

## Routine Hematological Assessment: A Window to Endothelial Health in Hospitalized COVID-19 Patients

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### ABSTRACT

**Background:** COVID-19 is associated with endothelial dysfunction and a hypercoagulable state that increases the risk of thrombotic complications. In our study we aim to investigate the correlation between routine hematological and coagulation parameters with circulating endothelial cells (CECs) and circulating endothelial progenitor cells (CEPs) in COVID-19 patients.

**Methods:** This study included Sixty adult COVID-19 patients and forty healthy controls. Complete blood counts, coagulation profile (PT, INR, PTT, fibrinogen, D-dimer), and flow cytometric analysis of CECs and CEPs were performed.

**Results:** The results showed that COVID-19 patients had significantly higher neutrophil counts, neutrophil/lymphocyte ratio, fibrinogen, D-dimer, CECs, and CEPs, while lymphocyte and platelet counts were significantly lower compared to controls. Strong positive correlations were observed between CECs/CEPs and markers of inflammation and coagulation.

**Conclusion:** In this study we reach to a conclusion that integrating endothelial biomarkers with routine laboratory tests provides valuable insight into endothelial injury and hypercoagulability in COVID-19 patients, supporting their role as potential predictors of thrombotic risk.

**Keywords:** COVID-19; Circulating endothelial cells (CECs); Circulating endothelial progenitor cells (CEPs); hypercoagulability; Coagulation markers; Inflammation; Complete blood count.

### INTRODUCTION

The pathogenesis of COVID-19 encompasses a constellation of immuno-inflammatory phenomena and endotheliopathy that culminate in a pronounced hypercoagulable state. Routine laboratory evaluations—such as complete blood count (CBC), activated partial thromboplastin time (aPTT or PTT), and prothrombin time (PT)—are crucial for classifying the severity of the illness and the risk of thrombosis in SARS CoV 2 infection <sup>(1)</sup>.

Within the CBC, leukocyte dynamics serve as key indicators: neutrophilia frequently accompanies severe COVID-19 and correlates with elevated systemic inflammation, whereas lymphopenia signifies adaptive immune exhaustion <sup>(2)</sup>. Either direct viral infection or inflammatory inhibition of erythropoiesis can lower red blood cell indices effects on erythroid precursors <sup>(2)</sup>. Thrombocytosis or evolving thrombocytopenia reflects the platelet activation milieu characteristic of COVID-associated coagulopathy, as platelets contribute directly to thrombus formation and endothelial cross-talk <sup>(3, 4)</sup>. PT and PTT frequently demonstrate mild prolongation; notably, an analysis identified PTT as the most reliable prognostic indicator, exhibiting an AUC of 0.68 and sensitivity of 83% for mortality prediction <sup>(4, 5)</sup>.

Advanced indicators of vascular damage and healing have been identified in both the circulation endothelial progenitor cells (CEPs) and the circulating endothelial cells (CECs). Mature CECs (CD146<sup>+</sup>) are shed from compromised endothelium, whereas CEPs (CD31<sup>+</sup>

CD34<sup>+</sup> CD146<sup>-</sup>, DNA<sup>+</sup> CD45<sup>-</sup>) originate from bone marrow and partake in endothelial regeneration <sup>(2)</sup>. Viable CEPs are noticeably higher in COVID-19 patients than in controls, with apoptotic CEP fractions correlating positively with SARS-CoV-2 RNAemia in severe disease <sup>(1)</sup>. Moreover, post-COVID cohorts exhibit altered endothelial colony-forming cell (ECFC) kinetics for up to 12 months, implying enduring vascular perturbation <sup>(6)</sup>.

Elevated levels of CECs indicate extensive endothelial disruption, whereas fluctuations in CEPs reflect impaired reparative responses. These perturbations potentiate endothelial procoagulant shifts—downregulation of thrombomodulin and EPCR, impaired plasminogen activation, and increased vWF and P-selectin—driving thrombin generation and fibrin deposition <sup>(7, 8)</sup>. Integration of routine laboratory parameters with endothelial biomarkers reveals notable correlations: higher leukocyte and platelet counts align with increased CEC shedding; prolonged PT and PTT accompany elevated CEC levels and reduced CEP viability, collectively reinforcing a hypercoagulable phenotype <sup>(1, 5, 9)</sup>.

