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ORIGINAL ARTICLE

Prognostic Value of Inflammatory and Coagulation Parameters in Covid-19 Without Comorbid Diseases: The Limited Role of PAI-1

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ABSTRACT

Background: **Coronavirus disease 2019 (Covid-19) is associated with endothelial injury, inflammation, and dysregulated coagulation. Plasminogen activator inhibitor-1 (PAI-1), a key regulator of fibrinolysis, may contribute to these abnormalities. This study aimed to evaluate the prognostic role of circulating PAI-1 and compare it with inflammatory and coagulation markers in Covid-19 patients without comorbid diseases.**

Methods: **In this prospective cross-sectional study, 106 hospitalized Covid-19 patients without comorbid diseases and 32 healthy controls were enrolled. Serum PAI-1, inflammatory (C-reactive protein (CRP), interleukin-6 (IL-6), neutrophils, lymphocytes, platelets), and coagulation parameters (D-dimer, fibrinogen, International Normalized Ratio (INR)) were measured within the first 3 days of admission. Disease severity, oxygen requirement, hospital length of stay (LoS), and mortality were analyzed in relation to these biomarkers.**

Results: **Plasma PAI-1 levels were significantly elevated in all Covid-19 groups compared with controls ($p < 0.001$), with the highest levels observed in moderate disease. However, PAI-1 was not predictive of disease severity, oxygen need, LoS, or mortality. In contrast, lymphocyte and neutrophil counts, CRP, IL-6, fibrinogen, D-dimer, and INR showed strong associations with worse outcomes. Lymphopenia ($< 0.9 \times 10^9$/L) independently predicted both oxygen requirement (area under the curve (AUC) = 0.790) and mortality (AUC = 0.847). Hospital LoS correlated negatively with lymphocyte count and positively with neutrophil count.**

Conclusions: **Although plasma PAI-1 levels were consistently higher in patients with Covid-19 without comorbid diseases compared to controls, they did not provide meaningful prognostic information regarding clinical course or outcomes. Traditional markers of inflammation and coagulation - most notably lymphocyte counts - proved to be more robust indicators of disease severity and survival.**

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KEYWORDS

Covid-19, plasminogen activator inhibitor-1, inflammation, coagulation markers, lymphopenia

LIST OF ABBREVIATIONS

ARDS - Acute respiratory distress syndrome
APTT - Partial thromboplastin time
AUC - Area under the curve
BMI - Body mass index
CI - Confidence interval (95% confidence interval)
Covid-19 - Coronavirus disease 2019
CrCl - Creatinine clearance
CRP - C-reactive protein
DIC - Disseminated intravascular coagulation
FiO_2 - Fraction of inspired oxygen
IL-6 - Interleukin-6
INR - International normalized ratio
IQR - Interquartile range
LMWH - Low molecular weight heparin
LoS - Length of stay
NETs - Neutrophil extracellular traps
NPV - Negative predictive value
PaO_2 - partial arterial oxygen pressure
PAI-1 - Plasminogen activator inhibitor-1
PIC - α_2-plasmin inhibitor-plasmin complex
PPV - Positive predictive value
RT-PCR - Real-time polymerase chain reaction
S. Error - Standard error
SC - Subcutaneous
SD - Standard deviation
SpO_2 - Oxygen saturation
tPA - Tissue-type plasminogen activator
t-PAIC - t-PA/PAI-1 complex
TM - Thrombomodulin
TAT - Thrombin-antithrombin complex
uPA - Urokinase-type plasminogen activator
VIF - Variance inflation factor

INTRODUCTION

Since its introduction, SARS-CoV-2, an RNA virus of zoonotic origin, has significantly impacted global health systems. Since the initial declaration of a positive COVID-19 case in Turkey on March 11, 2020, the total number of pandemic-related fatalities has been reported as 101,994 [1]. The majority of Covid-19 instances are mild or moderate; however, individuals with preexisting disorders including diabetes, lung ailments, or cancer sometimes encounter more serious consequences [2].
Matricardi et al. classify the disease into four separate stages, from early respiratory symptoms to severe illness. The first phase is marked by an upper respiratory tract infection, accompanied by fever, muscular exhaustion, and discomfort, with nausea, vomiting, and diarrhea being infrequent. The second stage is marked by the emergence of dyspnea and pneumonia, whilst the introduction of a cytokine storm in the third stage exacerbates the condition. In many cases, the hyperinflammatory response advances to significant lung damage akin to ARDS (acute respiratory distress syndrome), often associated with coagulation irregularities, thrombotic occurrences, and perhaps disseminated intravascular coagulation (DIC). The fourth and final stage is marked by the patient's recovery or death [3].
The post-mortem study of lung disorders revealed widespread alveolar damage, characterized by injury to the alveolar epithelial cells, development of hyaline membranes, fibrin deposition, and hyperplasia of type II pneumocytes [4]. Direct and synergistic mechanisms have been proposed for Covid-19 infection leading to macro and microvascular occlusions. Proposed mechanisms include cytokine-mediated activation of vascular endothelium, leukocytes, and platelets; hypoxic vasoconstriction; direct activation of endothelial cells by viral transduction; and enhancement of thrombosis by neutrophil extracellular traps (NETs) [5,6]. Conversely, hemorrhaging has also been identified in some Covid-19 patients. The disintegration of fibrin clots, termed fibrinolysis, is meticulously controlled and mostly facilitated by the transformation of plasminogen into plasmin [7]. Plasminogen activators and inhibitors govern this process, with fibrinolysis reliant only on the conversion of plasminogen to plasmin. The modulation of fibrinolytic activity results from the interplay between tissue-type plasminogen activators (tPA) and urokinase-type plasminogen activators (uPA) and their principal inhibitor, plasminogen activator inhibitor-1 (PAI-1) [7]. Impaired fibrinolysis has been implicated in the pathophysiology of Covid-19, potentially exacerbating the thrombotic risk in infected individuals.
Given that patients with Covid-19 may experience both hemorrhagic and coagulation abnormalities, especially during the hyperinflammatory phase, we postulated that the plasma of certain individuals may exhibit a hyper-fibrinolytic condition, while others would demonstrate fibrinolytic closure. The principal objective of our study was to assess the utility of serum PAI-1 levels in predicting disease prognosis, including severity, hospital length of stay, oxygen requirements, and mortality, across patients with varying severity. Additionally, we aimed to elucidate the impact of Covid-19 without comorbid diseases on the inflammation and coagulation cascade.

