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DIFFERENCES IN FIBRINOLYTIC PROFILE BETWEEN PATIENTS WITH DIFFERENT LOCALIZATIONS AND TYPES OF VENOUS THROMBOSIS

RAZLIKE U FIBRINOLIZNOM PROFILU IZMEĐU PACIJENATA SA RAZLIČITIM LOKALIZACIJAMA I TIPOVIMA VENSKIH TROMBOZA

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Summary

Introduction. The role of the fibrinolytic system in venous thrombosis remains incompletely understood. This study aimed to evaluate the effectiveness of the fibrinolytic system in patients with various types and locations of venous thrombosis compared to healthy controls. **Material and Methods.** The study included 100 patients with venous thrombosis and 100 healthy controls. Patients were stratified based on the type of venous thrombosis (spontaneous vs. provoked) and the location (distal, proximal, and atypical). Global fibrinolytic activity was assessed using euglobulin clot lysis time, while specific fibrinolytic components measured included plasminogen, tissue plasminogen activator, thrombin-activatable fibrinolysis inhibitor, and plasminogen activator inhibitor-1. **Results.** Patients with isolated distal and provoked venous thrombosis exhibited significantly prolonged euglobulin clot lysis time compared to healthy controls (218.3 ± 41.1 vs. 185.6 ± 42.3 min, p=0.001; 208.2 ± 48.5 min vs. 185.6 ± 42.3 min, p=0.018, respectively). Patients with provoked venous thrombosis demonstrated higher plasminogen (127.1 ± 27.7 vs. 117.1 ± 24.5%, p=0.044) and tissue plasminogen activator levels (20.0 ± 11.1 vs. 16.8 ± 8.1 ng/ml, p=0.042) compared to controls. Thrombin-activatable fibrinolysis inhibitor levels were significantly elevated in patients with both provoked (19.9 ± 4.0 vs. 17.1 ± 4.3 ng/ml, p=0.000) and spontaneous venous thrombosis (19.5 ± 6.0 vs. 17.1 ± 4.3 ng/ml, p=0.02), as well as in cases of isolated distal (20.7 ± 5.0 vs. 17.1 ± 4.3 ng/ml, p=0.001) and proximal (19.4 ± 5.3 vs. 17.1 ± 4.3 ng/ml, p=0.013) venous thrombosis when compared to healthy controls. **Conclusion.** The study reveals significant variations in the fibrinolytic process across different types and anatomical locations of venous thrombosis compared to healthy individuals.

Key words: Venous Thrombosis; Fibrinolysis; Plasminogen; Carboxypeptidase B2; Risk Factors

Sažetak

Uvod. Uloga fibrinolize u venskoj trombozi još uvek nije razjašnjena. Cilj studije je ispitivanje razlika u globalnoj funkcionalnosti fibrinoliznog mehanizma i njegovim pojedinačnim komponentama između pacijenata sa različitim tipovima i lokalizacijama venskih tromboza i zdravih osoba. **Materijal i metode.** Uzorak je obuhvatio 100 pacijenata sa venskom trombozom i 100 zdravih kontrola. Pacijenti su grupisani u odnosu na tip venske tromboze (spontana ili provocirana) i u odnosu na lokalizaciju (distalna, proksimalna i atipična). Euglobulinsko vreme lize koaguluma korišteno je za procenu globalne fibrinolizne funkcionalnosti, a određivane su i koncentracije plazminogena, tkivnog aktivatora plazminogena, trombinom aktiviranog fibrinoliznog inhibitora i inhibitora aktivatora plazminogena-1. **Rezultati.** Pacijenti sa izolovanom distalnom i sa provociranom venskom trombozom imaju značajno duže euglobulinsko vreme lize koaguluma u poređenju sa kontrolama (218,3 ± 41,1 vs. 185,6 ± 42,3 min, p = 0,001 i 208,2 ± 48,5 vs. 185,6 ± 42,3 min, p = 0,018). Pacijenti sa provociranom venskom trombozom imaju više nivoe plazminogena (127,1 ± 27,7 vs. 117,1 ± 24,5%, p = 0,044) i tkivnog aktivatora plazminogena (20 ± 11,1 vs. 16,8 ± 8,1 ng/ml, p = 0,042) u poređenju sa kontrolama. Trombinom aktivirani fibrinolizni inhibitor je značajno viši kod provociranih (19,9 ± 4 vs. 17,1 ± 4,3 ng/ml, p = 0,000), spontanih (19,5 ± 6 vs. 17,1 ± 4,3 ng/ml, p = 0,023), izolovanih distalnih (20,7 ± 5 vs. 17,1 ± 4,3 ng/ml, p = 0,001) i proksimalnih venskih tromboza (19,4 ± 5,3 vs. 17,1 ± 4,3 ng/ml, p = 0,013) u poređenju sa kontrolama. **Zaključak.** Postoje značajne razlike u fibrinoliznom mehanizmu između bolesnika sa različitim tipovima i lokalizacijama venskih tromboza u poređenju sa zdravim osobama.