In summary, the synergistic assessment of CBC, PT/PTT, and dynamic endothelial biomarkers such as CECs and CEPs enhances early detection of hypercoagulable risk in COVID-19. This multidimensional approach supports more nuanced prognostication and personalized anticoagulation strategies.

Aim of the study was to investigate the association between the severity of endothelial dysfunction in COVID-19 patients and routine hematological indices, including complete blood count and coagulation parameters and to elucidate the relationship between conventional hematological biomarkers and novel endothelial indicators, specifically circulating endothelial cells and their progenitor populations.

## MATERIALS AND METHODS

The present study was conducted on sixty adult patients from the isolation units in Kasr Al Ainy hospital and forty control adults that were enrolled from Kasr Al Ainy Hospital, Cairo University. The patients included twenty females and forty males with age range from eighteen to sixty years old.

COVID-19 was detected in patients with viral pneumonia who showed objective evidence on a chest X-ray or lung-CT scan and whose infection was verified by positive SARS-CoV-2 RT-PCR in a sputum, bronchoalveolar lavage, or nasopharyngeal swab. Patients were admitted to either the internal medicine ward or the intensive care unit (ICU) based on the severity of their medical conditions. Within 48 hours of the patient's admission, blood samples were collected.

Selection process was done with simple random selection by taking even patients file numbers. Reverse transcriptase-polymerase chain reaction (RT-PCR) for SARS-CoV-2 was used to diagnose the patients.

Inclusion and exclusion criteria were set as follows; inclusion criteria included age group (18-60) and positive RT-PCR for SARS COV 2 while exclusion criteria included cardiac patients and cancer patients <sup>(1)</sup>, for possible interference with test results regarding endothelial cell markers.

All participants were subjected to the following tests upon admission after recording their medical history; complete blood picture, fibrinogen assessment, D-Dimer assessment, (Prothrombin Time) PT and (Partial thromboplastin Time) PTT, flowcytometric assessment of circulating endothelial cells and their progenitors.

The D Dimer assay employs a two-step enzyme immunoassay sandwich approach in conjunction with final fluorescence detection (ELFA). An anti-FbDP monoclonal antibody is adsorbed on the surface of the pipetting device and in the Solid Phase Receptacle, which acts as the solid phase. Pre-dispensed, usable assay reagents are included in the sealed single-use reagent strips.

The addition of thrombin to fresh citrated plasma is necessary for the fibrinogen test to function. The concentration of fibrinogen is directly correlated with the

coagulation time. This makes it possible to use a functional clotting assay to estimate plasma fibrinogen.

Whole blood samples were obtained in EDTA tubes and subjected to the flowcytometric evaluation. Following blood lysis with ammonium chloride (NH<sub>4</sub>Cl), cells were treated with monoclonal antibodies for 15 minutes at 4 °C in the dark. Using 10-color flow cytometry (Navios EX, Beckman Coulter), a panel of monoclonal antibodies, comprising anti-CD45 (to rule out hematopoietic cells), anti-CD34, anti-CD31 (both from Beckman Coulter), and anti-CD146 (BD) as endothelial cell markers, were utilized to evaluate CD146 + CECs and CEPs and 7-AAD (BD) for viability. Following the capture of one million events, CECs and CEPs were counted using a suitable gating method (Kaluza software, Beckman Coulter). CD45-negative, CD31+, CD34+, and CD146+ were found to be CECs. With modifications, CD45-, CD31+, CD34+, and CD146- CEPs are listed by Mancuso *et al.* <sup>(1)</sup>. In accordance with normal protocols, a dual platform counting method based on the total number of leukocytes in peripheral blood as determined by a hemocytometer was used to determine the absolute quantity of the cells.

