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Association of Prothrombin G20210A (Factor II) and PAI-1 4G/5G polymorphisms with COVID-19 infection in emergency department patients

Acil servis hastalarında COVID-19 enfeksiyonu ile Protrombin G20210A (Faktör II) ve PAI-1 4G/5G polimorfizmlerinin ilişkisi

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Abstract

Purpose: This study aimed to investigate whether Prothrombin G20210A (Factor II) and PAI-1 4G/5G gene polymorphisms are associated with COVID-19 severity and clinical outcomes among patients admitted to the Emergency Department of Pamukkale University Hospital (Denizli, Türkiye) between June and December 2021.

Materials and methods: In this prospective, cross-sectional, observational study, 150 PCR-confirmed COVID-19 patients and 300 healthy volunteers were genotyped for Prothrombin G20210A and PAI-1 4G/5G polymorphisms by PCR and DNA sequencing. Laboratory parameters, disease severity indices, intensive care unit (ICU) admission, and mortality were analyzed.

Results: The genotype distribution among patients was GA 54.7% and GG 45.3% for Prothrombin G20210A, and 4G/4G 77.3% and 4G/5G 22.7% for PAI-1. There were no significant differences in Prothrombin G20210A variants except for lower lymphocyte counts in GA carriers (p=0.031). The PAI-1 4G/5G group showed a significantly higher monocyte count (p=0.012), while differences in urea and other biochemical parameters were not statistically significant (p>0.05). Patients carrying both PAI-1 and Prothrombin heterozygous variants (double heterozygotes, n=13) had lower hemoglobin (p=0.010) and lymphocyte counts (p=0.012), but higher monocyte (p=0.001) and ferritin levels (p=0.006). This subgroup also demonstrated lower oxygen saturation (p=0.049) and significantly higher intensive care unit admission (46.2% vs. 15.3%, p=0.005) and mortality (38.5% vs. 9.5%, p=0.002).

Conclusion: Prothrombin G20210A and PAI-1 4G/5G polymorphisms particularly their coexistence were associated with hematologic and inflammatory alterations, as well as increased ICU admission and mortality rates. These variants may have potential value for risk stratification and should be evaluated in larger studies.

Keywords: COVID-19, Prothrombin G20210A, PAI-1, gene polymorphism, thrombosis, risk factors.

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Öz

Amaç: Bu çalışma, Protrombin G20210A (Faktör II) ve PAI-1 4G/5G gen polimorfizmlerinin COVID-19 hastalığının şiddeti ve klinik sonuçları ile ilişkili olup olmadığını, Haziran-Aralık 2021 tarihleri arasında Pamukkale Üniversitesi Hastanesi Acil Servisine başvuran hastalarda araştırmayı amaçlamaktadır.

Gereç ve yöntem: Prospektif, kesitsel ve gözlemsel nitelikteki bu çalışmada, PCR ile doğrulanmış 150 COVID-19 hastası ve 300 sağlıklı gönüllüde Protrombin G20210A ve PAI-1 4G/5G polimorfizmleri PCR ve DNA dizileme yöntemiyle genotiplendi. Laboratuvar parametreleri, hastalık şiddeti indeksleri, yoğun bakım ünitesine (YBÜ) yatış ve mortalite oranları değerlendirildi.

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Bulgular: Hastalarda Protrombin G20210A genotip dağılımı GA %54,7 ve GG %45,3; PAI-1 genotip dağılımı ise 4G/4G %77,3 ve 4G/5G %22,7 olarak saptandı. Protrombin G20210A varyantları arasında anlamlı fark bulunmadı; yalnızca GA grubunda lenfosit sayısı daha düşüktü (p=0,031). PAI-1 4G/5G grubunda monosit sayısı anlamlı olarak daha yüksek bulundu (p=0,012), ancak üre ve diğer biyokimyasal parametrelerde fark yoktu (p>0,05). Her iki genin heterozigot varyantlarını taşıyan hastalarda (çifte heterozigot, n=13) hemoglobin (p=0,010) ve lenfosit (p=0,012) düzeyleri düşük, monosit (p=0,001) ve ferritin (p=0,006) düzeyleri ise yüksek bulundu. Bu alt grupta ayrıca oksijen satürasyonu daha düşük (p=0,049), YBÜ yatışı (%46,2'ye karşı %15,3; p=0,005) ve mortalite oranı (%38,5'e karşı %9,5; p=0,002) anlamlı olarak daha yüksekti.
Sonuç: Protrombin G20210A ve PAI-1 4G/5G polimorfizmleri, özellikle birlikte bulunduklarında, hematolojik ve inflamatuvar değişiklikler ile artmış YBÜ yatışı ve mortalite oranlarıyla ilişkilidir. Bu varyantlar risk sınıflandırması açısından potansiyel değer taşıyabilir ve daha büyük örneklemli çalışmalarda değerlendirilmelidir.