Ključne reči: venska tromboza; fibrinoliza; plazminogen; trombinom aktivirani fibrinolizni inhibitor; faktori rizika

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Introduction

Thrombosis is defined as the formation of a blood clot within a blood vessel, arising from an imbalance among procoagulant, anticoagulant, and fibrinolytic factors. The balanced function of the hemostatic system ensures that blood remains in a liquid state, allows for clot formation in response to injury, and prevents excessive clotting through three primary mechanisms: primary hemostasis, coagula-

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Abbreviations

VT – venous thrombosis
ECLT – euglobulin clot lysis time
t-PA – tissue plasminogen activator
TAFI – thrombin activatable fibrinolytic inhibitor
PAI-1 – plasminogen activator inhibitor – 1
u-PA – urokinase plasminogen activator
PI – plasmin inhibitor
PAI-2 – plasminogen activator inhibitor – 2
DVT – deep venous thrombosis
FV – factor V
CT – computed tomography
BMI – body mass index

tion, and fibrinolysis [1]. The fibrinolytic system, which is the final phase of the hemostatic process, plays a crucial role in breaking down fibrin and preventing the formation of fibrin cloths within circulation. The process involves a complex interplay of numerous factors. Central to fibrinolysis is plasmin, an enzyme that circulates in its inactive form, plasminogen. The conversion of plasminogen to plasmin is mediated by plasminogen activators, including tissue plasminogen activator (t-PA) and urokinase-type plasminogen activator (u-PA). Plasmin's proteolytic action involves cleaving arginine and lysine bonds within fibrin and fibrinogen molecules, leading to the generation of degradation products, such as fragments X, Y, D, and E [2]. Notably, several inhibitors regulate plasmin activity, including antiplasmin (PI), plasminogen activator inhibitor-1 (PAI-1), plasminogen activator inhibitor-2 (PAI-2), and thrombin-activatable fibrinolysis inhibitor (TAFI), which collectively prevent excessive fibrin breakdown [3].

The pathogenesis of thrombosis can be explained by Virchow's triad, which comprises three interrelated factors: alterations in the vessel wall, altered blood flow, and changes in blood composition. Among these, altered blood flow and blood composition are the most significant contributors to the development of venous thrombosis [4].Venous thrombosis typically presents as deep vein thrombosis (DVT) of the lower extremities or as a pulmonary embolism, although it can also affect other veins less frequently. Venous thromboses are categorized based on etiology into spontaneous and provoked types, and based on location, into distal, proximal, and atypical localizations.

The incidence of venous thrombosis is approximately 2 per 1,000 individuals per year in the general population, driven by a combination of genetic and acquired risk factors. In Europe, the annual incidence is around 108 per 100,000 individuals, while in the United States, approximately 250,000 new cases are reported annually among whites and 78 per 100,000 among African Americans [6]. The prevalence of venous thrombosis is similar between males and females, but a slightly higher incidence is observed in younger women, largely associated with hormone therapy, pregnancy, and the puerperium. The incidence also increases with age, from 1 per 100,000 annually in children to 1 per 100 annually in older adults [7]. It is important to note that approximately one-third of patients will experience a recurrence of thrombosis within 10 years of their initial episode. The risk of recurrence is highest within the first 6 to 12 months, particularly in men and following idiopathic thrombosis. The mortality rate associated with venous thrombosis ranges from 1% in the younger individuals to 20% in older patients, with the highest rates observed in those with malignancies, predominantly due to pulmonary embolism [8, 9].