## Ethical approval

**The study was accepted by the ethics committee of Cairo University and Helwan University (IRB: 40-2021). Every patient provided an informed written permission to accept the procedure. The study adhered to the Helsinki Declaration throughout its execution.**

## Statistical analysis

Version 20.0 of SPSS Inc.'s statistical software for social sciences (Chicago, Illinois, USA) was used to analyze the recorded data. The mean  $\pm$  standard deviation (SD) was used to express quantitative data. The Least Significant Difference (LSD) post-hoc test, the Mann Whitney U test, the Kruskal Wallis test, the one-way analysis of variance (ANOVA), the chi-square (x<sup>2</sup>) test of significance, and the independent-samples t-test of significance were all employed. A 95% confidence range and a 5% margin of error were defined, and a P value < 0.05 was deemed significant.

## RESULTS

In table number (1): Table demonstrates that the patients' group had a statistically significant higher mean value of neutrophils and the neutrophil/lymph ratio than the control group (p-value of p<0.05); the control group also had a highly statistically significant higher mean value of lymph (p-value of p<0.001), despite the fact that there was no statistically significant difference between the groups based on TLC (p-value of p>0.05).

**Table (1):** Comparison between groups according to TLC, Neutrophils, Lymph & Neut/ Lymph ratio.

	Patients Group (n=60)	Control Group (n=40)	Test value	P-value
<b>TLC</b>				
Mean±SD	8007.75±926.80	7125.00±938.38	1.161	0.284
<b>Neutrophils</b>				
Median (IQR)	4247.5 (2970-7749)	3800 (2861-5425)	4.521	0.036*
Range	1950-26950	1716-10353		
<b>Lymph</b>				
Mean±SD	1422.63±57.91	2432.00±53.54	56.79	<0.001**
<b>Neut/ Lymph ratio</b>				
Median (IQR)	3.49 (1.965-6.64)	1.65 (1.185-2.2225)	5.334	0.023*
Range	0.83-98	0.66-12.43		

*IQR: Interquartile range*

*Using: Mann-Whitney test for Non-parametric data "Median (IQR)"*

*Using: Independent Sample t-test for Mean±SD*

*p-value >0.05 is insignificant; \*p-value <0.05 is significant; \*\*p-value <0.001 is highly significant.*

In Table number (2) the patient group and the median platelet/lymph ratio was significantly higher than in the control group (p<0.001), and the mean platelet count was statistically significantly higher than in the control group (p<0.05).

**Table (2):** Comparison between groups according to Platelets and Plat/lymp ratio.

	Patients Group (n=60)	Control Group (n=40)	Test value	P-value
<b>Platelets</b>				
Mean±SD	216050.0±1607.0	257125.0±7915.8	5.715	0.019*
<b>Plat/lymp ratio</b>				
Median (IQR)	157.8 (111.8-227.2)	102.2 (84.6-130.4)	13.612	<0.001**
Range	57.14-468.75	8.8-385.35		

*IQR: Interquartile range, Using: Mann-Whitney test for Non-parametric data "Median (IQR)"*

*\*p-value <0.05 is significant; \*\*p-value <0.001 is highly significant.*

In table number (3), Although there is no statistically significant difference between the groups based on PTT, this table shows a highly statistically significant higher mean value of PT and INR in the patient group compared to the control group, with a p-value (p<0.001).

**Table (3):** Comparison between groups according to PT, INR & PTT.

	Patients Group (n=60)	Control Group (n=40)	Test value	P-value
<b>PT</b>				
Mean±SD	16.91±3.14	13.79±0.85	13.072	<0.001**
<b>INR</b>				
Mean±SD	1.15±0.14	1.06±0.07	13.29	<0.001**
<b>PTT</b>				
Median (IQR)	33 (30-36.8)	31 (29-35)	1.585	0.211
Range	28-180	26-41		

*IQR: Interquartile range*

*Using: Mann-Whitney test for Non-parametric data "Median (IQR)"*

*Using: Independent Sample t-test for Mean±SD*

*p-value >0.05 is insignificant; \*p-value <0.05 is significant; \*\*p-value <0.001 is highly significant*

*PT (Prothrombin Time), INR (International Normalized Ratio), and PTT (Partial Thromboplastin Time).*

In table number (4) in addition to the median D-Dimer value being significantly higher than the control group's (p-value p<0.001), the patient group's mean fibrinogen value was significantly higher than the control group's (p-value p<0.001).