MATERIALS AND METHODS

Study protocol

We conducted a prospective, cross-sectional analysis of patients admitted with Covid-19 to Gülhane Education and Research Hospital (University of Health Sciences Türkiye) between January and June 2021. The study was approved by the institutional ethics committee on October 2021. This clinical trial was registered before

patient enrollment (ClinicalTrials.gov identifier: NCT04997928)

A total of 106 participants were included in the experiment after meeting the inclusion criteria. Eligible participants were adults over 20 years with real-time polymerase chain reaction (RT-PCR) confirmed Covid-19 infection in at least one biological sample and compatible clinical symptoms (fever, cough, dyspnea, joint pain, etc.), with or without involvement in lung imaging. The exclusion criteria were an infectious disease other than Covid-19, previous or current autoimmune diseases, pregnancy, previous vascular endothelial damage due to secondary causes such as the history of thrombosis, thrombophlebitis and/or thromboembolism, presence of active cancer, history of chemotherapy, radiotherapy or bone marrow transplantation, vasculitis, familial inherited hereditary diseases, patients with chronic renal failure and/or requiring dialysis, patients receiving treatment with a diagnosis of bronchial asthma, hypertension, type 1 or type 2 diabetes mellitus patients with hemoglobin A1C levels > 6.4%, patients with diabetes for more than 5 years or currently taking oral insulin-sensitizing drugs (biguanides; metformin), patients using antithrombotic (Clopidogrel, low-dose aspirin, etc.) and anticoagulant [Low molecular weight heparin (LMWH), Warfarin, etc.] agents.

All patients enrolled in the study received supportive management of the most common complications of severe Covid-19. These were the treatment of pneumonia, hypoxemic respiratory failure, septicemia, cardiovascular complications (arrhythmia, cardiomyopathy), renal complications, (acute renal failure), complications from prolonged hospitalization, nosocomial infections, gastrointestinal bleeding, critical illness polyneuropathy, and heparin or LMWH prophylaxis. In the guideline of the Ministry of Health of the Republic of Türkiye, thrombosis prophylaxis is routinely recommended for all hospitalized Covid-19 patients unless there is active bleeding or thrombocytopenia (< 25,000 - 30,000/mL). If the D-dimer value is > 2 times higher than normal, the patient is at high risk for venous thromboembolism and should receive anticoagulant prophylaxis for at least 45 days. The prophylaxis dose guideline states that for those with a Body Mass Index (BMI) < 40 kg/m², enoxaparin is 40 mg/day, and for those with a BMI > 40 kg/m², enoxaparin is 40 mg twice daily subcutaneously (sc). It is not advised to use enoxaparin in patients whose creatinine clearance (CrCl) is less than 30 milliliters per minute. Standard heparin is recommended (5,000 U sc 2 x 1 or 3 x 1). In patients with severe Covid-19: If CrCl > 30 mL/minute, enoxaparin 40 mg 2 x 1 sc, or standard heparin 7,500 U is recommended at a dose of 3 x 1 [8].

On the basis of the clinical and radiological data in the study, the severity of Covid-19 disease was considered to be mild (group 1) when the patients had an oxygen saturation (SpO2) ≥ 92% in room air, no radiological lung involvement, or those with mild signs of pneumonia, to be moderate (group 2) when they had radiologically moderate pneumonia findings and/or SpO2 = 88 - 92% in room air, and to be severe (group 3) when the patients with radiological findings of severe pneumonia or ARDS and/or high fraction of inspired oxygen (FiO_2) requirement due to respiratory failure [8].

According to the degree of involvement by consolidation or ground-glass opacities, each lung was given a radiological severity score ranging from 0 to 4: 0 denoted no participation, 1 less than 25%, 2 25 - 49%, 3 50 - 75%, and 4 more than 75% involvement. The five lobe scores (which range from 0 to 20) were added up to provide a cumulative numerical score. A simplified category severity score was then used, which was divided into three categories: mild (< 7), moderate (8 - 17), and severe (18 or more) [9].

The medical history and clinical data of all patients were obtained from the hospital's electronic database and from e-Nabız, the national health data system. The study utilized PAI-1 for fibrinolysis, D-dimer, fibrinogen, and INR (International Normalized Ratio) for coagulation, while neutrophil, lymphocyte, and platelet counts, along with C-reactive protein (CRP) and interleukin-6 (IL-6) levels, served as inflammatory indicators. Laboratory parameters received from the patient's medical records within the first 3 days after hospitalization were D-dimer, CRP, IL-6, neutrophils, lymphocytes, platelets, fibrinogen, and INR.

All hemostasis assessments were conducted in the Hematology and Biochemistry Laboratories of Gülhane Education and Research Hospital. After receiving the patient's consent form of participation in the study, the initial 5 mL blood samples of all three groups were obtained on the day of admission or the following day. Peripheral blood was drawn into sodium citrate tubes and processed within two hours by centrifugation (2,000 × g, 15 minutes, room temperature), and the plasma aliquots were immediately frozen and stored at -80°C until testing. The blood samples were thawed under proper conditions at the end of the study after reaching a sufficient number of participants. Plasma PAI-1 antigen concentrations were quantified with a commercial ELISA kit (Molecular Innovations, USA), and absorbance was read on a Bio-Tek Synergy HT plate reader. An ROTEM Delta device (Werfen, Le-Pre-Sait-Gervais, France) with EXTEM reagent in the presence of 0.625 µg/mL tPA was used to measure clot formation and fibrinolysis (Actilyse, Paris, France; Boehringer-Ingelheim). Normal values were determined from a control group of 32 apparently healthy adult volunteers (19 men and 13 women) between 25 to 51 years of age (38.41 ± 7.39 years; mean ± SD). Healthy controls consisted of individuals who had not been previously diagnosed with Covid-19, had not had Covid-19 vaccinations, were non-smokers, were not taking substances known to influence the coagulation system, and had no history of venous thromboembolism or bleeding disorders. The exclusion criteria for controls were a history of a systemic autoimmune disease, the presence of any active infection, and pregnancy.

The sample size calculation

Sample size estimation, performed with Stata v16.0 (Stata Corp. College Station, Texas, USA), indicated that 60 participants would provide 99% power at a 5% significance level.