As noted, a range of genetic and acquired risk factors contribute to the onset of venous thrombotic events. Common acquired risk factors include advanced age, prolonged immobilization, pregnancy and puerperium, surgical procedures and trauma, malignancy, antiphospholipid syndrome, long-distance travel, lifestyle factors, and hormone therapy. Key hereditary risk factors encompass deficiencies in natural anticoagulants (protein C, protein S, and antithrombin), Factor V Leiden mutation, prothrombin gene mutation G20210A, fibrinogen γ-chain gene mutation, and an increased risk in individuals with non-O blood types [10–12].

While it is established that reduced fibrinolytic activity plays a role in thrombosis onset, many questions remain regarding the specific contributions of individual fibrinolytic factors in venous thrombosis and the overall role of the fibrinolytic system in different types and localizations of venous thrombotic disease.

This study aims to investigate the fibrinolytic system in patients with various types and locations of venous thrombosis and to compare these parameters with those in a healthy population.

Material and Methods

Between January 2022 and January 2024, the Clinical Center of Vojvodina conducted a study involving 100 patients, aged 18 to 88 years, who were newly diagnosed with venous thrombosis. The diagnosis of deep vein thrombosis was confirmed through Doppler ultrasonography, while pulmonary embolism was diagnosed using CT pulmonary angiography. Additionally, a control group of 100 individuals, aged 19 to 87 years, with no prior history of venous thrombosis, was selected. All participants provided written informed consent, and the study protocol was approved by the Medical Ethics Committee of the Clinical Center of Vojvodina in Novi Sad, Serbia.

To be eligible for inclusion in the study, participants had to be enrolled at least three months after their thrombosis diagnosis and at least two months after discontinuing anticoagulation therapy. They completed a standardized questionnaire that captured potential risk factors for venous thrombosis. The overall function of the fibrinolytic system was assessed using euglobulin clot lysis time (ECLT), with values between 120 and 240 minutes considered indicative of normal fibrinolytic activity, and values exceeding 240 minutes indicating reduced

fibrinolytic activity. Levels of TAFI and t-PA were measured using the ELISA method, while PAI-1 concentrations were determined using the chromogenic substrate method.

Out of the 176 patients initially considered for the study, 6 were excluded due to liver and kidney disease, 18 due to acute illness, 21 due to diagnosed malignancy, 12 due to the use of medications affecting the hemostatic mechanism, and 5 due to pregnancy at the time of diagnosis or within the preceding three months. Ultimately, 100 of the remaining 114 patients agreed to participate in the study. The control group also consisted of 100 participants who met the study's inclusion criteria after applying similar exclusion criteria.

All data were analyzed using SPSS software for Windows, version 24.0 (SPSS, Chicago, IL, USA). Descriptive statistics were used to summarize the characteristics of the study participants. To evaluate the statistical significance of differences in continuous variables, we employed the Student's t-test or the Mann-Whitney U test. Correlations were assessed using Pearson's linear correlation coefficient and Spearman's rank correlation coefficient. A P-value of less than 0.05 considered statistically significant.

Results

A total of 200 participants were included in the study after applying exclusion criteria, with 100 in the patient group and 100 in the control group**.**

The basic characteristics of the participants are summarized in **Table 1**. The patient group included 100 individuals, comprising 48 men and 52 women, with a mean age of 52 years. The control group also included 100 participants, with 51 men and 49 women, and a mean age of 50 years. Patients with venous thrombosis had a slightly higher BMI compared to the control group. Traditional risk factors for venous thrombosis were more common among patients (44%) than among controls (15%). Additionally, the patient group exhibited higher rates of obesity (22% vs. 16%), smoking (31% vs. 24%), hypertension (41% vs. 29%), hyperlipoproteinemia (69% vs. 54%), and hyperLp(a) lipoproteinemia (20% vs. 12%) compared to healthy controls.

Table 2 presents the characteristics of the patient group in terms of the type and location of venous thrombosis. Proximal venous thrombosis was observed in 63% of patients, with nearly equal distribution between left-sided and right-sided cases (49% vs. 51%). Distal venous thrombosis was found in 29% of patients, with a higher prevalence on the left side (59% vs. 41%). Thrombosis in atypical locations was seen in 8% of patients, including thrombosis in the deep veins of the arms (7 patients) and in the central retinal vein (1 patient).