**Table (4):** Comparison between groups according to coagulation test.

Coagulation test	Patients Group (n=60)	Control Group (n=40)	Test value	P-value
<b>Fibrinogen (g/L)</b>				
Mean±SD	2.69±0.93	2.15±0.34	12.503	<0.001**
<b>D DIMER 1 (ng/ml)</b>				
Median (IQR)	0.8 (0.4-1.1575)	0.2 (0.1-0.3)	13.946	<0.001**

*IQR: Interquartile range. Using: Mann-Whitney test for Non-parametric data "Median (IQR)"*

*Using: Independent Sample t-test for Mean±SD, \*\*p-value <0.001 is highly significant.*

Table number (5) shows that the median (IQR) of 146 negative "CEP" was statistically significantly greater in the patient group (1057) than in the control group (88.5), with a p-value of p<0.001. Additionally, the median (IQR) of 146 positive "CEC" was statistically significantly greater in the patient group (220; 141.25–314.5) than in the control group (15.5; p<0.001).

**Table (5):** Comparison between groups according to 146 negative (CEP) & 146 positive (CEC).

	Patients Group (n=60)	Control Group (n=40)	Test value	P-value
<b>146 negative (CEP)</b>				
Median (IQR)	1057 (677.5-1850.5)	88.5 (35-139.5)	59.929	<0.001**
Range	141-5308	14-231		
<b>146 positive (CEC)</b>				
Median (IQR)	220 (141.25-314.5)	15.5 (4-34.25)	59.758	<0.001**
Range	41-981	0-117		

*IQR: Interquartile range, Using: Mann-Whitney test for Non-parametric data "Median (IQR)"*

*\*\*p-value <0.001 is highly significant.*

In table number (6), 146 negative "CEP" and platelets had a statistically significant negative correlation (p-value p<0.001), whereas 146 negative "CEP" and 146 positive (CEC), age (years), neutrophils, neut/lymp ratio, fibrinogen (g/L), D-DIMER 1(on admission) (ng/ml), D-DIMER 5 (in 5<sup>th</sup> day of hospitalization), PT & INR, had a statistically significant positive correlation (p-value p<0.05). The p-value for the remaining variables was p>0.05, indicating an inconsequential connection. Furthermore, 146 positive "CEC" and lymph and platelets showed a statistically significant negative connection (p-value p<0.05); the other relationships were not significant (p-value p>0.05). Furthermore, a statistically significant positive connection was observed between 146 positive "CEC" and Neut/Lymp ratio, Plat/lymp ratio, D-DIMER 1 (ng/ml), D-DIMER 5, PT, INR, and PT in 5<sup>TH</sup> day of hospitalization.

**Table (6):** Correlation between 146 negative (CEP) and 146 positive (CEC) with different parameters among patients group, using Spearman's rank correlation coefficient (rs).

Parameters	146 negative (CEP)		146 positive (CEC)	
	Rs	p-value	Rs	p-value
146 negative (CEP)	--	--	0.302	0.019*
146 positive (CEC)	0.302	0.019*	--	--
Age (years)	0.341	0.008*	0.215	0.099
TLC	0.221	0.090	0.024	0.857
Neutrophils	0.271	0.036*	0.198	0.130
Lymph	-0.162	0.216	-0.554	<0.001**
Neut/ Lymph ratio	0.277	0.032*	0.450	<0.001**
Platelets	-0.434	<0.001**	-0.338	0.008*
Plat/lymp ratio	-0.116	0.379	0.349	0.006*
Fibrinogen (g/L)	0.454	<0.001**	0.116	0.377
D DIMER 1 (ng/ml)	0.422	<0.001**	0.558	<0.001**
D DIMER 5	0.547	<0.001**	0.517	<0.001**
PT	0.269	0.038*	0.418	<0.001**
INR	0.269	0.038*	0.410	<0.001**
PT 5TH DAY	0.215	0.098	0.308	0.017*
PTT	-0.030	0.821	0.033	0.805

