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High expression of angiotensin-converting enzyme 2 receptor (ACE-2), transmembrane protease serine (TMPRSS), and P-selectin in platelets lead to thrombosis formation in COVID-19 patients

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Abstract. – OBJECTIVE: The purpose of the present study is to see if the presence of angiotensin-converting enzyme 2 (ACE-2), transmembrane serine protease 2 (TMPRSS), and P-selectin in platelets increases the risk of thrombosis in COVID-19 patients.

PATIENTS AND METHODS: This was a cross-sectional study conducted in the COVID-19 isolation center between January and September 2021 and comprised 61 COVID-19-infected patients, 21 of whom were in the intensive care unit (ICU) and 40 of whom were non-ICU patients (non-ICUP) in the isolation center. The coagulation profile, as well as the ACE-2, TMPRES, and P-selectin receptors, were all assessed in addition to the complete blood count (CBC). A questionnaire was also utilized to collect social and demographic data.

RESULTS: All platelet indices and coagulation profiles were significantly altered in COVID-19 ICUP and non-ICUP in this research; additionally, there is a significant association between the presence of ACE-2, P-selectin, and TMPRRS2 in COVID-19 patients with coagulation profile and platelet indices leading to hypercoagulable state.

CONCLUSIONS: In summary, the interaction of ACE-2, TMPRSS, and P-selectin in platelets appears to be a key element contributing to COVID-19 severity *via* their impact on thrombus development. Further investigation into these pathways may provide possible treatment targets for reducing the severe consequences of the COVID-19 infection.

Key Words:
ACE-2, TMPRSS, P-selectin, Thrombosis, COVID-19.

Introduction

In hospitalized COVID-19 patients, thrombotic consequences have been identified, including high levels of D-dimer as well as macrothrombi and small vessel thrombosis in several tissues. The pathophysiologic cellular causes of these thrombotic problems, however, remain unknown[1-4]. COVID-19 enters host cells *via* its spike protein, which binds to angiotensin-converting enzyme 2 (ACE-2) on the host cell membrane[5-8]. Furthermore, transmembrane serine protease 2 (TMPRSS) proteolytically breaks down and stimulates the spike protein to allow COVID-19 virus-cell membrane fusions. Although the COVID-19 spike protein was shown to attach to ACE-2 and alter numerous cellular activities, it is unknown if platelets exhibit ACE-2 and TMPRSS2. Platelets from COVID-19 patients are hyperactive, exhibiting TMPRSS2 and ACE-2. COVID-19 and its spike protein bind to platelet ACE-2 directly and stimulate platelets *in vitro*. The spike protein induces the development of thrombus *in vivo*. In addition, it has been shown that SARS-CoV-2 and its spike protein effectively excite platelets, resulting in the secretion of coagulation factors, the generation of inflammation cytokines, and the development of leukocyte-platelet aggregates (LPAs). Lastly, it has been demonstrated that treating COVID-19 spike protein-induced platelet activation with recombinant human ACE-2 protein and an anti-spike monoclonal antibody may reverse it[9-13].

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P-selectin is involved in platelet and leukocyte adherence and movement to inflammatory and damaged sites. P-Selectin Glycoprotein Ligand 1 signaling in white blood cells (WBCs) and thrombocytes, as well as glycoprotein Ib (GPIb) signaling in thrombocytes, are critical in hemostasis and thrombosis. The von Willebrand factor (VWF) receptor, glycoprotein Iba (GPIba), was also shown to be a P-selectin counter-receptor, implying that platelet rolling on P-selectin may be promoted[14]. Platelet and leukocyte activity are quite similar in this circumstance; both cell types have to settle down before sticking securely to the area of damage. It is known that the binding proteins play a role in primary attachment in both WBCs and thrombocytes because they are kept in the identical organelle and are therefore constantly liberated, which indicates how strongly hemostatic and inflammation reactions are linked[15]. P-selectin levels are also increased in thrombotic consumptive diseases such as disseminated intravascular coagulation (DIC), thrombotic thrombocytopenic purpura (TTP), and heparin-induced thrombocytopenia (HIT)[16-18]. The goal of the research is to examine if ACE-2, TMPRSS, and P-selectin levels in platelets enhance the risk of thrombosis in patients with COVID-19.

Patients and Methods

This cross-sectional research was conducted in the COVID-19 isolation center in the period between January and September 2021 and included a total of 61 patients infected with COVID-19, of whom 21 were ICUP and 40 were non-ICUP in the isolation center. The complete blood count (CBC) was measured in addition to the coagulation profile, as well as the ACE-2, TMPRES, and P-selectin receptors. A questionnaire was also used to collect social and demographic information.

Blood samples are collected from the patient in EDTA and citrate anticoagulant using a venipuncture method for all tests: complete blood count (CBC), ACE-2, TMPRES, P-selectin receptors, and coagulation profile, respectively.

Blood Cell Auto-Analyzer

The blood sample is then prepared for analysis by being placed in a test tube or cartridge, and any necessary reagents are added. The blood cell auto-analyzer uses various methods, such as impedance, light scatter, and fluorescence, to analyze the different types of blood cells present in the sample. The analyzer generates data on the counts and characteristics of different blood cell types, including erythrocytes, leucocytes, and thrombocytes (Beckman Coulter, Riyadh, Saudi Arabia).