Regarding the type of venous thrombosis, 56% of patients had spontaneous venous thrombosis, while 44% had provoked venous thrombosis: 11% following surgical interventions, 27% due to plaster immobilization, 25% resulting from trauma, 23% occurring during pregnancy, and 14% associated with hormone therapy.

The results of global fibrinolytic system functionality testing demonstrated that patients with deep vein thrombosis had significantly prolonged euglobulin clot lysis times compared to the control group (204.3±51.2 min. vs. 185.6±42.3 min; p=0.01), as shown in **Graph 1.**

Patients with isolated distal venous thrombosis had significantly longer ECLT compared to the control group (218.3±41.1 minutes vs. 185.6±42.3 minutes; p=0.001). In contrast, there were no significant differences in fibrinolytic activity between patients with proximal venous thrombosis and healthy controls (194.7±54.0 minutes vs. 185.6±42.3 minutes; p=0.44).

Table 1. Clinical characteristics of the participants
Tabela 1. *Kliničke karakteristike ispitanika*

Patients/*Pacijenti* (n=100)		Controls/*Kontrole* (n=100)
General characteristics/*Opšte karakteristike*		
Male/*Muškarci*	48 (48)	51 (51)
Age, years/*Starost, godine*	52 (19-88)	50 (19-87)
Body mass index, kg/m²/*Indeks telesne mase, kg/m²*	27 (17-39)	26 (18-37)
Classical venous thrombosis risk factors†/*Klasični faktori rizika za nastanak venske tromboze†*		
Present/*Prisutni*	44 (44)	15 (15)
Absent/*Odsutni*	56 (56)	85 (85)
Arterial cardiovascular risk factors/*Klasični faktori rizika za nastanak arterijske tromboze*		
Obesity/*Gojaznost*	22 (22)	16 (16)
Smoking/*Pušenje*	31 (31)	24 (24)
Hypertension/*Hipertenzija*	41 (41)	29 (29)
Hyperlipoproteinemia/*Hiperlipoproteinemija*	69 (69)	54 (54)
HyperLp(a) lipoproteinemia/*HiperLp(a) lipoproteinemija*	20 (20)	12 (12)

†Classical risk factors include surgery, malignancy, immobility, trauma, plaster cast, immobilization, use of hormonal therapy, oral contraceptive therapy, long trips/*†Klasični faktori rizika uključuju hirurške intervencije, malignitet, nepokretnost, traumu, gipsanu imobilizaciju,upotrebu hormonskih preparata i duža putovanja*
Values are n (%) unless otherwise indicated/*Vrednosti su n (%) osim ako nije drugačije naznačeno*

Table 2. Characteristics of the patient group in relation to the type and localization of venous thrombosis
Tabela 2. *Karakteristike grupe bolesnika u odnosu na vrstu i lokalizaciju venske tromboze*

Localization of venous thrombosis/*Lokalizacija venske tromboze*			
Proximal venous thrombosis *Proksimalna venska tromboza*	63 (63)	Right-sided/*Desnostrana* Left-sided/*Levostrana*	32 (51) 31 (49)
Distal venous thrombosis *Distalna venska tromboza*	29 (29)	Right-sided/*Desnostrana* Left-sided/*Levostrana*	12 (41) 17 (59)
Atypical thrombosis localization *Atipično lokalizovana tromboza*	8 (8)	Deep veins of the arms/*Duboke vene ruku* Central retinal vein/*Centralna vena retine*	7 (88) 1 (12)
Type of venous thrombosis/*Tip venske tromboze*			
Provoked venous thrombosis *Provocirana venska tromboza*	44 (44)	Surgical interventions/*Hirurške intervencije*	5 (11)
		Plaster immobilization/*Gipsana imobilizacija*	12 (27)
		Trauma/*Povreda*	11 (25)
		Pregnancy/*Trudnoća*	10 (23)
		Hormone therapy/*Hormonska terapija*	6 (14)
Spontaneous venous thrombosis/*Spontana venska tromboza*	56 (56)		