Anahtar kelimeler: COVID-19, Protrombin G20210A, PAI-1, gen polimorfizmi, tromboz, risk faktörleri.

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Introduction

Hyperinflammatory response and systemic coagulation disorder frequently accompany the clinic of COVID-19, and this condition can turn into disseminated intravascular coagulopathy [1]. Local vascular damage caused by COVID-19, along with systemic activation of blood coagulation and pulmonary thrombo-inflammation, may increase the risk of venous thromboembolism and pulmonary artery thrombosis [2, 3]. Coagulopathy in COVID-19 patients has been associated with an increased risk of death. Unrecognized venous and arterial thromboembolic events develop in a significant proportion of patients with severe COVID-19 infection, which increases the importance of coagulation abnormalities. Examples of these complications include thrombocytopenia, increased D-dimer concentration, decreased fibrinogen, and prolonged prothrombin time [4].

In an autopsy study of patients who died due to COVID-19, deep vein thrombosis was found as the cause of death in 58% of the patients, and pulmonary embolism in 30% [5]. Other studies have confirmed that most COVID-19 deaths are associated with pulmonary embolism, despite the use of prophylactic anticoagulants [4-6]. Lodigiani et al. [7] described the complications and rates of acute coronary syndrome, deep vein thrombosis, myocardial infarction, pulmonary embolism, venous thromboembolism, proximal deep vein thrombosis, and distal deep vein thrombosis in symptomatic COVID-19 patients who were admitted to their hospitals in Milan, which belonged to the Red Zone and required hospitalization. Studies have shown that the rate of venous and arterial thromboembolic complications in hospitalized COVID-19 patients is approximately 8%, despite anticoagulant prophylaxis [8-10].

Considering the outcomes among COVID-19 populations in different countries, it is thought that there may be a genetic predisposition for thromboembolic complications triggered by COVID-19 pneumonia in patients carrying mutations of interacting factors. Accordingly, polymorphisms in genes associated with the coagulation process may induce a high risk of coagulopathy. Therefore, the incidence of COVID-19-related thromboembolism becomes significant in unaware population clusters carrying an undetected specific mutation [10]. For example, a study conducted to evaluate the effect of a hereditary prothrombin mutation, namely plasminogen activator inhibitor-1- 4G/5G, in COVID-19 infections revealed an association between PAI-1-4G/5G gene polymorphism and post-severe acute respiratory syndrome osteonecrosis mediated by thrombosis mechanisms caused by intravascular coagulation and leading to fibrin thrombus in the osteonecrosis area [11].

The polymorphism of the Prothrombin G20210A is a remarkable genetic variant located within the prothrombin gene (F2), leading to a single nucleotide alteration from Guanina (G) to Adenine (A) in position 20210. This mutation has attracted attention due to its association with an increased risk of thrombosis, particularly the venous thrombus.

The underlying mechanism is believed to imply an improvement in prothrombin levels in the bloodstream, which elevates the coagulation potential and therefore prepares individuals to thrombotic events. Studies suggest that this polymorphism can cause an increase of 30-50% of the prothrombin concentrations, contributing to a hypercoagulable state [12, 13]. The prevalence of the G20210A mutation varies between the different populations. For example, it has been reported to be particularly common in European populations, with load-bearing frequencies ranging from 1% to 5% [12]. On the contrary, it is relatively rare in Asian and African populations, in which frequencies can be lower than 0.2%. This geographical variation is probably influenced by a combination of genetic drift, selective pressures, and historical migration models. Elkattawy et al. (2022) [12] led an in-depth analysis of the genetic implications of the G20210A mutation, noting that individuals who transport this polymorphism have a significantly higher risk of developing thromboembolic disorders. In particular, the study highlighted that those who are compound heterozygous—carrying the G20210A mutation together with other thrombophilic factors such as the V Leiden—have an even greater predisposition to thrombosis. Further tests from Badulescu et al. (2022) [13] further strengthen this relationship, showing that patients with Covid-19 who host the G20210A variant present a greater incidence of thrombotic complications, underlining the importance of this genetic variant in the context of coagulation disorders related to Covid-19.

Therefore, while the G20210A variant is a critical factor in the understanding of thrombotic disorders, its implications in clinical contexts, in particular during pandemics that require a faded management of coagulopathy, cannot be overrated [12, 13].

