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THE INFLUENCE OF TISSUE PLASMINOGEN ACTIVATOR, PLASMINOGEN ACTIVATOR INHIBITOR-1, AND THROMBIN ACTIVABLE FIBRINOLYTIC INHIBITOR ON THE RISK OF VENOUS THROMBOSIS

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UTICAJ TKIVNOG AKTIVATORA PLAZMINOGENA, INHIBITORA AKTIVATORA PLAZMINOGENA-1 I TROMBINOM AKTIVIRANOG FIBRINOLIZNOG INHIBITORA NA RIZIK OD NASTANKA VENSKE TROMBOZE

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ABSTRACT

Objective. The role of the fibrinolytic mechanism in venous thrombosis risk remains controversial. The study aimed to examine the influence of tissue plasminogen activator (t-PA), plasminogen activator inhibitor-1 (PAI-1), and thrombin activatable fibrinolysis inhibitor (TAFI) on the risk of venous thrombosis.

Methods. The research included 100 participants with deep vein thrombosis and 100 participants with no previous history of venous thrombosis. Global fibrinolytic activity was estimated by euglobulin clot lysis time (ECLT). The ELISA method was used for the determination of t-PA and TAFI concentrations. The chromogenic substrate method was used for the determination of PAI-1 concentrations.

Results. A statistically significant difference was not obtained for t-PA and PAI-1 compared between the patients and controls. The patients had a significantly higher concentration of TAFI when compared to controls. Relative risk analysis showed a three-fold increase in the venous thrombosis risk connected with suppressed fibrinolytic activity. The crude OR was 2.70 (95% CI 1.22-5.98) and adjustments for confounding yielded an OR of 3.02 (95% CI 1.26-7.22). TAFI concentrations showed influence on venous thrombosis risk, as crude OR was 2.18 (95% CI 1.19-3.99) and fully adjusted one stayed in the same range (OR 2.25; 95% CI 1.16-4.35) indicating a two-fold increase in venous thrombotic risk associated with increased TAFI concentrations.

Conclusion. Suppressed functionality of the fibrinolysis mechanism triples the risk of occurrence of deep vein thrombosis while elevated TAFI level doubles the risk of this disease. There is no influence of plasminogen concentrations, t-PA, and PAI-1 levels on venous thrombosis risk.

Key words: *fibrinolysis; venous thrombosis; plasminogen activators; plasminogen activator inhibitor 1.*

SAŽETAK

Cilj. Uloga fibrinoliznog mehanizma za rizik za nastanak venske tromboze i dalje je kontroverzna. Cilj rada bio je da se ispita uticaj tkivnog aktivatora plazminogena (t-PA), inhibitora aktivatora plazminogena-1 (PAI-1) i trombinom aktiviranog fibrinoliznog inhibitora (TAFI) na rizik od nastanka prve epizode venske tromboze.

Metode. Istraživanje je obuhvatilo 100 ispitanika s trombozom dubokih vena i 100 ispitanika bez prethodne istorije venske tromboze. Globalna fibrinolizna aktivnost je procenjivana euglobulinskim vremenom lize koaguluma (EVLK). Za određivanje koncentracija t-PA i TAFI korišćena je ELISA metoda. Za određivanje koncentracije PAI-1 korišćen je metod hromogenog supstrata.

Rezultati. Nije dobijena statistički značajna razlika prilikom poređenja t-PA i PAI-1 između pacijenata i kontrola. Pacijenti su imali značajno veću koncentraciju TAFI u poređenju s kontrolama. Analizom relativnog rizika uočeno je trostruko povećanje rizika od venske tromboze povezano sa suprimiranom fibrinoliznom aktivnošću. Osnovni OR bio je 2,70 (95% CI 1,22–5,98), a nakon prilagođavanja za confounding OR iznosio je 3,02 (95% CI 1,26–7,22). Povišene koncentracije TAFI pokazale su uticaj na rizik od nastanka venske tromboze s obzirom na osnovni OR od 2,18 (95% CI 1,19–3,99) i prilagođeni OR od 2,25 (95% CI 1,16–4,35), što ukazuje na dvostruko povećanje rizika od nastanka venske tromboze povezano s povišenom koncentracijom TAFI.

Zaključak. Suprimirana funkcionalnost fibrinoliznog mehanizma trostruko povećava rizik od nastanka tromboze dubokih vena, dok povišen nivo TAFI dvostruko povećava ovaj rizik. Nivo plazminogena, t-PA i PAI-1 ne utiče na rizik od nastanka venske tromboze.

Ključne reči: *fibrinoliza; venska tromboza; aktivator plazminogena; inhibitor aktivatora plazminogena 1.*

INTRODUCTION

Venous thrombosis represents the process of formation of a blood clot inside a blood vessel because of a disturbed balance between the various links of the hemostasis mechanism (1). Clinically, it is most often manifested as deep vein thrombosis (DVT) or pulmonary embolism (PE), although it can also occur in atypical locations. Venous thromboses are classified according to the manner of occurrence into spontaneous and provoked; while

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concerning localization, we classify them into distal, proximal, and atypical thrombosis (2).