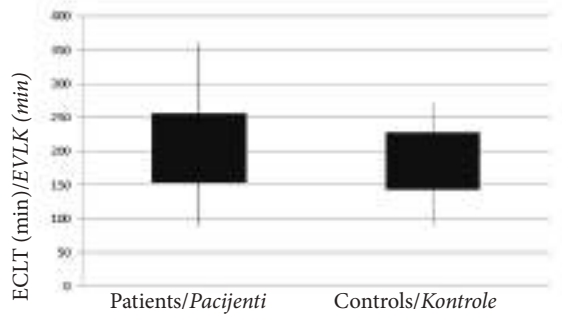
Table 3. Differences in global fibrinolytic mechanism functionality between individual subgroups of patients with venous thrombosis and healthy participants
Tabela 3. *Poređenje globalne funkcionalnosti fibrinoliznog mehanizma između različitih podgrupa bolesnika i zdravih ispitanika*

Euglobulin clot lysis time/*Euglobulinsko vreme lize koaguluma*	SV±SD	Range/*Raspon*	p/*p*
Patients/*Pacijenti*	204.3±51.2	90-360	0.01
Controls/*Kontrole*	185.6±42.3	90-270	
Patients – distal VT/*Pacijenti – distalna VT*	218.3±41.1	140-310	0.00
Controls/*Kontrole*	185.6±42.3	90-270	
Patients – proximal VT/*Pacijenti – proksimalna VT*	194.7±54.0	90-360	0.44
Controls/*Kontrole*	185.6±42.3	90-270	
Patients – atypical localization of VT/*Pacijenti – VT atipične lokalizacije*	229.4±46.2	160-310	0.01
Controls/*Kontrole*	185.6±42.3	90-270	
Patients – provoked VT/*Pacijenti – provocirana VT*	208.2±48.5	130-320	0.02
Controls/*Kontrole*	185.6±42.3	90-270	
Patients – spontaneous VT/*Pacijenti – spontana VT*	201.3±53.5	90-360	0.07
Controls/*Kontrole*	185.6±42.3	90-270	

Legend: VT – venous thrombosis/*Legenda*: *VT – venska tromboza*

Patients with atypical thrombosis locations had notably longer ECLT compared to controls (229.4±46.2 minutes vs. 194.7±54.0 minutes; p=0.01). Those with provoked venous thrombosis also exhibited significantly higher ECLT values compared to healthy controls (208.2±48.5 minutes vs. 185.6±42.3 minutes; p=0.02). However, no significant differences were found between patients with spontaneous thrombosis and the control group (201.3±53.5 minutes vs. 185.6±42.3 minutes; p=0.07) **(Table 3).**

A significant difference in euglobulin clot lysis time (ECLT) was observed among subgroups based on the localization of the venous thrombotic process (distal DVT 218.3 ± 41.1 minutes vs. proximal DVT 194.7 ± 54.0 minutes vs. rare localization DVT 229.4 ± 46.2 minutes; p=0.02). Patients with thrombosis in rare locations of deep venous thrombosis had the



Graph 1. Comparison of global fibrinolytic activity between patients and controls
Grafikon 1. *Poređenje globalne funkcionalnosti fibrinoliznog mehanizma između bolesnika i kontrola*

Table 4. Comparison of global fibrinolytic system functionality between different subgroups of patients
Tabela 4. *Poređenje globalne funkcionalnosti fibrinoliznog mehanizma između različitih podgrupa bolesnika*

Euglobulin clot lysis times/*Euglobulinsko vreme lize koaguluma*			
	SV±SD	Range/*Raspon*	p/*p*
Patients – distal VT/*Pacijenti – distalna VT*	218.3±41.1	140-310	
Patients – proximal VT/*Pacijenti – proksimalna VT*	194.7±54.0	90-360	**0.02**
Patients – atypical localizationof VT/*Pacijenti – VT atipične lokalizacije*	229.4±46.2	160-310	
Patients – provoked VT/*Pacijenti – provocirana VT*	208.2±48.5	130-320	0.66
Patients – spontaneous VT/*Pacijenti – spontana VT*	201.3±53.5	90-360	
Patients – isolated VT/*Pacijenti – izolovana VT*	205.2±51.3	90-360	0.39
Patients – pulmonary thromboembolism/*Pacijenti – plućna tromboembolija*	176.7±49.3	120-210	

