.8%)
Chest pain	4 (7.8%)
Diarrhea	3 (5.9%)
Headache	3 (5.9%)
Loss of consciousness	2 (3.9%)
Loss of taste and smell	
History of thrombosis	7 (13.7%)
Previous anticoagulant usage	18 (35.3%)
Antiplatelet	13 (25.5%)
LMWH*	2 (3.9%)
DOAC**	2 (3.9%)
Warfarin	1 (2%)
Positive Thorax CT findings	51 (100%)
Ground glass opacities	50 (98%)
Pulmonary embolism	1 (2%)
Oxygen demand	24 (47.1%)
None	22 (43.1%)
Mask oxygen	2 (3.9%)
Noninvasive	3 (5.9%)
Mechanical ventilation	
Treatment modality	51 (100%)
Anticoagulant	25 (49%)
Prophylactic dose	26 (51%)
Therapeutic dose	51 (100%)
Antiviral	36 (70.6%)
Steroids	1 (2%)
IVIG***	1 (2%)
Colchicine	5 (9.8%)
Plasmapheresis	45 (88.2%)
Antibiotics	

Patient characteristics	n (%)
Hospitalization type ICU**** Non-ICU	13 (25.5%) 38 (74.5%)
Thrombosis during hospitalization Present Absent	4 (7.8%) 47 (92.2%)
Outcome Alive Exitus	45 (88.2%) 6 (11.8%)

*LMWH:Low molecular weight heparin,**DOAC: Direct oral anticoagulant, ***IVIG: Intravenous immunoglobulin, ****ICU: Intensive care unit

Table 2

Patients' clinical findings and treatment according to ICU* vs Non-ICU groups

Patient characteristics	Non-ICU	ICU	p
Sex (F/M)	18/20 (47.4%/52.6%)	6/7(46.2%/53.8%)	0.940[a]
Comorbid disease	33 (86.8%)	12 (92.3%)	> 0.999[b]
Hypertension	22 (57.9%)	8 (61.5%)	0.818[a]
Diabetes mellitus	16 (42.1%)	5 (38.5%)	0.818[a]
Cardiac disease	11 (28.9%)	5 (38.5%)	0.730[b]
Chronic lung disease	8 (21.1%)	3 (23.1%)	> 0.999[b]
Cancer	6(15.8%)	3 (23.1%)	0.676[b]
Chronic renal disease	3(7.9%)	1(7.7%)	> 0.999[b]
Chronic liver disease	1 (2.6%)	1(7.7%)	0.449[b]
İmmune deficiency	0(0%)	1(7.7%)	0.255[b]
Admission complaint	18 (47.4%)	5 (38.5%)	0.577[a]
Cough	17 (44.7%)	4 (30.8%)	0.377[a]
Fatigue	13 (34.2%)	5 (38.5%)	> 0.999[b]
Fever	13 (34.2%)	4 (30.8%)	> 0.999[b]
Respiratory insufficiency	7 (18.4%)	3 (23.1%)	0.701[b]
Myalgia	4 (10.5%)	1 (7.7%)	> 0.999[b]
Chest pain	4 (10.5%)	0 (0%)	0.561[b]
Diarrhea	3 (7.9%)	0 (0%)	0.561[b]
Headache	2(5.3%)	1(7.7%)	> 0.999[b]
Loss of consciousness	2 (5.3%)	0(0%)	> 0.999[b]
Loss of taste and smell			
History of thrombosis			
Previous anticoagulant usage	12 (31.6%)	6(46.2%)	0.502[b]
Antiplatelet	9 (23.7%)	4 (30.8%)	0.547[c]
LMWH**	1 (2.6%)	1 (7.7%)	
DOAC***	1 (2.6%)	1 (7.7%)	
Warfarin	1 (2.6%)	0 (0%)	
Positive Thorax CT findings	38 (100%)	13 (100%)	-
Ground glass opacities	37 (97.4%)	13 (100%)	> 0.999[c]
Pulmonary embolism	1 (2.6%)	0 (0%)	
Oxygen demand	23 (60.5%)	1 (7.7.%)	< 0.001[c]
None	15 (39.5%)	7 (53.8%)	
Mask oxygen	0 (0%)	2 (15.4%)	
Noninvasive	0(0%)	3 (23.1%)	
Mechanical ventilation			