*Using: Spearman's rank correlation coefficient (rs, p-value >0.05 NS; \*p-value <0.05 S; \*\*p-value <0.001 HS.*

## DISCUSSION

Our investigation demonstrated that COVID-19 patients exhibit significantly elevated neutrophil counts compared to healthy controls, indicative of a systemic hyperinflammatory state and innate immune activation. Neutrophilia correlates with increased disease severity and poor outcomes, validating neutrophil count as a prognostic marker in recent COVID-19 cohorts <sup>(9)</sup>.

Conversely, lymphocyte and platelet counts were significantly reduced in COVID-19 patients. Lymphopenia reflects immune dysregulation specially When SARS CoV 2 infection is severe, T cell depletion while thrombocytopenia is likely secondary to platelet group. Lymphopenia, a hallmark of COVID-19, is attributed to T-cell exhaustion, apoptosis, and direct viral injury to lymphoid tissues <sup>(10)</sup>. Thrombocytopenia in COVID-19 may result from increased platelet consumption due to endothelial activation and microthrombus formation <sup>(11)</sup>.

Platelet-to-lymphocyte ratios (PLR) and neutrophil-to-lymphocyte ratios (NLR) were markedly elevated in COVID-19 patients. These ratios serve as accessible prognostic biomarkers, with NLR being a more sensitive marker of systemic inflammation, cytokine storm, and coagulopathy. PLR, while more variable, has also been associated with disease progression and hypoxemia <sup>(12)</sup>.

In terms of coagulation, Prothrombin time (PT) and international normalized ratio (INR) were significantly longer in patients than in controls, indicating hepatic impairment and extrinsic coagulation pathway activation. These findings are in line with COVID-associated coagulopathy (CAC), where elevated tissue factor expression and endothelial injury promote thrombin generation <sup>(3,13)</sup>. Notably, activated partial thromboplastin time (aPTT/PTT) showed no significant difference between groups, reinforcing that the extrinsic pathway is predominantly affected in CAC <sup>(13)</sup>.

Furthermore, D-dimer and fibrinogen levels were noticeably greater in COVID-19 individuals, which is consistent with hyperfibrinolysis and an acute-phase reactant response. D-dimer elevation reflects ongoing fibrin degradation and is a robust predictor of thrombotic risk and mortality <sup>(5,14)</sup>. Elevated fibrinogen, an acute-phase protein, contributes to a prothrombotic state by enhancing plasma viscosity and platelet aggregation <sup>(10,13)</sup>.

Critically, our study also found Endothelial progenitor cells (CEPs) and circulating endothelial cells (CECs) were markedly increased in the patient group. Elevated CECs are indicative of endothelial injury, while CEPs suggest a compensatory reparative response. However, excessive CEP mobilization may reflect dysfunctional endothelial repair, especially in severe disease <sup>(15-17)</sup>.

Strong correlations were identified between CEC and CEP levels and neutrophilia, lymphopenia, prolonged PT/INR, and reduced RBCs, indicating an intertwined pathophysiology linking inflammation, coagulopathy, and endothelial disruption. These results emphasize the significance of integrating endothelial biomarkers with routine laboratory parameters for comprehensive risk stratification in COVID-19.

## CONCLUSION

COVID-19 patients exhibit significant hematological and coagulation abnormalities that correlate with increased circulating endothelial cells and progenitors, reflecting endothelial injury and impaired repair mechanisms. The integration of these endothelial biomarkers with routine laboratory tests may enhance early detection of thrombotic risk and guide timely therapeutic interventions.