Legend: VT - venous thrombosis; *Legenda*: *VT – venska tromboza*

longest ECLT, while those with proximal deep vein thrombosis had the shortest. No significant differences in global fibrinolytic mechanism functionality were found when comparing patients with provoked deep vein thrombosis to those with primary deep vein thrombosis (208.2 ± 48.5 minutes vs. 201.3 ± 53.5 minutes; p=0.66), or when comparing patients with isolated deep vein thrombosis to those with pulmonary thromboembolism (205.2 ± 51.3 minutes vs. 176.7 ± 49.3 minutes; p=0.39) **(Table 4).**

The detailed results of the comparisons of plasminogen, t-PA, PAI-1, and TAFI concentrations between different patient subgroups and the control group are presented in **Table 5**. Patients with provoked deep vein thrombosis had significantly higher plasminogen levels compared to controls (127.1 ± 27.7% vs. 117.1 ± 24.5%; p=0.04). They also had significantly higher t-PA concentrations compared to the control group (20.0 ± 11.1 ng/ml vs. 16.8 ± 8.1 ng/ml; p=0.04). Regarding TAFI concentrations, patients with both distal deep vein thrombosis (20.7 ± 5.0 ng/ml vs. 17.1 ± 4.3 ng/ml; p=0.00) and proximal deep vein thrombosis (19.4 ± 5.3 ng/ml vs. 17.1 ± 4.3 ng/ml; p=0.01) had significantly elevated levels compared to controls. Similarly, patients with provoked deep vein thrombosis exhibited higher TAFI concentrations than healthy individuals (19.9 ± 4.0 ng/ml vs. 17.1 ± 4.3 ng/ml; p=0.00), as did those

Table 5. Comparison of plasminogen, t-PA, PAI-1, and TAFI concentrations between subgroups of patients and controls
Tabela 5. *Poređenje koncentracije plazminogena, t-PA, PAI-1, TAFI između bolesničkih podgrupa i kontrola*

	Plasminogen *Plazminogen* (%)		t-PA (ng/ml)		PAI-1 (ng/ml)		TAFI (ng/ml)	
	SV±SD	p/*p*	SV±SD	p/*p*	SV±SD	p/*p*	SV±SD	p/*p*
Patients DDVT/*Pacijenti DDVT*	129.9±29.7	0.06	18.6±8.4	0.27	5.4±2.7	0.89	20.7±5.0	0.00
Controls/*Kontrole*	117.1±24.5		16.8±8.1		5.4±2.7		17.1±4.3	
Patients PDVT/*Pacijenti PDVT*	121.3±24.7	0.43	18.3±9.8	0.17	5.0±2.9	0.10	19.4±5.3	0.01
Controls/*Kontrole*	117.1±24.5		16.8±8.1		5.4±2.7		17.1±4.3	
Patients ALDVT/*Pacijenti ALDVT*	122.6±38.0	0.51	21.7±15.3	0.51	6.0±2.6	0.48	18.6±4.6	0.38
Controls/*Kontrole*	117.1±24.5		16.8±8.1		5.4±2.7		17.1±4.3	
Patients PRDVT/*Pacijenti PRDVT*	127.1±27.7	0.04	20.0±11.1	0.04	5.5±3.0	0.83	20.0±4.0	0.00
Controls/*Kontrole*	117.1±24.5		16.8±8.1		5.4±2.7		17.1±4.3	
Patients SDVT/*Pacijenti SDVT*	121.4±27.1	0.55	17.6±8.8	0.49	5.0±2.6	0.16	19.5±6.0	0.02
Controls/*Kontrole*	117.1±24.5		16.8±8.1		5.4±2.7		17.1±4.3	
Patients DDVT/*Pacijenti DDVT*	129.9±29.7		18.6±8.4		5.4±2.7		20.7±5.0	
Patients PDVT/*Pacijenti PDVT*	121.3±24.7	0.41	18.3±9.8	0.94	5.0±2.9	0.28	19.4±5.3	0.32
Patients ALDVT/*Pacijenti ALDVT*	122.6±38.0		21.7±15.3		6.0±2.6		18.6±4.6	
Patients PRDVT/*Pacijenti PRDVT*	127.1±27.7	0.16	20.0±11.1	0.37	5.5±3.0	0.31	19.9±4.0	0.34
Patients SDVT/*Pacijenti SDVT*	121.4±27.1		17.6±8.8		5.0±2.6		19.5±6.0	
Patients IDVT/*Pacijenti IDVT*	123.3±27.3	0.15	18.7±10.0	0.98	5.2±2.8	0.26	19.7±5.2	0.94
Patients PTE/*Pacijenti PTE*	142.0±27.2		17.1±7.6		6.6±1.8		18.9±1.9	