Flow Cytometer

Blood samples are collected and prepared for analysis by labeling the cells with fluorescent dyes or antibodies specific to ACE-2, TMPRES, and P-selectin receptors. The prepared sample is then passed through a flow cytometer, where laser beams are used to detect the fluorescent signals emitted by the labeled cells. The labeled cells are then passed through a flow cytometer, which uses lasers to excite the fluorescent labels on the cells. The emitted fluorescence is then detected and measured by the flow cytometer. This allows for the quantification of the expression levels of ACE-2, TMPRES, or P-selectin receptors on the cell surface. The data obtained from flow cytometry can provide information about the expression levels of these receptors on platelets (Thermo Fisher Scientific, Waltham, MA, USA).

Fully Automated Coagulation Analyzer

The blood sample is then prepared for analysis by being mixed with reagents that initiate the coagulation process. The CS-5100 system includes an optical cable that emits light at five different wavelengths and a detector that can identify light at multiple wavelengths. With its multi-wavelength capabilities, the system handles not only analytical results but also pre-analytical issues. A fully automated coagulation analyzer measures the coagulation profile, including PT, PTT, fibrinogen levels, and D dimer (Sysmex CS-5100, Sysmex India Pvt. Ltd., Mumbai, India).

Statistical Analysis

The information was gathered from the COVID-19 patients. SPSS version 24 (IBM Corp., Armonk, NY, USA) was used to analyze the reported data. To ensure accuracy, the data was entered by two different people. For continuous data, descriptive statistics were used to calculate the mean, while proportions were utilized to assess categorical variables. To investigate the link, inferential statistics, specifically the Chi-square test and the Fisher's exact test, were used. In this setting, a p lower than 0.05 was deemed statistically significant.

Results

A total of 61 COVID-19 patients were admitted to the ICU, with 16.4% being males and 18.0% being females. While the majority of male and female patients were not admitted to the ICU, within the sample, 21.3% of the elder patients admitted to the ICU fell within the age range of more than 50 years. Among the 61 COVID-19 patients admitted to the hospital for more than 3 days, 14.8% were admitted to the ICU, and 27.9% of the patients were not admitted to the ICU. In terms of hemoglobin (HB) g/dl, 16.4% of female patients admitted to the ICU had less than 120 g/L, while 14.8% of male patients had less than 130 g/L. Concerning red blood cells (RBCs) and total white blood cells (TWBC) count cells, 26.2% of the COVID-19 patients admitted to the ICU have <3.93 cells/10^{12}/L, while 42.6% of patients not admitted to the ICU have an RBC count greater than 3.93 cells/10^{12}/L. 21.3% of patients admitted to the ICU have TWBC counts less than 3.3 cells/10^{9}/L. In contrast, 42.6% of patients non-admitted to the ICU have TWBC counts greater than 3.3 cells/10^{9}/L. Regarding hematocrit (HCT) level, 34.4% of patients admitted to the ICU had an HCT level of less than 38%. Meanwhile, the mean cell volume (MCV)/fL results showed that 62.3% of patients non-admitted to the ICU have >80 fL (Table I).

Table II illustrates that platelet indices such as platelet counts, mean platelet volume (MPV), platelet distribution width (PDW), and platelet large cell ratio (P-CLR) among patients with COVID-19 are statistically associated with admission status with a p lower than 0.05. This indicates that all 21 (34.4%) patients admitted to the ICU have platelet counts less than 150 cells/10^{9}/L, an MPV level greater than 13 fL, a PDW level greater than 16.8 fL, and a P-LCR greater than or equal to 12 fL.

Figure 1 illustrates that the coagulation profile of PT, PTT, fibrinogen, and D. dimer in COVID-19 is statistically associated with admission status with a p lower than 0.05. This indicates that in the majority (31.1%) of patients admitted to the ICU, PT is longer than 13 seconds, PTT is longer than 35 seconds, and D dimer is longer than one. Moreover, all of the patients admitted to the ICU had a fibrinogen level greater than 4 g/L.

Table I. Characteristics of the research subjects.

Variables	Response	COVID-19 Wards	
		ICUP (n/%)	Non-ICUP (n/%)
Age in year	20-29 years	2 (3.3)	12 (19.7)
	30-39 years	4 (6.6)	3 (4.9)
	40-49 years	2 (3.3)	12 (19.7)
	50-59 years	6 (9.8)	9 (14.8)
	>60 years	7 (11.5)	4 (6.6)
Gender	Male	10 (16.4)	30 (49.2)
	Female	11 (18.0)	10 (16.4)
Duration	One day	4 (6.6)	8 (13.1)
	2 days	4 (6.6)	7 (11.7)
	3 days	4 (6.6)	8 (13.1)
	>3 days	9 (14.8)	17 (27.9)
HB g/L	Female ≥120	1 (1.6)	3 (4.9)
	Female ≤120	10 (16.4)	7 (11.5)
	Male ≤130	9 (14.8)	12 (19.7)
	Male ≥130	1 (1.6)	18 (29.5)
Platelet counts (cell/10^{9}/L)	<150	21 (34.4)	25 (41.0)
	>150	0	15 (24.6)
RBCs count (cell/10^{12}/L)	<3.93	16 (26.2)	14 (23.0)
	>3.93	5 (8.2)	26 (42.6)
TWBCs count (cell/10^{9}/L)	< 3.3	13 (21.3)	14 (23.0)
	>3.3	8 (13.1)	26 (42.6)
HCT%	<38%	21 (34.4)	20 (32.8)
	>38%	0	20 (32.8)
MCV fL	<80	5 (8.2	2 (3.3)
	>80	16 (26.2)	38 (62.3)

HEV, hepatitis E virus; HBV, hepatitis B virus; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyltransferase; TBIL total bilirubin; ALB, albumin; TBA, total bile acid; PTA, prothrombin activity; PT, prothrombin time.