Statistical analysis

Data were analyzed with IBM SPSS V23. The Kolmogorov-Smirnov and Shapiro-Wilk tests were used to determine if the data followed a normal distribution. To compare paired groups' non-normally distributed data, Mann-Whitney U test was used. The factors affecting PAI-1 level in the patient group were analyzed by linear regression analysis. The factors affecting the length of stay were analyzed by linear regression analysis. A one-way analysis of variance was employed to compare normally distributed data across groups of three or more, with multiple comparisons assessed using Tamhane's T2 test and Duncan's test. The Kruskal-Wallis H test was used to compare data that were non-normally distributed according to groups of three or more, and multiple comparisons were examined with Dunn's test. ROC analysis was used to predict death in the outcome state and those in need of oxygen in the respiratory state. Analysis results were presented as mean ± standard deviation and median (minimum - maximum) for quantitative data. Significance level was set at $p < 0.050$.

RESULTS

The study enrolled 106 hospitalized patients (61 male, 45 female) with a mean age of 56.7 years (SD ±15.6). The distribution of the disease was mild in 39 patients (group 1), moderate in 34 patients (group 2), and severe in 33 patients (group 3). Clinical characteristics such as disease severity, respiratory status, concomitant treatments, length of stay (LoS), and final outcomes varied significantly between the groups ($p < 0.05$). Twenty-four patients were being treated in our hospital's Covid intensive care clinic, and 10 of them (41.6%) were under invasive mechanical ventilation. The remaining eighty-two patients were followed up and treated in the Covid internal medicine clinic.

Prophylactic LMWH anticoagulation was initiated and maintained during hospitalization in all patients included in the study. No demonstrable thrombosis was detected in any of our patients based on their clinical and appropriate radiological findings. The clinical features of the patients are summarized in Table 1.

Plasminogen activator inhibitor-1 levels

Figure 1 shows the plasma PAI-1 levels of Covid-19 patients grouped by disease severity. Across all severity categories, patients demonstrated markedly elevated PAI-1 concentrations (mean 84.5 ± 58.6 ng/mL) compared with healthy controls (37.5 ± 22.7 ng/mL, $p < 0.001$). They were found to be 83.91 ± 52.1 ng/mL ($p = 0.032$), 107.61 ± 63.44 ng/mL ($p < 0.001$), and 77.85 ± 57.48 ng/mL ($p = 0.007$) in mild, moderate, and severe disease groups, respectively. Pairwise analysis revealed higher PAI-1 values in moderate cases compared with both mild ($p = 0.007$) and severe disease ($p = 0.033$), while mild and severe cases showed no meaningful difference ($p = 0.561$) (Figure 1).

The independent variables affecting the PAI-1 level were examined by linear regression analysis. The distribution of data did not change when patients were subdivided according to the groups of age, sex, BMI, used methylprednisolone, favipiravir, and subcutaneous enoxaparin ($p > 0.05$). The PAI-1 level of those who use antibiotics is 50,903 lower than those who do not use antibiotics ($p = 0.001$) (Table 2).

The association of PAI-1 levels with the disease severity and worse outcomes

Based on the severity of the disease, there was a statistically significant difference in the median PAI-1 values ($p = 0.017$). The difference between group 1 and 2 (Figure 1, Table 3) is the cause of this disparity. Figure 2 shows that the median PAI-1 concentrations were similar for patients receiving room air (61.2 ng/mL, $p = 0.238$) and supplementary oxygen (70.8 ng/mL). Moreover, Figure 3 shows that the median PAI-1 levels did not alter statistically significantly in comparison to the final results ($p = 0.263$).

Correlations between PAI-1 levels and inflammatory and coagulation makers

Table 3 presents the increased levels of acute phase proteins and the presence of hematological and coagulation system abnormalities in Covid-19 patients of our study groups. Although the PAI-1 plasma levels were high in all three study groups in comparison to controls, it was highest in patients with moderate disease ($p < 0.001$) (Figure 1). PAI-1 levels only correlated with IL-6 ($r = -0.308$, $p = 0.05$).

Effect of inflammatory and coagulation markers on Covid-19 disease severity and worse outcomes

Covid-19 disease severity

A statistically significant difference was discovered between the groups when lymphocyte count, D-Dimer, and CRP median values were categorized in accordance with the severity of the disease ($p = 0.001$). This is due to the difference between group 1 and group 2 versus group 3. A statistically significant difference was found between the median values of IL-6 according to disease severity ($p = 0.026$). This is due to the difference between group 1 and group 3. According to the severity of the condition, there was a statistically significant difference between the mean neutrophil count values ($p < 0.001$). The difference between group 1 and group 2 against group 3 is the cause of this variation. There was found to be a statistically significant variation between the mean fibrinogen values depending on the disease severity ($p = 0.025$). The difference between group 1 and

Table 1. Demographic and clinical characteristics of Covid-19 patient groups.

	Group 1	Group 2	Group 3	p-value *
Age (mean ± SD, years)	49.72 ± 16.36	58.82 ± 16.21	61.61 ± 14.20	0.004
Gender (n %)				
Female	23 (59)	14 (41.2)	8 (24.2)	0.012
Male	16 (41)	20 (58.8)	25 (75.8)	
BMI (IQR, kg/m^2)	7.6	6.4	5.2	0.026
Medications (n %)				
Antibiotic	17 (43.6)	24 (70.6)	33 (100)	0.00
Methylprednisolone	8 (20.5)	19 (55.9)	32 (97)	0.00
Subcutaneous heparin	-	-	-	-
Subcutaneous enoxaparin	39 (100)	34 (100)	33 (100)	0.25
Favipiravir	32 (82.1)	29 (85.3)	33 (100)	0.04
Hydroxychloroquine	-	-	-	-
Respiratory status (n %)				
Room air	35 (89.1)	8 (23.5)	2 (6.1)	0.00
Nasal cannula	4 (10.3)	14 (41.2)	1 (3)	
Mask	-	10 (29.4)	6 (18.2)	
High flow oxygen	-	2 (5.9)	14 (42.4)	
Mechanical ventilation	-	-	10 (30.3)	
Hospital length of stay (LoS) (mean ± SD)	6.54 ± 4.07	8 ± 4.60	16 ± 18.58	0.02
Final outcomes (n %)				
Discharged	37 (94.9)	32 (94.1)	18 (54.5)	0.00
Transfer to the intensive care unit of another hospital	1 (2.6)	1 (2.9)	13 (39.4)	
Gave up treatment	1 (2.6)	-	-	
Death	-	1 (2.9)	2 (6.1)	
Remains hospitalized	-	-		

Mean ± SD: mean ± standard deviation, BMI: body mass index, IQR: interquartile range.
*** Statistically significant results are highlighted underlined.**

Table 2. Factors that may affect PAI-1 level in patient groups with linear regression.