The Plasminogen-1 (PAI-1) activator inhibitor, located on chromosome 7, exhibits remarkable polymorphism characterized by the presence of a 4G or 5G allele within its promoter region. This genetic variation has significant implications for the regulation of PAI-1 expression and, consequently, influences the fibrinolytic system. The 4G allele is associated with greater transcriptional activity, resulting in a greater production of PAI-1. In contrast, the 5G allele, which is quieter transcriptionally, leads to lower levels of PAI-1. In the context of COVID-19, an understanding of PAI-1 4G/5G polymorphism is essential, particularly because patients have altered coagulation profiles. The high levels of PAI-1 have been correlated with a hypercoagulable state, which leads to a higher risk of thromboembolic events that are frequently observed in severe cases of COVID-19. Studies have indicated that people who transport the 4G allele may be predisposed to a greater expression of PAI-1, resulting in deteriorated fibrinolysis and contributing to the abnormalities of coagulation observed in these patients [14].

Genetic predispositions, dictated by 4G/5G polymorphism, can elucidate why some individuals experience thrombosis and respiratory complications more serious than others after SARS-COV-2 infection [14].

Recent epidemiological studies strengthen the association between the 4G allele and the severe COVID-19 results. For example, a cohort analysis revealed that patients with the 4G/4G genotype exhibited significantly higher incidents of venous thromboembolism compared to those with the 5G/5G genotype. These data suggest that genetic detection for PAI-1 polymorphism can provide critical information on the management of patients with COVID-19, which allows the stratification of individuals based on their thrombotic risk [15]. This detection could guide therapeutic interventions, including the use of anticoagulants, in patients identified with the high-risk genotype. Based on these literature data, we aimed to investigate the relationship between these polymorphisms and clinical severity, based on the idea that the severity of COVID-19 disease may be related to the prothrombin G20210A and PAI-1-4G/5G gene polymorphisms, and that this relationship may provide insights into predicting the clinical outcomes.

Materials and methods

The study population consisted of patients with a confirmed COVID-19 diagnosis polymerase chain reaction (PCR) who presented with respiratory symptoms. The study sample comprised a total of 450 participants, including 150 PCR-positive patients and 300 healthy volunteers. Any participants whose conditions

could have an impact on their coagulation or inflammatory processes were excluded from the study. Among these excluded cases are those with known coagulopathies (for example, hemophilia, thrombophilia, or disseminated intravascular coagulation), chronic liver or kidney disease, autoimmune disorders, malignancy, infection other than COVID-19, pregnancy, or if they are currently on anticoagulant, antiplatelet, or corticosteroid therapy. 300 healthy volunteers with no past history of COVID-19 infection, chronic disease, or medication use during the last six months were chosen as a control group. These people were gathered for determining the normal genotype frequencies of Prothrombin G20210A and PAI-1 4G/5G polymorphisms in the general population. Control data were only used for genetic comparison with the group of COVID-19 patients in order to find out if the distribution of the polymorphisms under study varied significantly between patients and healthy individuals. Permission was obtained from the Pamukkale University Ethics Committee for the study (permission date: 22.06.2021, file/ permission number: E-60116787-020-67183).

The case report form evaluated the time of symptom onset, systolic and diastolic blood pressure values, vital signs, medications used for COVID-19 treatment, high-sensitivity troponin value, CK-MB value, hemogram parameters, blood gas parameters, liver enzyme levels, kidney function parameters, D-dimer level, ferritin level, pneumonia severity index, and comorbid diseases. The extent of the lung infection on computed tomography (CT) was categorized as light, middle, or heavy depending on the percentage of the lung parenchyma that was affected, in accordance with the radiological criteria that have been published earlier [16].

For the study, an average of 2 ml of blood was collected from volunteer participants into vacuum tubes containing the anticoagulant K3EDTA. Genomic DNA was then isolated using the standard phenol-chloroform extraction method. The genomic DNA obtained was subjected to PCR to amplify the gene-specific regions of interest. Polymorphic loci within these amplified regions were visualized on a high-resolution agarose gel. Finally, genotyping was performed based on DNA sequence analysis. Genomic DNA samples were subjected to PCR to amplify gene-specific regions. The polymorphic loci within these regions were visualized on high-resolution agarose gel, and genotyping was performed based on DNA sequence analysis.