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## REFERENCES

1. Mancuso P, Gidaro A, Gregato G *et al.* (2020): Circulating endothelial progenitors are increased in COVID 19 patients and correlate with SARS CoV 2 RNA in severe cases. *J Thromb Haemost.*, 18(11):2744–52.
2. Peñaloza H, Lee J, Ray P (2021): Neutrophils and lymphopenia, an unknown axis in severe COVID-19 disease. *PLoS pathogens*, 17(9): e1009850.
3. Apak B, Yuce G, Topcu I *et al.* (2023): Coagulopathy is initiated with endothelial dysfunction and disrupted fibrinolysis in patients with COVID 19 disease. *Indian J Clin Biochem.*, 38(2):220–230.
4. Bocatonda A, Campello E, Simion C *et al.* (2023). Long-term hypercoagulability, endotheliopathy and inflammation following acute SARS-CoV-2 infection. *Expert review of hematology*, 16(12): 1035–1048.
5. Job M, Chacko B, Selvarajan S *et al.* (2024): Biomarkers of coagulation, endothelial, platelet function, and fibrinolysis in patients with COVID-19: a prospective study. *Sci Rep.*, 14(1):2011. doi:10.1038/s41598-024-51908-9.
6. Poyatos P, Luque N, Sabater G *et al.* (2024): Endothelial dysfunction and cardiovascular risk in post COVID 19 patients after 6 and 12 months SARS CoV 2 infection. *Infection*, 52(4):1269–1285.
7. Fard M, Fard S, Ramazi S *et al.* (2021). Thrombosis in COVID-19 infection: Role of platelet activation-mediated immunity. *Thrombosis journal*, 19(1): 59-64.
8. Jerah A, Farasani A, Abu Tawil H *et al.* (2024): Unveiling coagulation dysfunction in patients with COVID 19: a retrospective analysis. *J Med Life.*, 17(9):886–891.

9. **Rong N, Wei X, Liu J (2024):** The Role of Neutrophil in COVID-19: Positive or Negative. *J Innate Immun.*, 16(1):80-95.
10. **Khalel M, Osman A, Ghobrial A *et al.* (2024):** Coagulation Profile in COVID-19 Patients. *Minia Journal of Medical Research*, 35(4): 87-98. doi: 10.21608/mjmr.2023.222077.1455
11. **El-Khaiat M, El-Lehlah M, Kesheita A *et al.* (2024):** Association between thrombocytopenia and the severity of Covid-19 infection among hospitalized Egyptian patients. *Ann Med Surg (Lond)*, 79:103973.
12. **Asperges E, Albi G, Zuccaro V *et al.* (2023):** Dynamic NLR and PLR in Predicting COVID-19 Severity: A Retrospective Cohort Study. *Infect Dis Ther.*, 12, 1625–1640.
13. **Ranucci M, Baryshnikova E, Anguissola M *et al.* (2023):** The Long Term Residual Effects of COVID-Associated Coagulopathy. *International Journal of Molecular Sciences*, 24(6):5514.
14. **Nemec M, Ferenczy A, Christie D *et al.* (2022):** Correlation of D-dimer and Outcomes in COVID-19 Patients. *Am Surg.*, 88(9):2115-2118. doi: 10.1177/00031348221091940.
15. **Mancuso P, Antoniotti P, Quarna J *et al.* (2009):** Validation of a standardized method for enumerating circulating endothelial cells and progenitors: flow cytometry and molecular and ultrastructural analyses. *Clin Cancer Res.*, 15(1):267-73.
16. **Guervilly C, Burtsey S, Sabatier F *et al.* (2020):** Circulating Endothelial Cells as a Marker of Endothelial Injury in Severe COVID -19. *J Infect Dis.*, 222(11):1789-1793.
17. **Boos J, Lip Y, Blann D (2006):** Circulating endothelial cells in cardiovascular disease. *J Am Coll Cardiol.*, 48(8):1538-47.