	β_0 (95% CI)	S. Error	β_1	t	p *	r^1	r^2	VIF
Invariant	-7.386 (-134.178 - 119.406)	63.892		-0.116	0.908			
Age	-0.278 (-0.96 - 0.404)	0.344	-0.078	-0.809	0.420	-0.062	-0.081	1.060
Gender	-0.554 (-25.252 - 24.143)	12.445	-0.005	-0,045	0.965	-0.076	-0.005	1.278
BMI	1.84 (-0.84 - 4.52)	1.350	0.142	1.363	0.176	0.091	0.136	1.244
Antibiotic	-50.903 (-80.29 - -21.517)	14.808	-0.400	-3.438	0.001	-0.246	-0.328	1.561
Methylprednisolone	24.927 (-0.835 - 50.689)	12.982	0.212	1.920	0.058	0.048	0.190	1.405
Subcutaneous enoxaparin	67.458 (-50.836 - 185.751)	59.610	0.112	1.132	0.261	0.109	0.114	1.122
Favipiravir	13.272 (-23.176 - -49.719)	18.366	0.072	0.723	0.472	0.131	0.073	1.144

F = 2.439, p = 0.024, R2 = 0.148, adjusted R2 = 0.088, β_0: unstandardized beta coefficient, S. Error: standard error, β_1: standardized beta coefficient, r^1: zero-order correlation, r^2: partial correlation, VIF: variance inflation factor.
*** Statistically significant results are highlighted underlined.**

Table 3. Laboratory parameters of the study groups.

	Group 1		Group 2		Group 3		
Laboratory parameters	mean ± SD	median	mean ± SD	median	mean ± SD	median	p-value ǂ
PAI-1 (ng/mL)	83.91 ± 52.1	72.95 (19.49 - 158.17) [b]	107.61 ± 63.44	101.33 (24.54 - 254.93) [a]	77.85 ± 57.48	61.21 (13 - 229.52) [ab]	0.017 *
Lymphocyte count (x 10^9/L)	1.18 ± 0.54	1.1 (0.3 - 2.2) [b]	0.95 ± 0.4	0.85 (0.3 - 1.8) [a]	0.86 ± 0.89	0.7 (0.2 - 4.5) [a]	< 0.001 *
D-dimer (mg/L)	0.59 ± 0.62	0.37 (0.19 - 2.16) [b]	1.77 ± 3.15	0.83 (0.2 - 16.75) [a]	2.08 ± 2.88	0.96 (0.2 - 11.24) [a]	< 0.001 *
INR	1.14 ± 0.25	1.07 (0.93 - 1.6)	1.16 ± 0.32	1.03 (0.92 - 2.36)	1.12 ± 0.17	1.08 (0.85 - 1.6)	0.788 *
CRP (mg/L)	19.4 ± 19.05	14.89 (4 - 74) [b]	98.24 ± 84.62	94 (3 - 305) [a]	120.77 ± 108.21	102.5 (0 - 531) [a]	< 0.001 *
IL-6 (pg/mL)	549.16 ± 927.44	25 (2 - 1,620) [b]	45.98 ± 69.6	23.4 (3 - 259) [ab]	188.89 ± 407	68 (3 - 1620) [a]	0.026 *
Neutrophil count (x 10^9/L)	4.52 ± 4.09 [a]	3.2 (1.5 - 18.7)	5.28 ± 3 [a]	4.5 (0.9 - 12.4)	8.43 ± 4.26 [b]	8.1 (2.4 - 2.4)	< 0.001 **
Platelet count (x 10^9/L)	201.24 ± 62.1	196 (110 - 386)	230.26 ± 85.78	201.5 (123 - 472)	238.64 ± 128.37	210 (46 - 607)	0.932 **
Fibrinogen (g/L)	440.24 ± 129.81 [b]	438 (240 - 827)	480.33 ± 144.14 [ab]	454.5 (222 - 756)	551.52 ± 211.48 [a]	525 (174 - 900)	0.025 **

*** Kruskal-Wallis H test, ** one-way test of variance, a - b: there is no difference between groups with the same letter.**
ǂ Statistically significant results are highlighted underlined.

Table 4. Regression analysis of laboratory parameters in predicting the oxygen requirements of study groups.

	Cut-off	AUC (95% CI)	p *	Sensitive	Specificity	PPV	NPV
PAI-1 (ng/mL)	---	0.475 (0.267 - 0.683)	0.815	---	---	---	---
D-dimer (mg/L)	---	0.392 (0.190 - 0.594)	0.312	---	---	---	---
INR	---	0.342 (0.130 - 0.554)	0.139	---	---	---	---
Fibrinogen (g/L)	≥ 469	0.142 (0.007 - 0.276)	0.001	53.57%	75%	73.17%	55.93%
CRP (mg/L)	≥ 17.9	0.252 (0.057 - 0.447)	0.021	85%	61.11%	75.86%	73.33%
IL-6 (pg/mL)	≥ 11.6	0.269 (0.087 - 0.451)	0.031	79.17%	70.59%	79.17%	70.59%
Lymphocyte count (x 10^9/L)	≥ 1	0.790 (0.694 - 0.885)	< 0.001	78.43%	67.65%	78.43%	67.65%
Neutrophil count (x 10^9/L)	≥ 5.2	0.140 (0 - 0.284)	0.001	65.57%	75.56%	78.43%	61.82%
Platelet count (x 10^9/L)	---	0.538 (0.414 - 0.662)	0.554	---	---	---	---

AUC: Area under the ROC curve, NPV: Negative predictive values, PPV: Positive predictive value.
*** Statistically significant results are highlighted underlined. --- There is no observation.**

group 3 is what led to this outcome (Table 3).