For the PAI-1 4G/5G polymorphism, the following primers were used:

- **Forward primer:** 5’-AAGCTTTTACCA TGGTAACCCCTGGT-3’
- **Reverse primer:** 5’-TGCAGCCAGCCACGTGATTGTCTAG-3’

For the **Prothrombin G20210A (Factor II) polymorphism**, the primers were as follows:

- **Forward primer:** 5’-TCTAGAAACAGTTGCCTGGC-3’
- **Reverse primer:** 5’-ATAGCACTGGGAGCATTGAAGC-3’

Statistical analysis

The Shapiro-Wilk normality test was first used to assess the distribution of continuous variables. Descriptive characteristics of the data were presented as number (n) and percentages (%) for categorical variables and as mean, standard deviation, median, minimum, and maximum values for numerical variables. For comparisons between two independent groups, the Independent Samples t-test was applied to normally distributed variables, whereas the Mann-Whitney U test was used for non-normally distributed variables. The Pearson’s Chi-square test was used to evaluate the association between categorical variables. A *p*-value <0.05 was considered statistically significant in all analyses. All statistical analyses were performed using Stata 16.1 software (StataCorp LLC, College Station, TX, USA).

Results

The study included 150 PCR-confirmed COVID-19 patients, with a mean age of 53.40±20.66 years, of whom 87 (58%) were male. The mean systolic and diastolic blood pressures were 126.15±22.28 mmHg and 75.65±12.58 mmHg, respectively, and 83

patients (55.3%) had at least one comorbid condition. Regarding genotype distribution, 82 patients (54.7%) carried the GA genotype and 68 (45.3%) the GG genotype for *Prothrombin G20210A*, whereas 116 patients (77.3%) had the 4G/4G genotype and 34 (22.7%) the 4G/5G for *PAI-1*. Clinical characteristics according to combined heterozygous status are summarized in Table 4. The mean body temperature was 36,8°C, mean oxygen saturation (SpO_2) 94.23%, median CURB-65 score 1 (0-5), and median PSI score 64 (8-217). The mean hospital stay among survivors was 5.51 days, with 80% of patients showing pneumonia on CT imaging. A total of 108 patients (72%) were hospitalized, including 27 (18%) in intensive care, and 18 patients (12%) died during hospitalization. As shown in Table 1, no significant differences were observed between *Prothrombin G20210A* GA and GG carriers in comorbidities or laboratory parameters (all $p>0.05$), except for lymphocyte count, which was significantly lower in GA carriers ($p=0.031$).

Table 1. Comparison of patients' laboratory data with Prothrombin G20210A mutation

	Prothrombin G20210A GA (n=82)		Prothrombin G20210A GG (n=68)		*p* (test statistic)
	Mean±SD	Median (min-max)	Mean±SD	Median (min-max)	
WBC (×10³/μL)	10.32±16.18	7.44 (0.73-147.98)	10.28±8.14	8.15 (2.38-61.30)	0.223 (z=-1.219)
Hemoglobin (g/dL)	13.17±2.37	13.35 (5.4-17.5)	13.18±2.29	13.20 (5.5-17.4)	0.929 (z=0.089)
Neutrophil count (×10³/μL)	6.46±4.67	5.22 (0.51-28.88)	7.57±7.38	5.11 (0.96-52.57)	0.595 (z=-0.532)
Lymphocyte count (×10³/μL)	1.55±0.84	1.36 (0.15-3.78)	1.91±1.06	1.74 (0.14-5.50)	0.031* (z=-2.155)
Platelet count (×10³/μL)	237.38±93.01	232.5 (32-472)	234.26±66.74	232.5 (94-370)	0.818 (t=0.231)
Monocyte count (×10³/μL)	2.68±3.27	0.76 (0.04-12.10)	2.47±3.35	0.81 (0.10-16.00)	0.696 (z=0.391)
CRP (mg/L)	51.47±61.49	24.47 (0-280.77)	66.88±94.01	20.02 (0-350.89)	0.983 (z=0.021)
Urea (mg/dL)	38.23±27.28	29 (11-149)	41.04±38.00	30 (11-276)	0.949 (z=-0.064)
Creatinine (mg/dL)	1.07±1.06	0.88 (0.40-9.80)	0.95±0.48	0.83 (0.22-3.29)	0.522 (z=0.640)
D-dimer (μg/mL)	435.26±577.62	230 (30-2914)	490.60±675.64	192.5 (12-3293)	0.830 (z=0.215)
Ferritin (ng/mL)	382.05±519.00	139.5 (8.44-2000)	354.41±695.65	136.5 (3-4866)	0.426 (z=0.797)
Troponin (ng/L)	51.83±285.18	7.5 (3-2547)	16.13±23.10	5.17 (3-126.7)	0.274 (z=1.093)
Fibrinogen (mg/dL)	367.33±172.73	312 (85-872)	386.56±184.21	327.5 (78-942)	0.409 (z=-0.825)
INR (ratio)	1.24±0.98	1.09 (0.82-9.40)	1.15±0.29	1.08 (0.88-2.49)	0.907 (z=-0.117)

t:Independent samples t-test, z:Mann-Whitney U test, *$p<0.05$ was considered statistically significant

For *PAI-1* genotypes (Table 2), patients with the 4G/5G variant exhibited a significantly higher monocyte count (p=0.013), while other biochemical parameters, including urea, CRP, ferritin, and fibrinogen, did not differ significantly (p>0.05).