Patient characteristics	Non-ICU	ICU	p
Treatment modality	38 (100%)	13 (100%)	> 0.999[c]
Anticoagulant use	31 (81.5%)	3 (23.1%)	-
Prophylactic dose	7 (18.5 %)	10 (76.9%)	0.006[b]
Therapeutic dose	38 (100%)	13 (100%)	0.255[b]
Antiviral	23 (60.5%)	13 (100%)	0.255[b]
Steroids	0 (0%)	1 (7.7%)	> 0.999[b]
IVIG****	0 (0%)	1 (7.7%)	> 0.999[b]
Colchicine	4 (10.5%)	1 (7.7%)	
Plasmapheresis	33(86.8%)	12 (92.3%)	
Antibiotics			
Thrombosis during hospitalization	2 (5.3%)	2 (15.4%)	0.266[b]
Present	36 (94.7%)	11(84.6%)	
Absent			

a: Pearson X-square test; n(%)

b: Fisher's Exact Test; n(%)

c: Fisher-Freeman-Halton Exact Test; n(%)

*ICU: Intensive care unit, **LMWH: Low molecular weight heparin, ***DOAC: Direct oral anticoagulant, IVIG: Intravenonous immunoglobulin

Table 3
Initial laboratory parameters in patient and control groups

Laboratory parameter	Patient group	Control group	p
Hb (g/dl)	12.83 ± 2.02	12.35 ± 1.13	0.210[a]
Platelet (/mm3)	192.78 ± 76.36	196.52 ± 45.20	0.798 [a]
Fibrinogen	420.41 ± 130.18	233.14 ± 21.46	< 0.001 [a]
EPCR*	1680.32 ± 423.10	894.19 ± 447.56	< 0.001 [a]
HMGBP1**	351481.56 ± 60912.71	377817.14 ± 39530.08	0.072 [a]
WBC***(/mm3)	7.04 (2.30-18.96)	6.50(3.12–8.62)	0.027[b]
D-dimer	0.80(0.01–14.91)	0.19(0.19–0.39)	< 0.001 [b]
PT¶	12.6(10.2–24.3)	11.2(10.2–12.0)	< 0.001 [b]
aPTT¶¶	26.2(20.0-48.2)	24.7(22.4–30.6)	0.088 [b]
sE-selectin	8129.0(689.3-79840.0)	2856.0(58.3-17150.0)	0.043 [b]
PAI-1¶¶¶	1053.0(8.0-4664.0)	187.3(8.0-3215.0)	< 0.001 [b]
TPA¶¶¶¶	0.193(0.005–2.774)	0.100(0.001–0.273)	0.001 [b]
Soluble fibrin monomer complex (SFMC)	4.106(1.934–68.220)	55.700(3.761–74.610)	< 0.001 [b]
β-thromboglobulin	396800.00(17870.00-2156000.00)	799900.00(44750.00-8577000.00)	0.055 [b]

Laboratory parameter	Patient group	Control group	p
sP-selectin	38690.00(5025.00-165400.00)	16455.00(1445.00-48945.00)	< 0.001 b
PDMP****	392.400(137.200-1044.600)	720.200(135.400-1161.600)	0.009 b
Thrombomodulin	30.26(4.98–106.5)	32.71(8.92–118.6)	0.916 b
AT	86.30(28.30-122.30)	93.100(80.30-113.10)	0.007 b
Protein C	87.30(32.20–150.00)	125.20(66.20–150.00)	< 0.001 b

*a: Student's t test; mean ±SD, b: Mann-Whitney U test; median (minimum-maximum)

*EPCR: Endothelial protein C receptor, **HMGBP1: High molecular weight group box prrotein-1, ***WBC: White blood cell, ¶ PT: Prothrombin time, ¶¶ aPTT: Activated partial thromboplastin time, ¶¶¶: PAI-1: Plasminogen avitvator inhibitor, ¶¶¶¶: TPA: Tissue plasminogen activator, ****PDMP: Platelet derived microparticles, AT: Antithrombin