Oxygen requirement

The AUC values of lymphocyte and neutrophil counts, CRP, IL-6, and fibrinogen values were found to be statistically significant in predicting Covid-19 patients in need of oxygen ($p < 0.001$, 0.001, 0.021, 0.031, and 0.001, respectively) (Table 4).

ROC was drawn to analyze the oxygen requirement of coagulation and inflammatory parameters in Covid-19

Table 5. The linear regression analysis of the effect of all the laboratory parameters on hospital length of stay.

	β_0 (95% CI)	S. Error	β_1	t	p *	r^1	r^2	VIF
Constant	4.611 (0.448 - 8.773)	1.981		2.327	0.032			
PAI-1 (ng/mL)	0.002 (-0.004 - 0.008)	0.003	0.111	0.582	0.568	0.079	0.136	2.039
D-dimer (mg/L)	0.084 (-0.042 - 0.21)	0.06	0.265	1.398	0.179	0.421	0.313	2.035
INR	-0.337 (-1.742 - 1.068)	0.669	-0.079	-0.504	0.621	0.183	-0.118	1.387
Fibrinogen (g/L)	0 (-0.002 - 0.002)	0.001	0.035	0.175	0.863	0.36	0.041	2.243
CRP (mg/L)	-0.002 (-0.006 - 0.001)	0.002	-0.356	-1.298	0.211	0.193	-0.292	4.259
IL-6 (pg/mL)	0 (-0.001 - 0.001)	0	-0.055	-0.342	0.736	0.138	-0.08	1.458
Lymphocyte count (x 10^9/L)	-0.59 (-1.033 - -0.147)	0.211	-0.548	-2.799	0.012	-0.683	-0.551	2.164
Neutrophil count (x 10^9/L)	0,1 (0.025 - 0.175)	0.036	0.498	2.793	0.012	0.523	0.55	1.796
Platelet count (x 10^9/L)	-0.002 (-0.006 - 0.002)	0.002	-0.173	-0.95	0.355	-0.247	-0.218	1.883

F = 3,21, p = 0,013, r^2 = 0,682, adjusted R2 = 0.469, β_0: standardized beta coefficient, S. Error: standard error, β_1: standardized beta coefficient, r^1: zero-order correlation, r^2: partial correlation.
*** Statistically significant results are highlighted underlined.**

Table 6. Regression analysis of laboratory parameters in predicting the mortality of study groups.

	Cut-off	AUC (95% CI)	p *	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
PAI-1 (ng/mL)	---	0.583 (0.397 - 0.770)	0.361	---	---	---	---
D-dimer (mg/L)	≥ 1.32	0.758 (0.485 - 1)	0.038	69.23%	77.78%	36%	93.33%
INR	≥ 1.2	0.827 (0.627 - 1)	0.009	63.64%	84.31%	46.67%	91.49%
Fibrinogen (g/L)	---	0.615 (0.385 - 0.846)	0.355	---	---	---	---
CRP (mg/L)	---	0.695 (0.483 - 0.907)	0.118	---	---	---	---
IL-6 (pg/mL)	---	0.714 (0.475 - 0.954)	0.086	---	---	---	---
Lymphocyte count (x 10^9/L)	≤ 0.9	0.847 (0.737 - 0.958)	< 0.001	100%	54.29%	27.27%	100%
Neutrophil count (x 10^9/L)	≥ 6	0.843 (0.707 - 0.980)	0.006	85.71%	74.71%	35.29%	97.01%
Platelet count (x 10^9/L)	---	0.541 (0.334 - 0.748)	0.215	---	---	---	---

AUC: Area under the ROC curve, NPV: Negative predictive values, PPV: Positive predictive value.
*** Statistically significant results are highlighted underlined.**

patients; the AUC of lymphocyte and neutrophil counts, CRP, IL-6, and fibrinogen were 0.790 [95% confidence interval (95% CI) = 0.694 - 0.885], 0.140 [95% CI = 0 - 0.284], 0.252 [95% CI = 0.057 - 0.447], 0.269 [95% CI = 0.087 - 0.451], and 0.142 [95% CI = 0.007 - 0.276] (Table 4) (Figure 4a, 4b). It was shown that the lymphocyte was independently more closely predicting the oxygen requirement of COVID-19 patients. The sensitivity and specificity of lymphocytes in predicting oxygen requirement respectively were 78.43% and 67.65% with the cutoff lower than 1 (x 109/L), and the positive predictive value and negative predictive value were 78.43% and 67.65%.