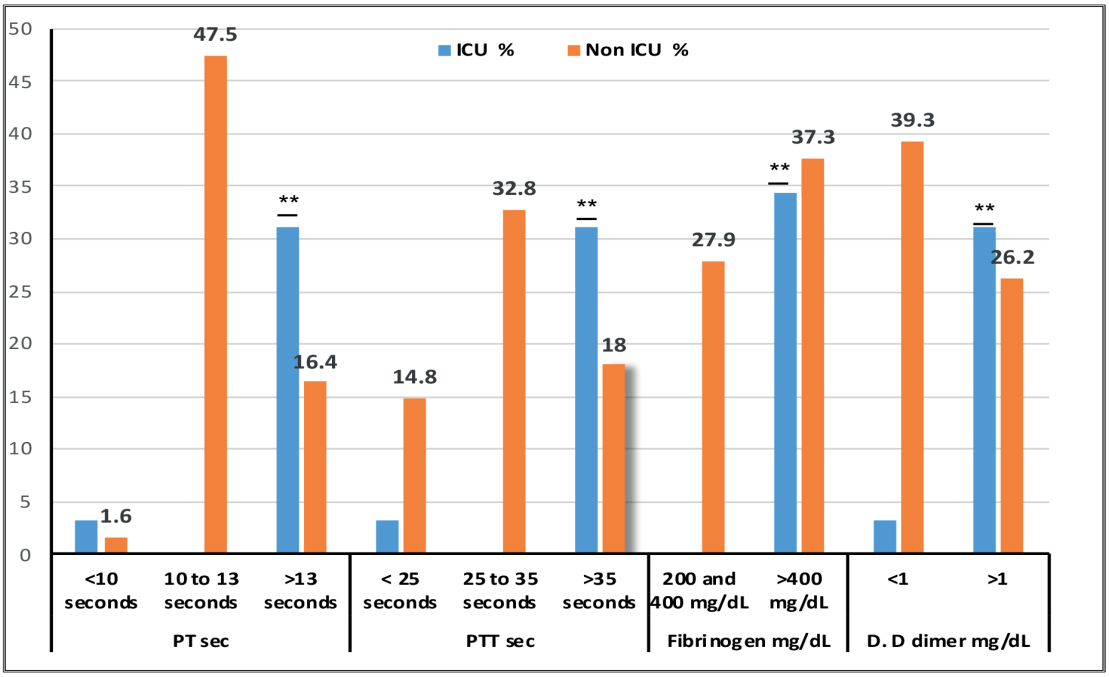

Figure 1. Coagulation profile PT, PTT, Platelet count, Fibrinogen, D-dimer in COVID-19 patients admitted ICU, and Non-ICU. **Statistically highly significant $p<0.05$.

Table II. Platelet indices among patients with COVID-19. Platelet count, MPV, PDW, P-LCR in COVID-19 patients admitted ICU, and non-ICU.

Platelet indices	Measurement	COVID-19 Wards	
		ICU (n/%)	Non-ICU (n/%)
Platelet counts (cell/10^9/L)	<150	21 (34.4)	25 (41.0)*
	>150	0	15 (24.6)
MPV (fL)	7-12	0	14 (23.0)*
	>13	21 (34.4)	26 (42.6)
PDW (%)	≤16.8	0	8 (13.1)*
	>16.8	21(34.4)	32 (52.5)
P-LCR (fL)	<12	0	15 (24.6)*
	>12	21 (34.4)	25 (41.0)

*Statistically significant $p<0.05$; MPV: mean platelet volume, PDW: platelet distribution width, P-LCR: platelet-large cell ratio.

Figure 2 illuminates that the existence of ACE-2, TMPRRS2, and P-selectin among patients with COVID-19 was associated with admission status with a $p<0.05$. Also, the results showed that the patients admitted to the non-ICU were highly positive for ACE-2, TMPRRS2, and P-selectin in comparison with ICU patients.

Table III shows the association between ACE-2, TMPRRS2, and P-selectin in COVID-19 patients with a coagulation profile. The results indicate there is a significant association between the existence of ACE-2 and the coagulation profile ($p<0.05$). Specifically, 47.5% of those with negative ACE-2 had the PT within 10 to 13 seconds; 32.8% of those with negative ACE-2 had the partial thromboplastin time of 25 to 35 seconds; 39.3% of them had platelet counts <150 cells/10^9/L; and 39.3% of them had D-dimer mg/L.

47.5% of those with negative TMPRRS2 have their PT within 10 to 13 seconds, and 32% of those with negative ACE-2 have their PTT between 25 and 35 seconds. 49.2% of them have platelet counts <150 cells/10^9/L. 45.9% of them have a fibrinogen concentration >4 g/L, and 39.3% of them have a D dimer of less than one.