The incidence of venous thrombosis is 2/1000 persons per year in the general population, representing the joint action of several genetic and acquired risk factors (3). The most common acquired risk factors include age, long-term immobilization, pregnancy and puerperium, surgical interventions and trauma, oncological and hematological diseases, long-distance trips, lifestyle habits, and the use of hormone therapy (4,5). The most common genetic risk factors are deficiency of natural coagulation inhibitors (protein C, protein S, and antithrombin), FV Leiden mutation, FII G20210A mutation, a mutation in the gene for the synthesis of the γ-chain of fibrinogen, and the risk is increased in people with no-0 blood group (6-8). The great epidemiological importance of the disease stems from the extremely high mortality rate, which ranges from 1% in the younger population of patients to 10% in the older population and is the highest in people with malignancy (9, 10). Current data show that the mortality rate within the first 30 days after the occurrence of the first episode of venous thrombosis is 6.4%, and the one-year mortality is 21.6% when all patients who experienced venous thrombosis are considered (11). Epidemiological importance additionally arises from the high percentage of recurrence that develops in a third of patients within the first 10 years of a thrombotic event, as well as the frequent occurrence of post-thrombotic syndrome in the chronic course of the disease, which significantly affects the reduction of workability and quality of life (12).

The basis for understanding the pathophysiological mechanism of thrombosis was established in 1856. by Rudolf Virchow and still represents a cornerstone in the field (13). Virchow's triad includes three basic components of the pathophysiological mechanism responsible for thrombosis: changes in the blood vessel wall, changes in the blood flow, and changes in the blood composition, whereby venous stasis and hypercoagulability contribute the most to the process of venous thrombosis (14). For the occurrence of venous thrombosis itself, it is important to highlight multicausality while its occurrence requires the simultaneous presence of several risk factors that are combined according to the principle of multiplication of the effect.

The fibrinolytic system, as an integral part of the hemostasis mechanism, ensures the recanalization of the blood vessel and retention of the blood clot at the site of the blood vessel injury (15). We distinguish between primary fibrinolysis, which is a physiological process in the body, and secondary fibrinolysis, which is a consequence of the effect of a drug, medical disorder, or some other cause (16). In the very process of fibrinolysis, numerous factors take part, whose roles are constantly intertwined with each other. The central place is occupied by plasmin, which is found in the circulation in the form of its inactive precursor, the proenzyme plasminogen (17). The conversion of plasminogen to plasmin occurs under the action of plasminogen activators, such as tissue plasminogen activator (t-PA) and urokinase-type plasminogen activator (u-PA) (18). Plasmin exhibits its proteolytic activity by breaking down arginine and lysine bonds, primarily in fibrin and fibrinogen molecules, resulting in numerous degradation products, i.e. fragments X, Y, D, and E (19). It is also important to mention the existence of plasmin inhibitors, dominated by antiplasmin (PI) in the circulation, and plasminogen activator inhibitor-1 (PAI-1), plasminogen activator inhibitor-2 (PAI-2), and thrombin-activated fibrinolysis inhibitor (TAFI) in the thrombus, all to prevent the breakdown of fibrin (20).

Previous research in the field of venous thrombosis was primarily focused on the coagulation system, and lately on endothelial dysfunction. The influence of the global functionality of the fibrinolysis mechanism, as well as its components, on the risk of venous thrombosis, is the subject of a significantly smaller number of studies, and the results of the research so far are inconsistent.

Our study aimed to examine the influence of t-PA, PAI-1, and TAFI, as the most significant factors of the fibrinolysis mechanism, on the risk of the first episode of venous thrombosis.

PATIENTS AND METHODS

Study design

The research was conducted as a retrospective, case-control study, in the Clinical Center of Vojvodina in the period between January 2022 and January 2024. All the necessary information was obtained from the medical records of the Department for Prevention of Thrombosis, Clinic of Hematology. The study included a total of 200 patients classified into two groups. The first group of 100 patients consisted of respondents, aged 19-88 years, diagnosed with deep vein thrombosis for the first time. For the control group, 100 participants were selected, aged 19-87 years, with no previous history of venous thrombosis. All the patients in the control group were randomly selected from the population examined in the Clinical Center of Vojvodina during routine systematic exams. All participants gave written informed consent. The study was approved by the Medical Ethics Committee of the Clinical Center of Vojvodina, Novi Sad, Serbia.

Data collection

The date of informed consent signing was defined as an index date. Participants completed a questionnaire regarding thrombosis risk factors. Inclusion of patients

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had to be at least 3 months after venous thrombosis and oral anticoagulation had to be stopped at least 2 months before the study began. The diagnosis of deep vein thrombosis must have been confirmed with Doppler ultrasonography.