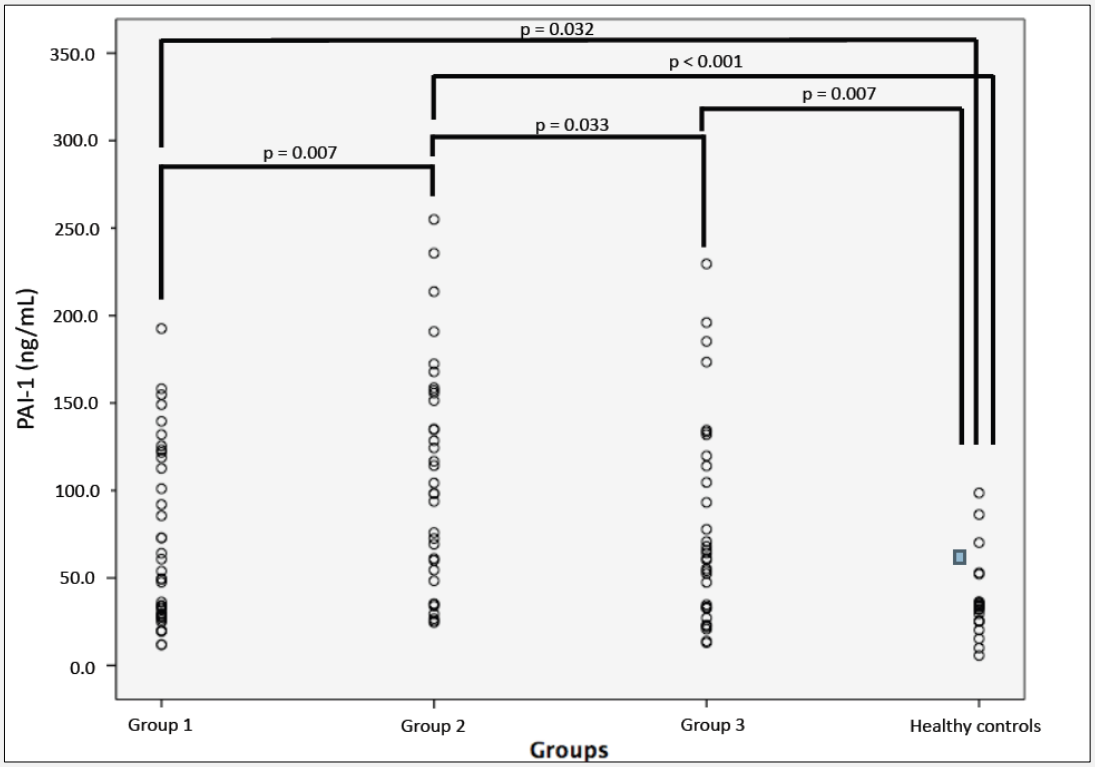


Figure 1. Serum PAI-1 levels of patient groups and healthy controls.

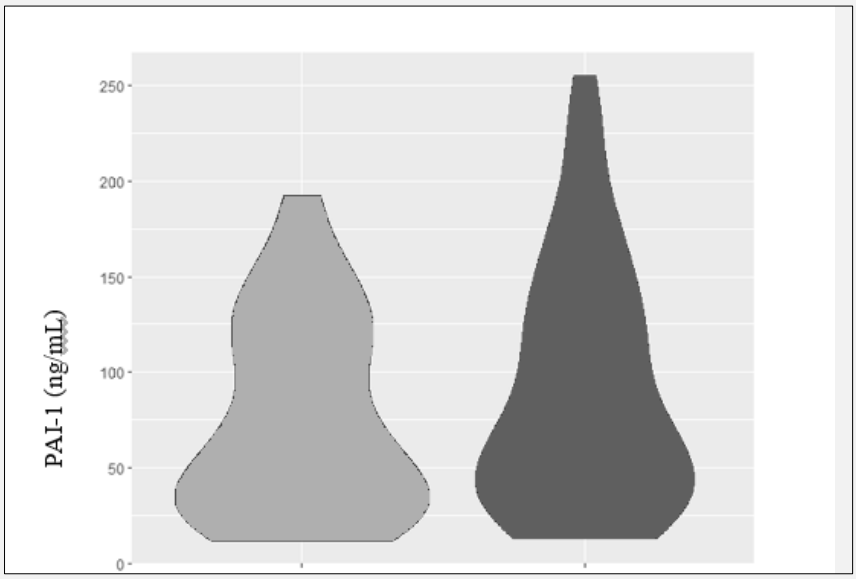


Figure 2. PAI-1 levels according to respiratory status.

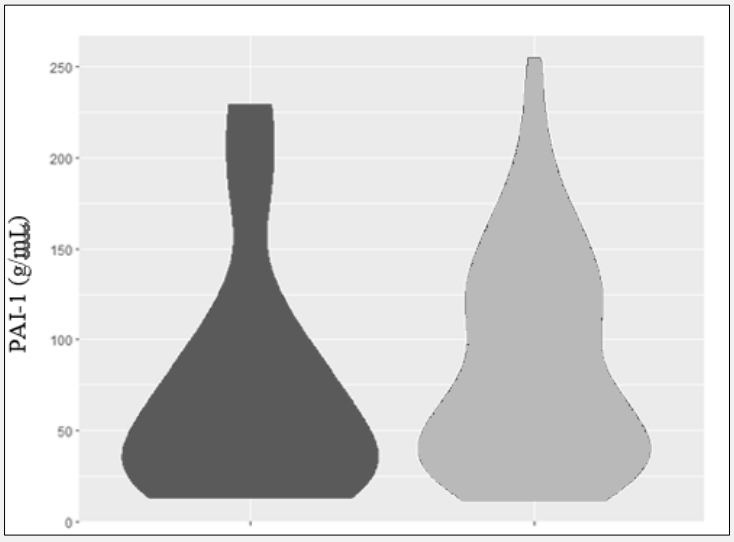


Figure 3. PAI-1 levels according to final outcomes.

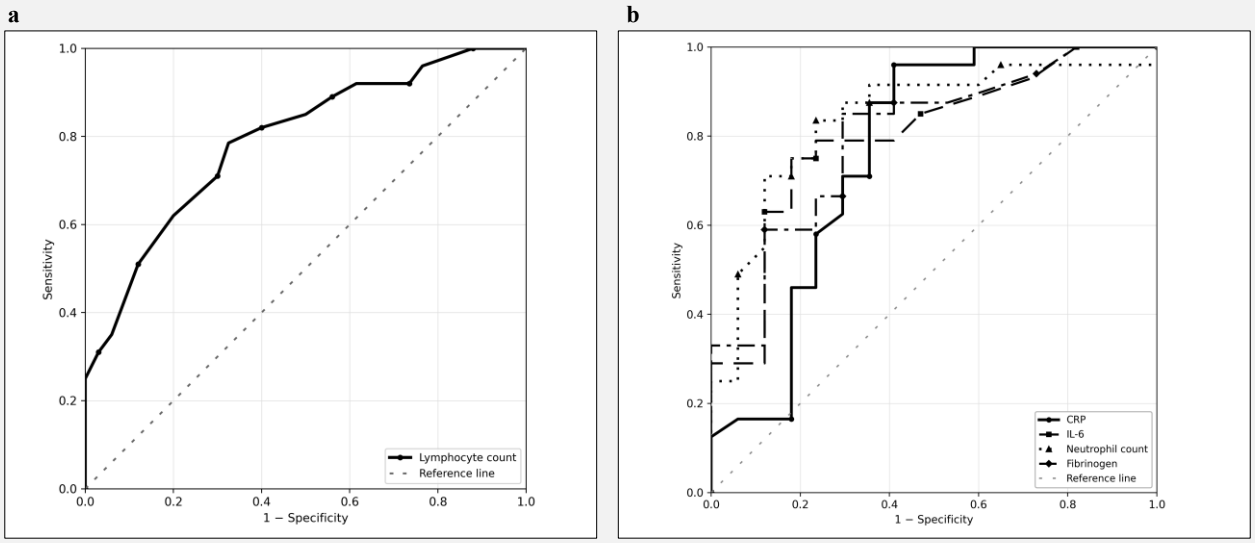


Figure 4. a: ROC curve of lymphocyte count in COVID-19 patients for predicting oxygen requirements. b: ROC curves of CRP, IL-6, neutrophil count and fibrinogen in COVID-19 patients for predicting oxygen requirements.