Related to the association of P-selectin with the coagulation profile. The results showed it is associated with the coagulation profile: 45.9% of those with positive P-selectin blood clots within 10 to 13 seconds. 49.2% of their blood clots within more than 35 seconds, 73.8% of their platelet counts less than 150 cells/10^9/L, 72.1% of their fibrinogen level more than 4 g/L, and 55.7% of their D dimer level more than one mg/L.

Table IV demonstrates that ACE-2 is significantly associated with platelet count, MPV, PDW, and P-LCR (p<0.05). 39.3% of COVID-19 patients with negative ACE-2 have a platelet count less than 150 cell/10^9/L, 41.0% of them have an MPV count >13 fL; 50.8% of them have a PDW concentration greater than 16.8 fL; and 39.3% have a P-LCR greater than 12 fL.

TMPRRS2 has a significant association with platelet indices (platelet count, MPV, PDW, and P-LCR) (p<0.05). This is reflected in the fact that 49.2% of patients with negative TMPRRS2 had a platelet count less than 150 cell/10^9/L, 50.8% had a MPV concentration >13 fL, 60.7% had a PDW concentration >16.8 fL, and 49.2% of them had a P-LCR concentration greater than 12 fL.

Concerning the patients with positive P-selectin, it was reported that 73.8% of patients had a platelet count <150 cell/10^9/L, 75.4% of them had a MPV concentration >13 fL, 82.0% of them had a PDW concentration >16.8 fL, and 73.8% of them had a P-LCR concentration >12 fL.

Discussion

The most common symptoms of COVID-19 are ARDS and hypoxemic respiratory failure. Substantial coagulation cascade abnormalities, including higher D-dimers, fibrinogen, and von Willebrand factor levels, have been seen in critically ill COVID-19 patients[19]. Thrombosis, including pulmonary embolism (PE), deep venous thrombosis (DVT), and ischemic stroke, is very common in critically ill patients[20]. Platelets were indicated to have a crucial role in the expansion of various thrombotic problems. Furthermore, platelets serve as a vital link between the hemostatic system and immunological defense, especially in viral diseases.

In this study, all platelet parameters, such as platelet counts, PDW, P-CLR, and MPV, among patients with COVID-19 have significantly changed statistically associated with COVID-19 disease. Platelet indices have been found to be altered in patients with COVID-19. It was shown that patients with COVID-19 often present with decreased platelet counts, increased MPV, and increased PDW. Additionally, the P-CLR has been found to be elevated in patients with COVID-19. The decreased platelet count in patients with COVID-19 may be indicative of thrombocytopenia, which is a common finding in severe cases of the disease. Thrombocytopenia can lead to an increased risk of bleeding and could be

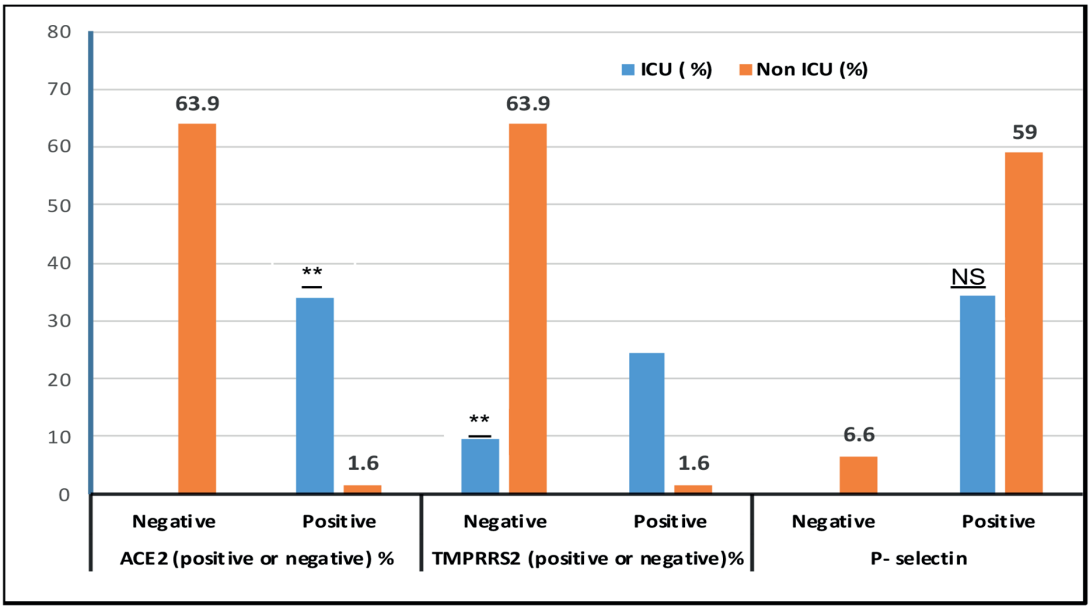

Figure 2. Existence of ACE-2, TMPRRS2, and P-selectin in COVID-19 patients admitted in ICU, and Non-ICU. **Statistically highly significant p<0.05; NS, non-significant.

Table III. Displays the association between ACE-2, TMPRRS2, and P-selectin in COVID-19 (ICUP, Non-ICUP) and coagulation profile (PT, PTT, Platelet count, Fibrinogen and D-dimer).