Table 2. Comparison between patients' laboratory parameters and PAI-1 gene mutation

	PAI-1 4G/5G (n=34)		PAI-1 4G/4G (n=116)		*p* (test statistic)
	Mean ± SD	Median (min-max)	Mean ± SD	Median (min-max)	
WBC (×10³/μL)	12.62±24.59	6.72 (0.73-147.98)	9.63±6.89	7.93 (2.67-61.30)	0.326 (z=-0.983)
Hemoglobin (g/dL)	12.70±2.22	12.95 (7.5-16.4)	13.32±2.35	13.40 (5.4-17.5)	0.174 (z=-1.358)
Neutrophil count (×10³/μL)	6.65±5.43	4.47 (0.51-25.77)	7.06±6.24	5.21 (1.48-52.57)	0.463 (z=-0.734)
Lymphocyte count (×10³/μL)	1.45±0.83	1.49 (0.15-3.26)	1.79±0.99	1.50 (0.14-5.50)	0.114 (z=-1.582)
Platelet count (×10³/μL)	217.94±88.61	211 (32-452)	241.25±79.46	235.5 (51-472)	0.145 (t=-1.465)
Monocyte count (×10³/μL)	4.11±3.76	2.90 (0.15-11.00)	2.15±3.04	0.72 (0.04-16.00)	0.013* (z=2.496)
CRP (mg/L)	56.62±75.09	32.64 (0.27-350.07)	58.99±79.18	20.02 (0-350.89)	0.423 (z=0.801)
Urea (mg/dL)	49.21±49.21	30.5 (11-276)	36.66±25.24	29 (11-149)	0.256 (z=1.136)
Creatinine (mg/dL)	1.07±0.59	0.90 (0.48-3.29)	1.01±0.91	0.85 (0.22-9.80)	0.308 (z=1.019)
D-dimer (μg/mL)	642.82±851.94	286.5 (50-3293)	406.62±529.60	214 (12-2914)	0.191 (z=1.307)
Ferritin (ng/mL)	412.43±543.53	204.5 (15.2-2000)	356.94±621.64	117 (3-4866)	0.151 (z=1.436)
Troponin (ng/L)	31.08±87.93	10.04 (3-520)	36.99±236.18	6 (3-2547)	0.345 (z=0.945)
Fibrinogen (mg/dL)	390.85±183.39	372.5 (78-735)	371.71±176.55	318.5 (85-942)	0.436 (z=0.779)
INR (ratio)	1.15±0.31	1.07 (0.83-2.49)	1.21±0.83	1.09 (0.82-9.40)	0.868 (z=-0.166)

t:Independent samples t-test, z:Mann-Whitney U test, *p<0.05 was considered statistically significant

When both *Prothrombin* and *PAI-1* polymorphisms coexisted (double heterozygotes, n=13), significant alterations in hematologic and inflammatory markers were noted (Table 3). These patients had lower hemoglobin (p=0.010) and lymphocyte counts (p=0.012) but higher monocyte (p=0.001) and ferritin levels (p=0.006).

Table 3. Comparison of patients' laboratory findings with PAI-1 and G20210A polymorphisms