The effect of laboratory parameters on the hospital length of stay (LoS)

To identify the laboratory parameters that influence the length of hospitalization of our patients, a linear regression analysis of all the laboratory parameters already presented in the descriptive analysis was performed (Table 5).

As the severity of the disease increased, the length of hospital stay increased ($p = 0.02$). As the lymphocyte count increases, the length of stay decreases by 0.548

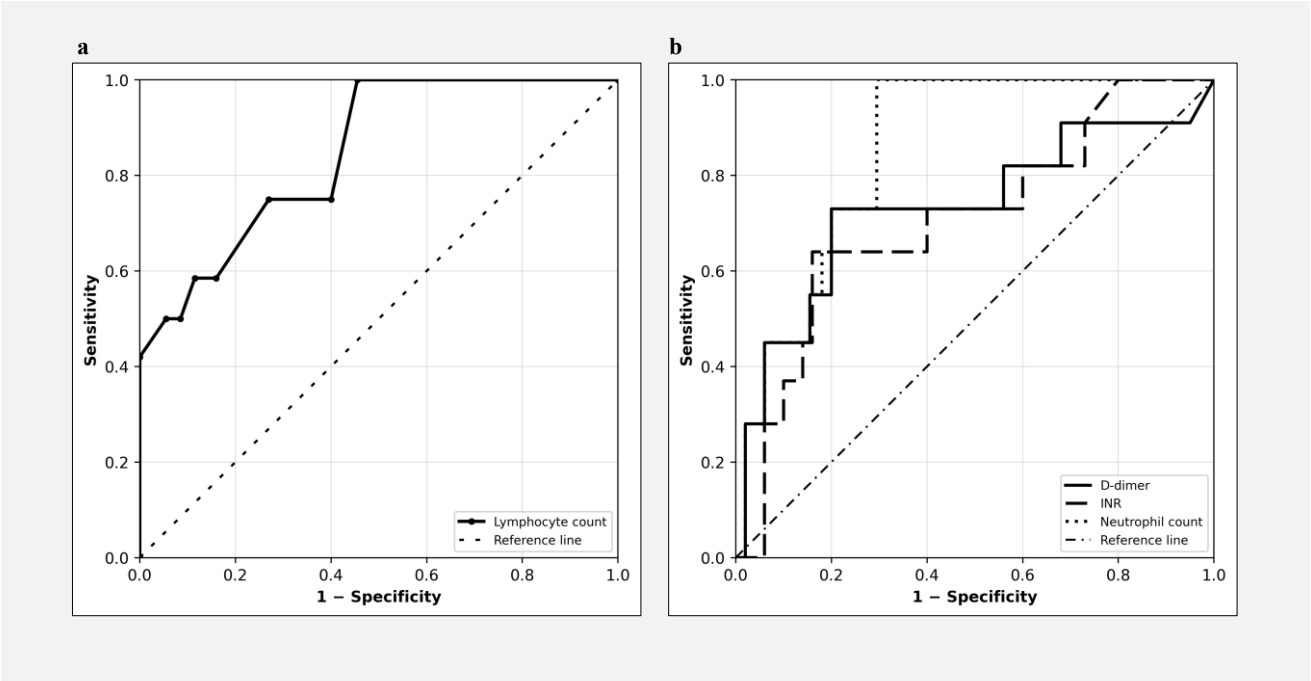


Figure 5. a: ROC curve of lymphocyte count in COVID-19 patients for predicting mortality. b: ROC curves of D-dimer, INR, and neutrophil count in COVID-19 patients for predicting mortality.

days (p = 0.012). The length of stay rises by 0.498 days (p = 0.012) as the number of neutrophils rises. There were no statistically significant other factors (p > 0.050).

Mortality

Subsequent study utilizing logistic regression identified lymphocyte and neutrophil counts, D-dimer, and INR as independent risk factors for mortality in patients (Table 6). The ROC curve was utilized to evaluate the prognostic significance of inflammatory and coagulation parameters in COVID-19 patients. AUC for lymphocyte and neutrophil counts, INR, and D-dimer were 0.847 [95% confidence interval (95% CI) = 0.737 - 0.958], 0.843 [95% CI = 0.707 - 0.980], 0.827 [95% CI = 0.627 - 1], and 0.758 [95% CI = 0.485 - 1], respectively (Figure 5a, 5b).

It was shown that the lymphocyte count was independently best at predicting the mortality risk of Covid-19. The sensitivity and specificity of lymphocyte count in predicting death respectively were 100% and 54.29%, with the cutoff lower than 0.9 (x 109/L), and the positive predictive value and negative predictive value were 27.27% and 100%.

DISCUSSION

Our findings demonstrated that circulating PAI-1 concentrations were markedly elevated in Covid-19 patients without comorbid diseases, relative to the healthy control group. In particular, the levels were even higher than those observed in trauma patients with exaggerated fibrinolytic activity and in patients with hantavirus cardiopulmonary syndrome who are at high risk of alveolar hemorrhage [10]. Unexpectedly, PAI-1 values showed no clear relationship with disease severity and were not useful for predicting hospital LoS, oxygen demand, or survival outcomes. Consistent with previous studies performed in patients with various levels of Covid-19 illness [11-13], we found that inflammatory and coagulation parameters including lymphocyte and neutrophil count, CRP, IL-6, D-Dimer, INR, and fibrinogen, previously reported to have prognostic significance in Covid-19 patients, can be used to predict hospital LoS, oxygen requirements, and mortality in Caucasian people. Both inflammatory and coagulation parameters were statistically different between disease severity groups, but none of the parameters differentiated between mild-moderate-severe Covid-19 disease. This statistical difference between the groups is due to the difference between the paired groups.