Parameters	Measurement	ACE-2 (No.) %		χ^2	*p*	TMPRRS2 (No.) %		χ^2	*p*	P-selectin (No.) %		χ^2	*p*
		-ve	+ve			-ve	+ve			-ve	+ve		
PT (second)	<10	0 (0.0)	3 (4.9)	32.6	0.001*	0 (0.0)	3 (4.9)	23.9	0.001*	0 (0.0)	3 (4.9)	1.4	0.510
	10 to 13	29 (47.5)	0 (0.0)			29 (47.5)	0 (0.0)			3 (4.9)	26 (42.6)		
	>13	10 (16.4)	19 (31.1)			16 (26.2)	13 (21.3)			1 (1.6)	28 (45.9)		
PTT (second)	<25	8 (13.1)	3 (4.9)	21.3	0.001*	8 (13.1)	3 (4.9)	11.7	0.003*	0 (0.0)	11 (18.0)	8.8	0.012*
	25 to 35	20 (32.8)	0 (0.0)			20 (32.6)	0 (0.0)			4 (6.6)	16 (26.2)		
	>35	11 (18.0)	19 (31.1)			17 (27.9)	13 (21.3)			0 (0.0)	30 (49.2)		
Platelet counts (cell/10^9/L)	<150	24 (39.3)	22 (36.1)	11.2	0.001*	30 (49.2)	16 (26.2)	7.1	0.005*	1 (1.6)	45 (73.8)	5.9	0.043*
	>150	15 (24.6)	0 (0.0)			15 (24.6)	0 (0.0)			3 (4.9)	12 (19.7)		
Fibrinogen (g/L)	2-4	17 (27.9)	0 (0.0)	13.3	0.001*	17 (27.9)	0 (0.0)	8.4	0.002*	4 (6.6)	13 (21.30	11.1	0.005*
	>4	22 (36.1)	22 (36.1)			28 (45.9)	16 (26.2)			0 (0.0)	44 (72.1)		
D dimer (mg/L)	<1	24 (39.3)	2 (3.3)	15.8	0.000*	24 (39.3)	2 (3.30)	8.0	0.004*	3 (4.9)	23 (37.7)	1.8	0.203
	>1	15 (24.6)	20 (32.8)			21 (34.4)	14 (23.0)			1 (1.6)	34 (55.7)		

Some cells have expected count less than 5 Fisher exact test was performed, *statistically significant $p<0.05$; NS: non-significant, ACE-2: angiotensin-converting enzyme, TMPRRS2: transmembrane protease serine, PT: prothrombin time, PTT: partial thromboplastin time.

Table IV. the association between ACE-2, TMPRRS2, and P-selectin in COVID-19 (ICUP, Non-ICUP) and Platelet indices (Platelet count, MPV, PDW, P-LCR).

Platelet indices	Measurement	ACE-2 (No.) %		χ^2	*p*	TMPRRS2 (No.) %		χ^2	*p*	P-selectin (No.) %		χ^2	*p*
		-ve	+ve			-ve	+ve			-ve	+ve		
Platelet counts (cell/10^9/L)	<150	24 (39.3)	22 (36.1)	11.2	0.000*	30 (49.2)	16 (26.2)	7.1	0.005*	1 (1.6)	45 (73.8)	5.9	0.015*
	>150	15 (24.6)	0 (0.0)			15 (24.6)	0 (0.0)			3 (4.9)	12 (19.7)		
MPV (fL)	7-12	14 (23.0)	0 (0.0)	10.3	0.001*	14 (23.0)	0 (0.0)	6.5	0.007*	3 (4.9)	11 (18.0)	6.6	0.035*
	>13	25 (41.0)	22 (36.1)			31 (50.8)	16 (26.2)			1 (1.6)	46 (75.4)		
PDW (%)	≤16.8	8 (13.1)	0 (0.0)	5.2	0.021*	8 (13.1)	0 (0.0)	3.3	0.070	1 (1.6)	7 (11.5)	0.53	0.439
	>16.8	31 (50.8)	22 (36.1)			37 (60.7)	16 (26.2)			3 (4.9)	50 (82.0)		
P-LCR (fL)	<12 fL	15 (24.6)	0 (0.0)	11.2	0.000*	15 (24.6)	0 (0.0)	7.08	0.008*	3 (4.9)	12 (19.7)	5.8	0.043
	>12 fL	24 (39.3)	22 (36.1)			30 (49.2)	16 (26.2)			1 (1.6)	45 (73.8)		

Some cells have expected count less than 5 Fisher exact test was performed, *statistically significant $p<0.05$; NS non-significant, ACE-2: angiotensin-converting enzyme, TMPRRS2: transmembrane protease serine, MPV: mean platelet volume, PDW: platelet distribution width, P-LCR: platelet-large cell ratio.

linked to poor results in patients with COVID-19. The increased MPV and PDW in patients with COVID-19 suggest that there is increased platelet activation and turnover. This may be related to the inflammatory response and coagulopathy that are commonly seen in severe cases of COVID-19. The elevated P-CLR in patients with COVID-19 may also indicate increased platelet activation and consumption, as well as potential endothelial damage and microthrombosis. Overall, these platelet indices can serve as important indicators of the severity of COVID-19 and may help guide clinical management and treatment decisions for affected patients.