Figures

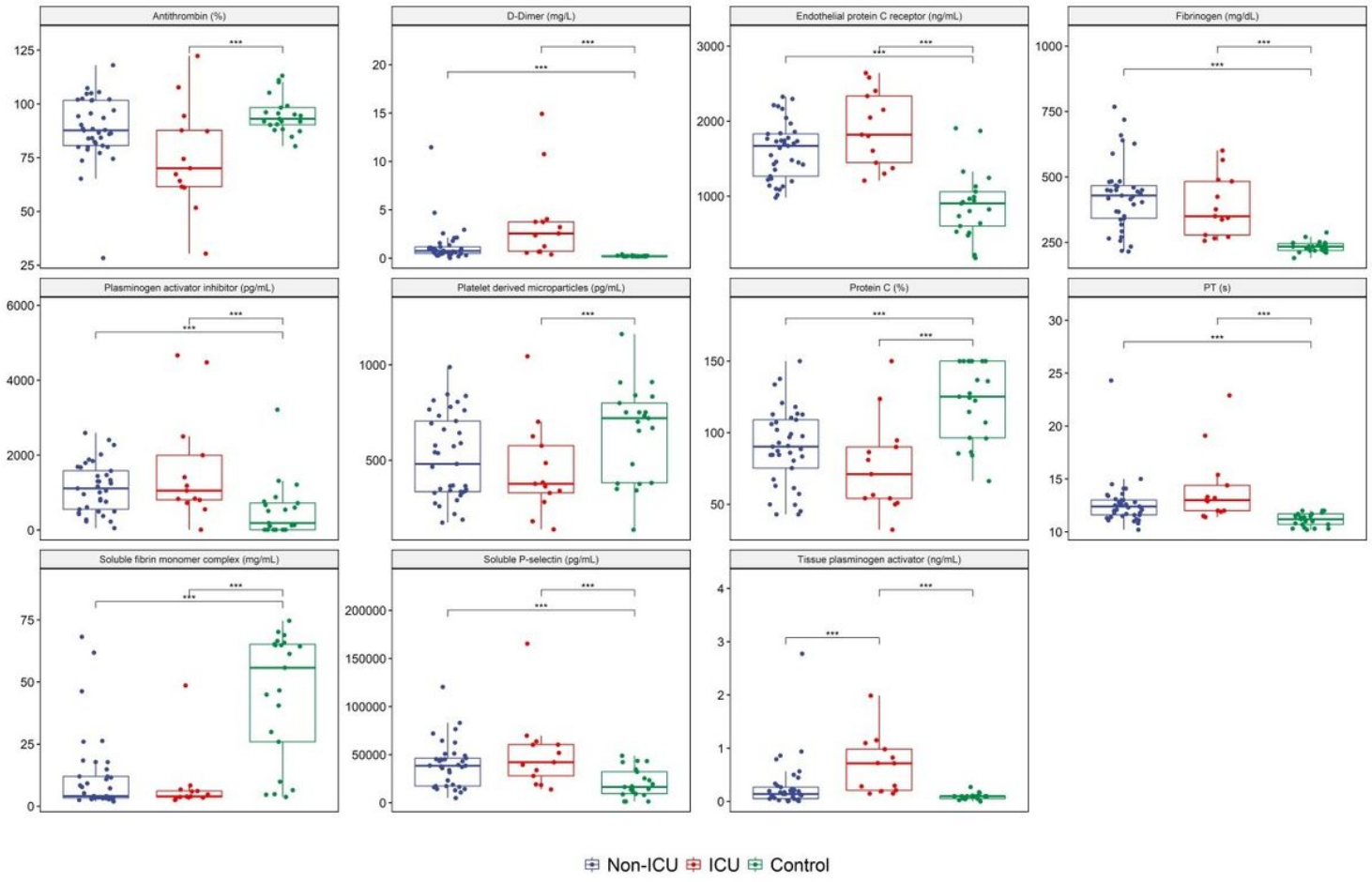

Figure 1

Plasma hemostatic markers in ICU*, non-ICU and control groups. Statistically significant parameters were given at the figure. All data points are presented as individual values (dots), median,and interquartile range (IQR)(horizontal bars). Mann-Whitneyy U test was performed to determine the statistical significance (*** p<0.05). *ICU: Intensive care unit, PT: Prothrombin time