Legend/Legenda: DDVT – distal deep venous thrombosis/*DDVT – distalna tromboza dubokih vena;* PDVT – proximal deep venous thrombosis/*PDVT – proksimalna tromboza dubokih vena;* ALDVT – atypical localization deep venous thrombosis/*ALDVT – atipična lokalizacija tromboze dubokih vena;* PRDVT – provoked deep venous thrombosis/*PRDVT – provocirana tromboza dubokih vena*; SPDVT – spontaneous deep venous thrombosis/SPDVT – spontana tromboza dubokih vena; IDVT – isolated deep venous thrombosis/ IDVT – izolovana tromboza dubokih vena; PTE – pulmonary thromboembolism/PTE – plućna tromboembolija

with primary deep vein thrombosis (19.5 ± 6.0 ng/ml vs. 17.1 ± 4.3 ng/ml; p=0.02).

Discussion

The role of fibrinolysis in the pathophysiological mechanism underlying thrombosis formation is an evolving area of research with numerous aspects not fully understood. This study aimed to evaluate the functionality of the fibrinolytic system and its components in patients with various types and locations of VT compared to a healthy control group. In our cohort, 63% of patients had proximal venous thrombosis, while isolated distal venous thrombosis was present in 29% of cases, as aligning with the findings of other studies in this field [14–19]. Similar to the study by Ouriel et al., which reported a left-sided to right-side ratio of 1.3:1 for distal deep vein thrombosis [20], our study found a ratio of 1.4:1 favoring left-sided localization. However, we did not observe this difference in cases of proximal deep vein thrombosis. In our study, 56% of patients had spontaneous venous thrombosis, while 44% had provoked venous thrombosis. This classification is clinically significant, as evidence suggests that the risk of recurrent venous thromboembolism after 3-6 months of anticoagulant therapy is approximately 50% lower in patients with provoked thrombosis compared to those with spontaneous deep vein thrombosis.

Our analysis of the fibrinolytic system's global functionality demonstrated that patients with deep vein thrombosis had significantly prolonged ECLT, indicating reduced fibrinolytic activity compared to healthy participants. The first study to investigate the association between decreased fibrinolytic activity and venous thrombosis was published in 1991, concluding that the evidence for this link was inconclusive [22]. However, the authors noted that while reduced fibrinolytic activity may not predict venous thromboembolism overall, there is likely an association between suppressed fibrinolysis and postoperative venous thrombosis.

When we compared the fibrinolytic system's functionality between specific patient subgroups and the control group, we found no significant difference in ECLT between patients with proximal deep vein thrombosis and healthy controls. However, this difference became apparent when comparing patients with isolated distal venous thrombosis to healthy participants. A comparative analysis of the fibrinolytic system's functionality among patients with different localizations of deep vein thrombosis revealed significant differences in ECLT, with a notable prolongation of ECLT in patients with isolated distal deep vein thrombosis compared to those with proximal thrombotic localization. Additionally, we found that patients with thrombosis in rare locations had the longest ECLT. Given that thrombosis in rare locations is often accompanied by inflammation, increased PAI-1 activity during inflammation may explain these findings [23].