Covid-19 has been recognized as a systemic illness capable of triggering endothelial dysfunction, activating coagulation pathways, and promoting intravascular fibrin formation. Increasing evidence in Covid-19 indicates that a frequent pathogenic factor is endotheliopathy, corroborated by recent findings of elevated plasma biomarkers of vascular damage [14]. Consequently, we detected a significant elevation in plasma levels of PAI-1 across all three patient groups, with the most elevated values seen in those with moderate illness. Cugno et al.

similarly found elevated levels of PAI-1 and tissue-type plasminogen activator (t-PA) in all patients, irrespective of illness severity; these are recognized indicators of endothelial damage in cardiovascular diseases and other vasculopathies [15]. During cytokine release syndromes (CRS), including bacteria, viral, and in severe Covid-19, IL-6 signaling contributes to a self-sustaining inflammatory loop that stimulates endothelial cells to produce PAI-1 [16]. In our study, which included not only severe but also mild and moderate Covid-19 patients, PAI-1 level was only correlated with IL-6. It supports the fact that the release of IL-6 and PAI-1 is at every stage of the Covid-19 disease.

The fibrinolytic homeostasis in Covid-19 is probably intricate and affected by several variables. Normal lung physiology exhibits a pro-fibrinolytic inclination [17]. During ARDS, compromised fibrinolysis leads to fibrin buildup, which facilitates hyaline membrane development and alveolar damage [18]. Plasmin eliminates fibrin. Depressed fibrinolysis in ARDS is thought to be largely influenced by elevated circulating PAI-1, which adversely affects the plasminogen activation system [17]. In the 2002 SARS-CoV outbreak, elevated PAI-1 levels and a corresponding hypo-fibrinolytic condition were noted, but subsequent analyses of Covid-19 patients have indicated compromised global fibrinolysis [19]. Interestingly, although PAI-1 levels were found to be increased in all study groups in our study, we observed that increased PAI-1 levels were similar in mild and severe disease groups. This may be due to the fact that all patients in the severe disease group were administered antibiotics, which are the only independent factors affecting the PAI-1 level.

A prior investigation involving 118 hospitalized patients reported that increases in PAI-1 and D-dimer levels were strongly associated with reduced oxygenation capacity [5]. Contrarily, in our study, lymphocyte and neutrophil counts, CRP, IL-6 and fibrinogen values, which are inflammatory markers, were found to be statistically significant in predicting Covid-19 patients in need of oxygen. The lymphocyte count was even the laboratory parameter that most closely predicted the oxygen need of Covid-19 patients. The lymphocyte AUC value in predicting those in need of oxygen was found to be statistically significant at 0.790. The sensitivity was 78.43% and the specificity was 67.65% when the cutoff value was set to ≤ 1.

In severe Covid-19 patients, coagulation activation might result in thrombus development and even DIC [20]. Patients exhibit thrombocytopenia, higher D-Dimer levels, and extended activated partial thromboplastin time (APTT), indicating that mortality in Covid-19 patients may be linked to DIC [21,22]. The thrombin-antithrombin complex (TAT), α2-plasmin inhibitor-plasmin complex (PIC), thrombomodulin (TM), and t-PA/PAI-1 complex (t-PAIC) serve as markers for the early prediction and monitoring of DIC, whereas D-Dimer indicates the presence or continuation of a thrombus. In the final results, the laboratory parameters that were statistically significant in predicting death were lymphocyte and neutrophil counts, and INR and D-dimer levels (AUC values were 0.847, 0.843, 0.827, and 0.758, respectively) in this study, which included mild - moderate and severe patients. Out of these, lymphocyte and neutrophil counts were most closely at independently predicting the risk of death from Covid-19. The sensitivity for the lymphocyte count (cutoff value 0.9) was 100% and the specificity was 54.29%, and the sensitivity for the neutrophil count (cutoff value 6) was 85.71% and specificity was 74.71%. A recent research including severe and critical patients has shown that the assessment of t-PAIC and D-Dimer can independently predict the risk of mortality with Covid-19, achieving AUC values of 0.92 and 0.94, respectively [23]. The sensitivity and specificity of t-PAIC in predicting mortality were 90.0% and 91.2%, respectively, with a threshold exceeding 20.6 ng/mL. The D-Dimer results were 100.0% and 86.0%, with a cutoff value exceeding 2.78 mg/L.

The discharge criterion in our study was two negative PCR specimens at least 24 hours before discharge, which is recommended by the guidelines [24,25]. However, differences between settings may arise as a result of local capacity and strain on the health system. In our study, while the median hospital LoS was 10, a systematic review of hospital stay in patients infected with Covid-19 reported higher (mean hospital LoS for China 14 (interquartile range (IQR) 10 - 19) days) [26]. In this study, as the severity of the disease increased, hospital LoS increased statistically. Two important laboratory parameters affecting hospital LoS in our study were neutrophil and lymphocyte counts. As the lymphocyte count increased, the hospital LoS decreased by 0.548 days, whereas as the neutrophil count increased, the hospital LoS increased by 0.498 days. A recent cohort study utilizing univariate analysis revealed that hospitalization length of stay is influenced by specific laboratory parameters: an admission CRP value exceeding 300 mg/L, a lymphocyte count below 1,000/mL at admission, and partial arterial oxygen pressure (PaO_2) levels under 60 mmHg, along with SpO_2 values of less than 90% in arterial gasometry at admission [27].

CONCLUSION

The strength of our study is that it was conducted in Covid-19 patients without comorbid diseases that could cause endothelial damage and coagulation activation. Our results may directly reflect the effect of endothelial damage, inflammatory, and coagulation processes, which are assumed to play a role in the pathogenesis of Covid-19 disease, on blood PAI-1 level, inflammatory, and coagulation parameters.

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Ethical Approval Statement:
The trial protocol adhered to the Declaration of Helsinki, and this study protocol was reviewed and approved by the Ethics Committee of Gülhane Education and Research Hospital (project/decision no. 2021/10). This clinical trial was registered before patient enrollment.

Clinical Trial Registration:
Clinical trial number was obtained, NCT04997928.

Patient Consent Statement:
All patients provide informed consent when applying to our university hospital, permitting the use of their information for research purposes. Written informed consent was obtained from the participants themselves and next of kin to participate in the study before study inclusion.

Permission to Reproduce Material from Other Sources:
No material from other sources was used in this study.

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Data Availability Statement:
Data will be shared upon reasonable request to the corresponding author.

Declaration of Generative AI in Scientific Writing:
This study did not utilize generative artificial intelligence (ChatGPT, OpenAI) support for language editing, formatting, or improvement of the readability of the article.

Declaration of Interest:
All authors have no conflicts of interest to declare.

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