Previous research[21] has shown that viral infections such as dengue, influenza, and sepsis are related to dynamic alterations in platelet function. Platelets in COVID-19 patients were considerably altered, as in other infectious disorders, as compared to healthy donors. Interestingly, gene expression alterations in non-ICUP and ICUP COVID-19 individuals were similar. Although thrombocytopenia has been found in certain patients with COVID-19 and is associated with mortality[22], patients recruited with COVID-19 (ICUP and non-ICU) had normal platelet numbers and sizes, which is consistent with at least one prior study[23].

The current study shows a significant alteration in PT, PTT, fibrinogen, and D-dimer in patients with COVID-19. There have been reports of abnormalities in the coagulation profile in patients with COVID-19, including prolonged PT and PTT, increased fibrinogen levels, and elevated D-dimer levels. Prolonged PT and PTT indicate impairment in the extrinsic and intrinsic coagulation pathways, respectively. This can lead to an increased risk of bleeding in COVID-19 patients. Increased fibrinogen levels can also contribute to a higher risk of thrombosis formation, as fibrinogen is essential for blood clot formation. Elevated D-dimer levels are indicative of increased fibrinolysis and can be a marker of hypercoagulability and potential thrombotic events in patients with COVID-19. These abnormalities in the coagulation profile suggest that COVID-19 can lead to both bleeding and thrombotic complications, highlighting the importance of monitoring and managing coagulation abnormalities in these patients.

Other research has found that patients with COVID-19 have coagulation issues such as increased D-dimer, prolonged PT and PTT, and other coagulation profile abnormalities. Thrombosis is detected in severe cases with a high risk of death. Endothelial cells are vulnerable to the new COVID-19. The presence of the virus, for example, has been found to be an indication of inflammation and malfunction. Endothelial cell activation and dysfunction are important factors in COVID-19 patients' hypercoagulation status. In addition to direct blood exposure of subendothelial tissue, Weibel-Palade bodies harboring coagulants within the endothelium can be discharged into the circulation. Endothelial nitric oxide synthase could be inhibited, allowing platelets to adhere. Furthermore, anti-2-glycoprotein I antibodies could lead to COVID-19 coagulopathy by upregulating proinflammatory mediators and adhesion molecules[24].

In this study, the significant presence of ACE-2 in platelets has been linked to increased platelet activation and aggregation, which can contribute to thrombosis and inflammation, both of which are associated with severe COVID-19. As well, TMPRSS plays a key role in priming the spike protein of SARS-CoV-2, allowing it to bind to ACE-2 and enter host cells. TMPRSS is also involved in platelet activation and aggregation, and its expression has been linked to increased thrombotic events in COVID-19 patients. The correlation between ACE-2 and TMPRSS in platelets and the severity of COVID-19 lies in their combined effects on platelet activation, aggregation, and thrombosis. When these proteins are upregulated or dysregulated, they can contribute to a prothrombotic state that exacerbates the severity of COVID-19 by increasing the risk of clot formation in blood vessels throughout the body. This can lead to complications such as pulmonary embolism, stroke, heart attack, and multiorgan failure.

Several prior investigations produced conflicting results. The first demonstrated that proteomic techniques based on mass spectrometry on isolated human platelets likewise failed to find ACE-2 or TMPRSS2 proteins[25]. RNA-seq investigations of mouse platelets and megakaryocytes show a paucity of ACE-2 and TMPRSS2 expression[26]. In contrast, Zhang et al[27] revealed that *TMPRSS2* mRNA, *ACE-2*, and protein levels were shown to be high in platelets from normal humans and animals. These seemingly contradictory data about whether platelets and megakaryocytes express ACE-2 and TMPRSS2. Differences in ethnicity could explain the disparities in *ACE-2* and *TMPRSS2* RNA expression[28]. Nevertheless, the genetic basis of ACE-2 expression and functionality in association with SARS-CoV-2 in diverse populations remains

mainly unclear. A systematic comparison of *ACE-2* coding-region variants and expression quantitative trait loci (*eQTL*) variants across different populations revealed that East Asian populations had higher allele frequencies of *eQTL* variants correlated with higher ACE-2 tissue expression levels than European populations. This could imply that various populations are more susceptible to SARS-CoV-2 infection[29].

This study revealed that P-selectin was found on the outermost layer of activated thrombocytes and endothelial cells. It is essential for regulating interactions between platelets and between platelets and endothelial cells. P-selectin concentrations have been found to be elevated in COVID-19 patients, both ICUP and non-ICUP, and are linked to hypercoagulability and thrombotic events.

This finding contradicts a previous study[30], which indicated that P-selectin and other platelet and endothelial markers were significantly higher in ICUP in comparison to controls, as well as considerably greater in ICUP compared to non-ICUP. The elevation of such markers was found to be substantially associated with mortality. Another two investigations[28,31] found no difference in P-selectin levels between ICUP and non-ICUP.

Conclusions

Overall, the interaction of ACE-2, TMPRSS, and P-selectin in platelets appears to be a key element contributing to COVID-19 severity *via* their impact on thrombus development. Further investigation into these mechanisms may provide possible treatment targets for reducing the severe consequences of the COVID-19 infection.

Conflict of Interest
The authors declared that there is no conflict of interest in this research.

Informed Consent
All participants signed informed consent forms.