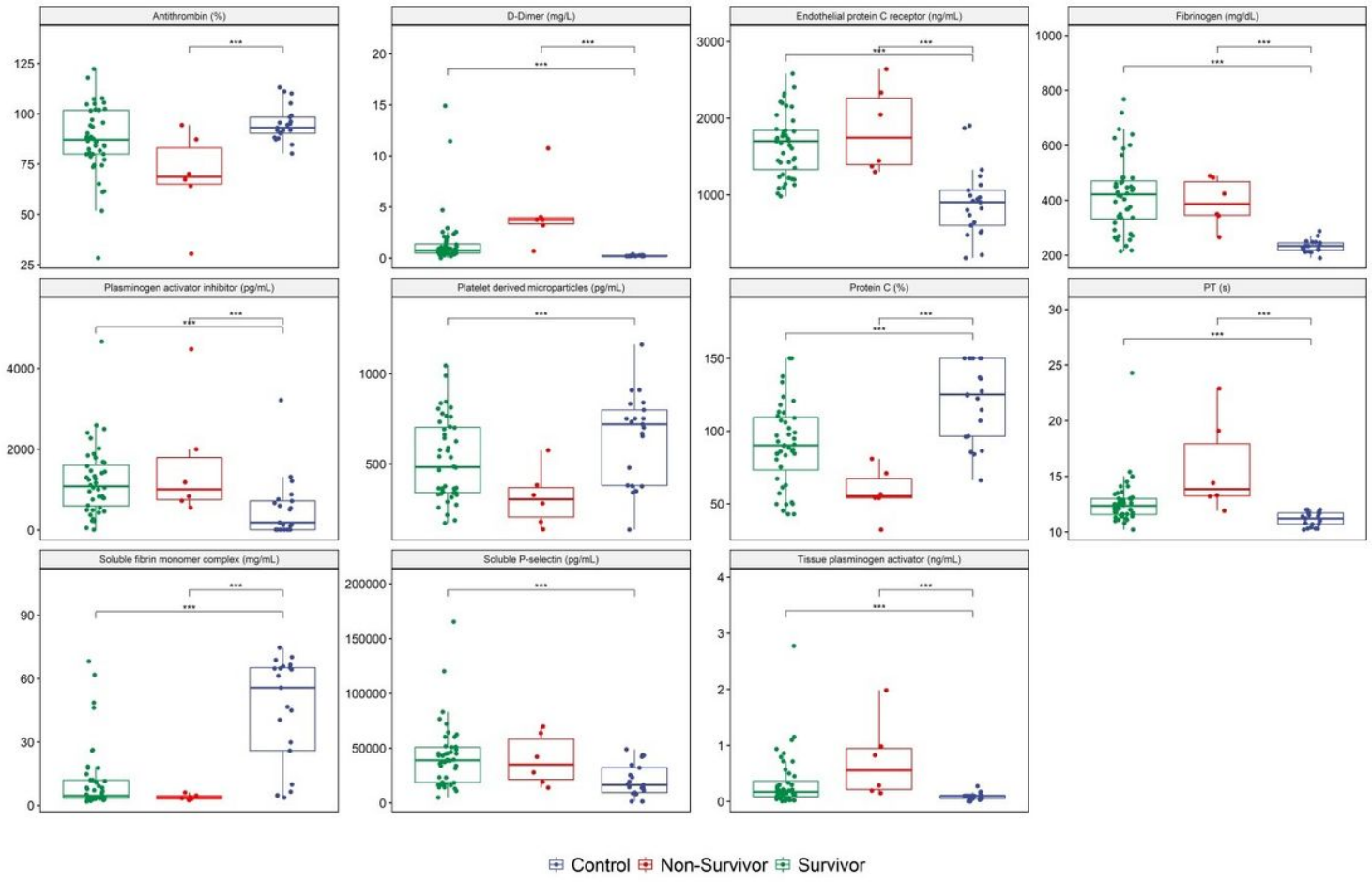

Figure 2

Plasma hemostatic markers in survivor, non-survivor and control groups. Statistically significant parameters were given at the figure. All data points are presented as individual values (dots), median,and interquartile range (IQR)(horizontal bars). Mann-Whitneyy U test was performed to determine the statistical significance (*** $p<0.05$). PT: Prothrombin time

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