	(PAI-1)- (G20210A) Heterozygous (-) (n=137)		(PAI-1)- (G20210A) Heterozygous (+) (n=13)		p (test statistic)
	Mean ± SD	Median (min-max)	Mean ± SD	Median (min-max)	
WBC (×10³/μL)	9.59±6.79	7.90 (2.38-61.30)	17.81±39.34	5.49 (0.73-147.98)	0.252 (z=1.146)
Hemoglobin (g/dL)	13.33±2.29	13.40 (5.4-17.5)	11.56±2.14	11.90 (7.5-15.0)	0.010* (z=2.566)
Neutrophil count (×10³/μL)	7.07±6.23	5.28 (0.96-52.57)	5.84±3.81	4.36 (0.51-12.54)	0.491 (z=0.688)
Lymphocyte count (×10³/μL)	1.77±0.96	1.57 (0.14-5.50)	1.08±0.83	0.84 (0.15-2.71)	0.012* (z=2.515)
Platelet count (×10³/μL)	238.61±76.96	234 (51-472)	208.08±123.49	212 (32-452)	0.200 (t=1.287)
Monocyte count (×10³/μL)	2.28±3.14	0.72 (0.04-16.00)	6.02±3.26	6.35 (0.54-10.50)	0.001* (z=-3.638)
CRP (mg/L)	58.93±80.60	19.83 (0-350.89)	53.45±44.09	49.66 (9.72-145)	0.193 (z=-1.303)
Urea (mg/dL)	39.04±32.77	29 (11-276)	44.38±30.17	38 (16-124)	0.365 (z=-0.906)
Creatinine (mg/dL)	1.02±0.88	0.86 (0.22-9.80)	0.99±0.40	0.88 (0.48-1.85)	0.726 (z=-0.351)
D-dimer (μg/mL)	448.10±615.19	200.5 (12-3293)	590.38±710.46	348 (65-2651)	0.089 (z=-1.699)
Ferritin (ng/mL)	342.88±596.66	125.6 (3-4866)	650.23±628.01	422 (38.7-2000)	0.006* (z=-2.725)
Troponin (ng/L)	33.69±217.43	6 (3-2547)	56.25±139.88	16.2 (3-520)	0.050 (z=-1.958)
Fibrinogen (mg/dL)	371.74±180.88	317 (78-942)	421.38±137.02	458 (185-631)	0.142 (z=-1.470)
INR (ratio)	1.20±0.78	1.08 (0.82-9.40)	1.15±0.29	1.14 (0.83-2.02)	0.952 (z=-0.060)

t:Independent samples t-test, z:Mann-Whitney U test, *p<0.05 was considered statistically significant

In terms of clinical outcomes (Table 4), the double heterozygous subgroup demonstrated lower oxygen saturation (p=0.049) and markedly higher intensive care unit (ICU) admission (46.2% vs. 15.3%, p=0.005) and mortality (38.5% vs. 9.5%, p=0.009) compared with non-carriers.

Table 4. Comparison between patients' clinical manifestations and PAI-1 and G20210A polymorphisms

Variable	(PAI-1)-(G20210A) Heterozygous (-) (n=137)	(PAI-1)-(G20210A) Heterozygous (+) (n=13)	*p* (test statistic)
Fever (°C)	36.82±0.60; 36.7 (36-39.7)	36.59±0.44; 36.6 (36-37.6)	0.242 (z=-1.169)
SpO_2 (%)	94.47±4.93; 96 (70-100)	91.69±7.10; 94 (71-98)	0.049* (z=1.959)
CURB-65 Score	1.16±1.02; 1 (0-5)	1.92±1.61; 2 (0-4)	0.093 (z=1.679)
PSI Score	73.85±45.53; 64 (8-217)	103.54±55.67; 101 (22-200)	0.064 (z=1.852)
Length of hospital stay (days)	5.44±5.62; 5 (0-32)	6.31±11.79; 1 (0-42)	0.350 (z=-0.935)
Imaging			
Normal	30 (21.9)	0 (0.0)	0.128 (χ^2=2.321)
Pneumonia	107 (78.1)	13 (100.0)	
CT Severity			
Normal	39 (28.5)	6 (46.2)	0.167 (χ^2=3.578)
Mild-Moderate	69 (50.4)	3 (23.1)	
Severe	29 (21.2)	4 (30.8)	
COVID-19 Symptom			
No	15 (10.9)	0 (0.0)	0.439 (χ^2=0.599)
Yes	122 (89.1)	13 (100.0)	
Symptom duration (days)			
Asymptomatic	9 (6.6)	0 (0.0)	0.606 (χ^2=1.002)
1-10 days	112 (81.8)	11 (84.6)	
≥10 days	16 (11.7)	2 (15.4)	
Hospitalization			
Discharged	37 (27.0)	5 (38.5)	0.578 (χ^2=0.309)
Hospitalized	100 (73.0)	8 (61.5)	
Hospital ward			
No	37 (27.0)	5 (38.5)	0.005* (χ^2=10.755)
Ward	79 (57.7)	2 (15.4)	
Intensive Care	21 (15.3)	6 (46.2)	
Outcome			
Dead	13 (9.5)	5 (38.5)	0.009* (χ^2=6.894)
Alive	124 (90.5)	8 (61.5)	

Values for continuous variables are presented as mean ± standard deviation; median (min-max)
Values for categorical variables are presented as n (%), z: Mann-Whitney U test, χ^2: Chi-square test
*p<0.05 was considered statistically significant