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Ethics Approval
This investigation was carried out after receiving ethical approval from the Gebra isolated center administration (MOH/GIC/2021/3/1/467).

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Authors' Contributions
All the authors are responsible for the conception and design of the study responsible for the enrolment of patients, and the collection and analysis of data; all the authors contributed to data analysis and interpretation, writing, revision of the manuscript, and final approval of the manuscript.

Data Availability
All data are available upon request.

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References

1) Bikdeli B, Madhavan MV, Jimenez D, Chuich T, Dreyfus I, Driggin E, Nigoghossian C, Ageno W, Madjid M, Guo Y, Tang LV, Hu Y, Giri J, Cushman M, Quéré I, Dimakakos EP, Gibson CM, Lippi G, Favaloro EJ, Fareed J, Caprini JA, Tafur AJ, Burton JR, Francese DP, Wang EY, Falanga A, McLintock C, Hunt BJ, Spyropoulos AC, Barnes GD, Eikelboom JW, Weinberg I, Schulman S, Carrier M, Piazza G, Beckman JA, Steg PG, Stone GW, Rosenkranz S, Goldhaber SZ, Parikh SA, Monreal M, Krumholz HM, Konstantinides SV, Weitz JI, Lip GYH; Global COVID-19 Thrombosis Collaborative Group, Endorsed by the ISTH, NATF, ESVM, and the IUA, Supported by the ESC Working Group on Pulmonary Circulation and Right Ventricular Function. 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: 2950-2973.
2) McFadyen JD, Stevens H, Peter K. The emerging threat of (Micro) thrombosis in COVID-19 and its therapeutic implications. Circ Res 2020; 127: 571-587.
3) Levi M, Thachil J, Iba T, Levy JH. Coagulation abnormalities and thrombosis in patients with COVID-19. Lancet Haematol 2020; 7: e438-e440.

4) Al-Samkari H, Karp Leaf RS, Dzik WH, Carlson JCT, Fogerty AE, Waheed A, Goodarzi K, Bendapudi PK, Bornikova L, Gupta S, Leaf DE, Kuter DJ, Rosovsky RP. COVID-19 and coagulation: bleeding and thrombotic manifestations of SARS-CoV-2 infection. Blood 2020; 136: 489-500.

5) Zhou P, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, Si HR, Zhu Y, Li B, Huang CL, Chen HD, Chen J, Luo Y, Guo H, Jiang RD, Liu MQ, Chen Y, Shen XR, Wang X, Zheng XS, Zhao K, Chen QJ, Deng F, Liu LL, Yan B, Zhan FX, Wang YY, Xiao GF, Shi ZL. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature 2020; 579: 270-273.

6) Xu X, Chen P, Wang J, Feng J, Zhou H, Li X, Zhong W, Hao P. Evolution of the novel coronavirus from the ongoing Wuhan outbreak and modeling of its spike protein for risk of human transmission. Sci China Life Sci 2020; 63: 457-460.

7) Wrapp D, Wang N, Corbett KS, Goldsmith JA, Hsieh CL, Abiona O, Graham BS, McLellan JS. Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. Science 2020; 367: 1260-1263.

8) Walls AC, Park YJ, Tortorici MA, Wall A, McGuire AT, Veesler D. Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein. Cell 2020; 181: 281-292.

9) Chen IY, Chang SC, Wu HY, Yu TC, Wei WC, Lin S, Chien CL, Chang MF. Upregulation of the chemokine (C-C motif) ligand 2 via a severe acute respiratory syndrome coronavirus spike-ACE-2 signaling pathway. J Virol 2010; 84: 7703-7712.

10) Lin HX, Feng Y, Wong G, Wang L, Li B, Zhao X, Li Y, Smaill F, Zhang C. Identification of residues in the receptor-binding domain (RBD) of the spike protein of human coronavirus NL63 that are critical for the RBD-ACE-2 receptor interaction. J Gen Virol 2008; 89: 1015-1024.

11) Heurich A, Hofmann-Winkler H, Gierer S, Liepold T, Jahn O, Pohlmann S. TMPRSS2 and ADAM17 cleave ACE-2 differentially and only proteolysis by TMPRSS2 augments entry driven by the severe acute respiratory syndrome coronavirus spike protein. J Virol 2014; 88: 1293-1307.

12) Kuba K, Imai Y, Rao S, Gao H, Guo F, Guan B, Huan Y, Yang P, Zhang Y, Deng W, Bao L, Zhang B, Liu G, Wang Z, Chappell M, Liu Y, Zheng D, Leibbrandt A, Wada T, Slutsky AS, Liu D, Qin C, Jiang C, Penninger JM. A crucial role of angiotensin converting enzyme 2 (ACE-2) in SARS coronavirus-induced lung injury. Nature Medicine 2005; 11: 875-879.

13) Wong SK, Li W, Moore MJ, Choe H, Farzan M. A 193-amino acid fragment of the SARS coronavirus S protein efficiently binds angiotensin-converting enzyme 2. J Biol Chem 2004; 279: 3197-3201.

14) Polgar J, Matuskova J, Wagner DD. The P-selectin, tissue factor, coagulation triad. J. Thromb. Haemost 2005; 3: 1590-1596.

15) Furie, B. P-selectin, and blood coagulation: It's not only about inflammation anymore. Arterioscler Thromb Vasc Biol 2005; 25: 877-878.