When examining differences in fibrinolytic mechanism functionality, as indicated by ECLT, between patients with provoked venous thrombosis and control participants, and between patients with spontaneous thrombosis and controls, we found that patients with provoked venous thrombosis had significantly higher ECLT values compared to controls. In contrast, there was no significant difference in ECLT between patients with spontaneous thrombosis and healthy participants. A comparative analysis between patients with provoked venous thrombosis and those with spontaneous deep vein thrombosis revealed no significant differences in fibrinolytic mechanism's functionality. Trauma is among the primary triggers of provoked venous thrombosis, as it leads to the release of PAI-1 from the damaged endothelium, potentially suppressing overall fibrinolytic activity [24]. Following the assessment of the overall functionality of the fibrinolytic mechanism, we further analyzed data on specific components of this system. Our findings indicated no substantial differences in plasminogen levels between patients with venous thrombosis and healthy participants, consistent with most of the existing literature. Indeed, aside from a few isolated case reports, there is limited evidence linking plasminogen deficiency to venous thrombosis. A comprehensive population-based case-control study by Okamoto et al. found comparable prevalence rates of plasminogen deficiency among venous thrombosis patients and healthy controls [25]. Subsequent analysis, which stratified patients into various subgroups and compared plasminogen levels with those of healthy controls, revealed no significant differences in patients with isolated distal deep vein thrombosis, proximal thrombosis, atypical venous thrombosis localization, or spontaneous deep vein thrombosis compared to the control group. However, our findings indicate that patients with provoked deep vein thrombosis had significantly elevated plasminogen concentrations compared to healthy individuals.

When comparing t-PA concentrations between all patients and controls, no statistically significant difference was observed, which aligns with most reports in the literature. A case-control study within the Physicians' Health Study cohort, which included 55 patients and controls and followed them for five years, concluded that circulating t-PA levels do not predict venous thrombosis [26]. However, similar to plasminogen, our study showed that after classifying patients and analyzing the comparison of t-PA levels between different patient subgroups and healthy individuals, patients with provoked deep vein thrombosis had significantly higher t-PA concentrations compared to healthy participants.

According to our study results, patients with venous thrombosis did not differ from healthy participants in terms of PAI-1 concentration. Similar results were obtained when comparing individual patient subgroups and healthy participants regarding this fibrinolysis inhibitor level. Other authors also believe that circulating PAI-1 levels do not influence the risk of venous thrombosis. The Physicians' Health Study did not find differences in PAI-1 levels at the study's start between participants who developed venous thrombosis and those who did not. These results were confirmed by a

grouped case-control study from the LITE cohort [27], which included 308 patients and 640 controls, and by a cohort study by Crowther et al. [28], which found no connection between PAI-1 activity or antigen levels and recurrent venous thromboembolism. However, some studies have reported higher PAI-1 antigen or activity levels in patients with recurrent venous thrombosis compared to healthy controls [27, 29, 30].

In contrast to the findings related to other fibrinolysis parameters, our analysis of TAFI concentration revealed that patients with venous thrombosis had significantly elevated levels of this fibrinolysis inhibitor compared to individuals without a history of venous thrombosis. Similar results were observed when comparing TAFI concentrations among various patient subgroups categorized by the type and location of venous thrombosis, in comparison to healthy controls. These findings are consistent with other studies suggesting a direct correlation between TAFI levels and the occurrence of venous thrombosis. For example, a case-control study conducted by Verdu et al. [31], involving 60 patients and 62 controls, reported a fourfold increase in the risk of deep vein thrombosis among participants whose TAFI levels exceeded the 90th percentile.

Conclusion

In conclusion, our results demonstrate that patients with isolated distal deep vein thrombosis and those with provoked deep vein thrombosis show a markedly reduced fibrinolytic potential compared to healthy individuals. Levels of tissue plasminogen activator antigen and plasminogen are significantly elevated in patients with provoked venous thrombosis compared to healthy controls, while plasminogen activator inhibitor-1 concentrations remain similar between these groups. No significant differences in any specific fibrinolytic factor were observed between patients with primary deep vein thrombosis and healthy individuals. However, thrombin-activatable fibrinolytic inhibitor levels are significantly increased in patients with both provoked and spontaneous deep vein thrombosis, as well as in those with isolated distal and proximal deep vein thrombosis, compared to healthy controls. These results underscore notable variations in the fibrinolytic system's functionality depending on the types and locations of venous thrombosis. This information provides a robust foundation for re-evaluating venous thrombosis risk and opens avenues for exploring potential new therapeutic strategies.

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