Discussion

Genetic polymorphisms (especially hemostatic genetic candidates) are believed to be one of the main factors responsible for individual susceptibility to thrombotic events and the severity of coagulopathy in COVID-19 patients. Polymorphisms such as prothrombin G20210A and PAI-1-4G/5G have been linked to changes in plasma levels of factors of coagulation and fibrinolytic activity, respectively. G20210A prothrombin is a mutation caused by single base substitution, which, most probably, will elevate prothrombin levels and thus will increase the risk of thrombotic episodes. Likewise, the polymorphism of the PAI-1 gene affects the level of the plasminogen activator-1 (PAI-1) that is the main regulator of the fibrinolytic system. Overproduction of PAI-1 may lead to a hypercoagulable state and contribute to thromboembolic complications in COVID-19 patients. In our cohort, the 4G/5G genotype was associated with higher monocyte counts but not with significant differences in urea or other biochemical markers, suggesting that the effect of this polymorphism may act primarily through inflammatory and fibrinolytic mechanisms rather than metabolic changes [17].

The knowledge concerning the function of these polymorphisms is primary in the context of COVID-19, as variations in the genotype can result in differential clinical expression of the disease. In patients with the G20210A prothrombin variant, the production of excessive thrombin can lead to them being elevated in the occurrence of thromboembolic events, thus associating with negative clinical outcomes. In contrast, those individuals who have PAI-1-4G/5G polymorphism may become fibrinolytic activity disturbed, which results in the prolongation of coagulase formation and a worsened resolution of thrombi, thereby increasing the risk of such complications as acute respiratory difficulty syndrome (ARDS) [17]. (In contrast, individuals carrying the PAI-1 4G/5G polymorphism may exhibit impaired fibrinolytic activity, which can prolong coagulation and hinder thrombus resolution, thereby increasing the risk of complications such as acute respiratory distress syndrome (ARDS)).

Identifying high-risk genotypes may provide important prognostic details that can be utilized in clinical settings to modify therapeutic strategies. For instance, those patients that present the above-mentioned polymorphisms might be the ones that profit the most from a closer follow-up and a more intensive anticoagulation plan that can lower the risk of thromboembolic events. Moreover, the comprehension of the genetic factors that are behind coagulation abnormalities can be a great help in the process of uncovering the pathophysiological mechanisms that lead to the severity and progression of COVID-19. This may pave the way for personalized medicine tailored to the patient's genetic profile.

The disclosure of high-risk genotypes can provide essential prognostic information that can quite easily be used in clinical settings to modify therapeutic treatment plans. As an instance, patients who express these variations can repeatedly enjoy the benefits of not only close supervision but also of the more frequent use of anticoagulation to reduce the risk of thromboembolic events. Moreover, knowing the genetic basis of abnormalities in the blood coagulation process may unravel the pathophysiological connections that explain the severity of the disease and are fueling its progression. Thus we would be paving the road for personalized medicine that tailors the treatment to one's genetic profile. Besides, by understanding the repercussions of these gene markers, the need to include genetic detection in regular evaluations for patients with COVID-19, especially those at high risk of severe disease manifestations, is emphasized. This would enable a timely identification of individuals with a predisposition to complications related to coagulation as the event unfolds, thus facilitating clinical decision-making and, ultimately, patient outcomes in a disease with great variability in clinical presentation and treatment response. Firstly, the study of genetic predispositions such as the Prothrombin G20210A and the PAI-1-4G/5G polymorphisms has the potential to help identify the abnormalities of coagulation that are consistent with clinical implications in COVID-19. The interaction between PAI-1, gene polymorphisms, and COVID-19 pathology is one of many examples showing how genetic factors and clinical symptoms in infectious diseases

depend closely on each other. To put it simply, understanding the detailed nature of the action of PAI-1 in relation to coagulation would be very helpful both in terms of clinical outcomes for COVID-19 patients and in terms of public health strategies for dealing with coagulation disorders after viral infections [14]. Thus, as such genetic comparisons become increasingly apparent, the idea of directed therapies for the modular control of PAI-1 activity, hence improving fibrinolytic responses and reducing thrombotic complications in vulnerable populations, moves even closer to becoming a reality.