16) Laursen MA, Larsen JB, Larsen KM, Hvas AM. Platelet function in patients with septic shock. Thromb Res 2020: 185: 33-42.

17) Widemann A, Pasero C, Arnaud L, Poullin P, Loundou AD, Choukroun G, Sanderson F, Lacroix R, Sabatier F, Coppo P, Dignat-George F, Kaplanski G; ENDO-13 study group. Circulating endothelial cells and progenitors as prognostic factors during autoimmune thrombotic thrombocytopenic purpura: Results of a prospective multicenter French study. J Thromb Haemost 2014: 12: 1601-1609.

18) Campello E, Radu CM, Duner E, Lombardi AM, Spiezia L, Bendo R, Ferrari S, Simioni P, Fabris F. Activated Platelet Derived and Leukocyte-Derived Circulating Microparticles and the Risk of Thrombosis in Heparin-Induced Thrombocytopenia: A Role for PF4-Bearing Microparticles? Cytometry Part B (Clinical Cytometry) 2018; 94B: 334-341.

19) Griffin DO, Jensen A, Khan M, Chin J, Chin K, Saad J, Parnell R, Awwad C, Patel D. Pulmonary embolism and increased levels of d-dimer in patients with coronavirus disease. Emerg Infect Dis 2020; 26: 1941-1943.

20) Llitjos JF, Leclerc M, Chochois C, Monsallier JM, Ramakers M, Auvray M, Merouani K. High incidence of venous thromboembolic events in anticoagulated severe COVID-19 patients. J Thromb Haemost 2020; 18: 1743-1746.

21) Middleton EA, Rowley JW, Campbell RA, Grissom CK, Brown SM, Beesley SJ, Schwertz H, Kosaka Y, Manne BK, Krauel K, Tolley ND, Eustes AS, Guo L, Paine R 3rd, Harris ES, Zimmerman GA, Weyrich AS, Rondina MT. Sepsis alters the transcriptional and translational landscape of human and murine platelets. Blood 2019; 134: 911-923.

22) Liu Y, Sun W, Guo Y, Chen L, Zhang L, Zhao S, Long D, Yu L. Association between platelet parameters and mortality in coronavirus disease 2019: retrospective cohort study. Platelets 2020; 31: 490-496.

23) Fan BE, Chong VCL, Chan SSW, Lim GH, Lim KGE, Tan GB, Mucheli SS, Kuperan P, Ong KH. Hematologic parameters in patients with COVID-19 infection. Am J Hematol 2020; 95: E131-E134.

24) Chen AT, Wang CY, Zhu WL, Chen W. Coagulation Disorders and Thrombosis in COVID-19 Patients and a Possible Mechanism Involving Endothelial Cells: A Review. Aging Dis 2022; 13: 144-156.

25) Burkhart JM, Vaudel M, Gambaryan S, Radau S, Walter U, Martens L, Geiger J, Sickmann A, Zahedi RP. The first comprehensive and quantitative analysis of human platelet protein composition allows the comparative analysis of structural and functional pathways. Blood 2012; 120: e73-e82.

26) Machlus KR, Wu SK, Stumpo DJ, Soussou TS, Paul DS, Campbell RA, Kalwa H, Michel T, Bergmeier W, Weyrich AS, Blackshear PJ, Hartwig JH,

Italiano JE Jr. Synthesis and dephosphorylation of MARCKS in the late stages of megakaryocyte maturation drive proplatelet formation. Blood 2016; 127: 1468-1480.

27) Zhang S, Liu Y, Wang X, Yang L, Li H, Wang Y, Liu M, Zhao X, Xie Y, Yang Y, Zhang S, Fan Z, Dong J, Yuan Z, Ding Z, Zhang Y, Hu L. SARS-CoV-2 binds platelet ACE-2 to enhance thrombosis in COVID-19. J Hematol Oncol 2020; 13: 120.

28) Manne BK, Denorme F, Middleton EA, Portier I, Rowley JW, Stubben C, Petrey AC, Tolley ND, Guo L, Cody M, Weyrich AS, Yost CC, Rondina MT, Campbell RA. Platelet gene expression and function in patients with COVID-19. Blood 2020; 136: 1317-1329.

29) Cao Y, Li L, Feng Z, Wan S, Huang P, Sun X, Wen F, Huang X, Ning G, Wang W. Comparative genetic analysis of the novel coronavirus (2019-nCoV/SARS-CoV-2) receptor ACE-2 in different populations. Cell Discov 2020; 6: 11.

30) Hottz ED, Azevedo-Quintanilha IG, Palhinha L, Teixeira L, Barreto EA, Pão CRR, Righy C, Franco S, Souza TML, Kurtz P, Bozza FA, Bozza PT. Platelet activation and platelet-monocyte aggregate formation trigger tissue factor expression in patients with severe COVID-19. Blood 2020; 136: 1330-1341.

31) Agrati C, Bordoni V, Sacchi A, Petrosillo N, Nicastri E, Del Nonno F, D'Offizi G, Palmieri F, Marchioni L, Capobianchi MR, Antinori A, Ippolito G, Bibas M. Elevated P-Selectin in Severe COVID-19: Considerations for Therapeutic Options. Mediterr J Hematol Infect Dis 2021; 13: e2021016.