The prothrombin polymorphisms G20210A and PAI-1-4G/5G interaction is a really fascinating case that can help to better understand the situation of thrombotic risk, especially that of COVID-19. Both genes apart are related to the hemostatic balance, and their combined influence may lead to the worsening of the coagulation abnormalities that are observed in the patients with COVID-19. The G20210A mutation in the prothrombin gene has been identified as one of the causes of increased prothrombin levels and, consequently, the risk of thrombotic events. In patients with the G20210A mutation, the prothrombin activation that is elevated can go on to induce a hypercoagulable state, and thus, venous thromboembolism is made easier, a condition that is turning out to be one of the severest complications of COVID-19 in the last two years [15].

On the contrary, PAI-1-4G/5G polymorphism controls the levels of the Plasminogen-1 (PAI-1) activator, a key regulator of fibrinolysis. The 4G allele is related to more significant PAI-1 levels, which may result in lowered fibrinolysis, thus making thrombosis more probable. Many studies have emphasized the association of high PAI-1 levels with the thromboembolic complications' nature of COVID-19. Pai-1 expression seems to be induced by inflammatory cytokines released during viral infections, which implies that carriers of the 4G allele may become additionally susceptible to thromboembolic events due to increased fibrin deposition [14]. The combined effect of these polymorphisms is believable due to their individual contributions to the thrombotic pathways. The hypothesis argues that those individuals who are of the G20210A and PAI-1-4G prothrombin genotypes might present an aggravated thrombotic phenotype that is the result of the conjunction of enhanced thrombin generation and impaired fibrinolytic capacity. Clinical studies have started investigating this possible interaction, revealing that COVID-19 patients with both genetic predispositions have considerably higher rates of thrombosis than patients with only one variation or those without such polymorphisms [15].

The purpose of this research was to investigate the link between Prothrombin G20210A and PAI-1 4G/5G polymorphisms and clinical outcome in patients with COVID-19. The results we got point to specific genetic changes that may affect the severity of the disease, various laboratory parameters, and clinical progression. Eighty percent of pneumonia among 150 patients was recognized by imaging, and the mortality rate was 12% overall. This data corresponds well with the previous studies about the different manifestations of severe COVID-19 and death of the hospital-admitted patients, which differ from 10-20% in different cohorts all over the world [16]. Regarding distribution of genes, we found that 54.67% of the Prothrombin G20210A samples were of the GA variant and 22.67% of patients had PAI-1 4G/5G. It was noted that the occurrence of both heterozygous variants (double mutation) had a close relationship with high monocyte (6.02 vs. 2.28; p=0.001) and ferritin levels (650.23 vs. 342.88; p=0.006), and also low hemoglobin (11.56 vs. 13.33; p=0.010) and lymphocyte counts (1.08 vs. 1.77; p=0.012). The data in parentheses indicate the levels of the parameters in the groups with the mutations and without the mutations, respectively, followed by the p-values. Here we see that the changes in blood parameters are indicative of the initial phase of cytokine storm as well as a possible coagulopathy. Similar to that, Zuo et al. [17] found that patients with prothrombotic mutations developed stronger thrombi and had elevated markers of hyperinflammation. Besides that, a study by Whyte et al. [18] puts the attention on PAI-1 action in fibrinolytic shutdown, resulting in the hardened clinical condition and subsequent death of the patient.

Interestingly, those with the double heterozygous genotype were found to have significantly greater rates of ICU admission (46.2% vs. 15.3%; p=0.005) and mortality (38.5% vs. 9.5%; p=0.009), thus suggesting that

genes may be useful as a prognostic biomarker in COVID-19. This is supported by Helms et al. [10], who found coagulopathy and inflammation, among other clinical variables, to be major determinants of the critical state in SARS-CoV-2 infection. Moreover, no statistically significant differences in CRP, D-dimer, creatinine, and other standard inflammation markers were noted between the groups. This would seem to indicate that genetic factors may modulate the clinical course independently of these parameters. Indeed, a notable absence of pneumonia was observed in the double mutation patients despite a severe clinical decline. This could be conceptualized as extra-pulmonary complications of systemic thromboinflammation that, according to Ackermann et al. [19], are caused by diffuse endothelial damage and microvascular thrombosis and have been described in COVID-19 autopsies. To be sure, our study is constrained by sample size, in particular, the heterozygous double group (n=13), which may result in insufficient power for the statistical analysis. That said, the alignment of trends with the literature breathes life into our conclusions.

Taken together, Prothrombin G20210A and PAI-1 4G/5G polymorphisms appear to be key determinants influencing inflammatory and hematological profiles in COVID-19. Particularly, their coexistence was linked to higher ferritin and monocyte levels, lower hemoglobin and lymphocyte counts, and markedly increased ICU admission and mortality. These findings underscore the potential clinical utility of genetic testing for early risk stratification and personalized management in COVID-19.

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