Global fibrinolytic activity was estimated by euglobulin clot lysis time (ECLT). Normal fibrinolytic activity was considered for participants with ECLT between 120 and 240 minutes, while decreased fibrinolytic activity was considered for those with ECLT longer than 240 minutes. The ELISA method was used for the determination of tissue plasminogen activator (t-PA) and thrombin activatable fibrinolysis inhibitor (TAFI) concentrations. The chromogenic substrate method was used for the determination of plasminogen activator inhibitor-1 (PAI-1) concentrations.

Inclusion/exclusion criteria

The basic criteria for exclusion from the study were: acute illness at the time of blood sampling or anytime within 6 weeks before blood sampling, existence of a previous disorder of the hemostasis mechanism, taking drugs that can affect the hemostasis functionality, malignancy, pregnancy, autoimmune disease, and refusal of the subject to sign the informed consent questionnaire.

Statistical analysis

For statistical analysis we used SPSS (Statistical Package for the Social Science) for Windows, release 24.0. (SPSS, Chicago, IL, USA). Descriptive statistical methods were used to present the characteristics of the subjects. Continuous variables were presented as mean (range) while categorical variables were presented as frequencies (percentages). For testing the statistical significance of differences for continuous variables we used Student's t-test or Mann-Whitney's U test. $\chi 2$ test was used for comparison of categorical variables. For all tests, two-sided p values were calculated, and statistical significance was defined as $p < 0.05$. For calculating odds ratio (OR) as a measure of relative risk, with 95% CI, unconditional logistic regression was used.

RESULTS

The research initially included a total of 339 participants. After excluding subjects who did not meet the criteria, a total of 200 subjects were included in the study, divided into two groups - the patient group and the control group.

The basic clinical characteristics of the patients are shown in Table 1. The group of patients, which included 100 subjects, consisted of 48 men and 52 women with an average age of 52 years (range 19-88 years). There were also 100 respondents in the control group, among whom there were 51 men and 49 women with an average age of 50 years (range 19-87 years).

Table 1. Clinical characteristics of the subjects.

Variable	Patients (n=100)	Controls (n=100)
General characteristics		
Men	48 (48)	51 (51)
Age, year	52 (19-88)	50 (19-87)
Body mass index, kg/m2	27 (17-39)	26 (18-37)
Classical vein thrombosis risk factors		
Present*	44 (44)	15 (15)
Absent*	56 (56)	85 (85)
Arterial cardiovascular risk factors		
Obesity	22 (22)	16 (16)
Smoking	31 (31)	24 (24)
Hypertension	41 (41)	29 (29)
Hyperlipoproteinemia	69 (69)	54 (54)
Hyper-Lp(a) lipoproteinemia	20 (20)	12 (12)

*classical risk factors include surgery, malignancy, immobility, trauma, plaster cast, immobilization, use of hormonal therapy, oral contraceptive therapy, long trips Values are n (%) unless otherwise indicated

Table 2. Comparison of individual components of the fibrinolysis mechanism.

Variable	Patients (n=100)	Controls (n=100)
Plasminogen (%)		
Mean±SD	123.90±27.37	117.09±24.49
Range	62-199	58-160
p	0.126	
t-PA (ng/ml)		
Mean±SD	18.65±9.86	16.78±8.08
Range	2-56	8-44
p	0.110	
PAI-1 (ng/ml)		
Mean±SD	5.23±2.76	5.42±2.73
Range	1-13	1-13
p	0.329	
TAFI (ng/ml)		
Mean±SD	19.70±5.17	17.13±4.25
Range	11-39	8-27
p	0.001	

SD-standard deviation; t-PA - tissue plasminogen activator; PAI-1-plasminogen activator inhibitor-1; TAFI-thrombin activable fibrinolytic inhibitor

Acknowledged risk factors for venous thrombosis were more often present in patients, compared to the control group (44% vs. 15%). Also, in the group of patients compared to healthy controls obesity was observed more often (22% vs. 16%), the percentage of smokers was higher (31% vs. 24%), hypertension was more often registered (41% vs. 29%) as well as hyperlipoproteinemia (69% vs. 54%) and hyperLp(a)-lipoproteinemia (20% vs. 12%).

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Table 3. Correlation between individual fibrinolysis parameters and the global functionality of the fibrinolysis mechanism.

Variable	Patients (n=100)	Controls (n=100)	All subjects (n=200)
ECLT – Plasminogen			
r	0.17	0.12	0.17
p	0.093	0.254	0.018
ECLT - t-PA			
r	-0.10	-0.03	-0.05
p	0.324	0.794	0.500
ECLT - PAI-1			
r	0.22	0.15	0.18
p	0.030	0.130	0.012
ECLT – TAFI			
r	-0.13	0.06	0.01
p	0.202	0.532	0.961

ECLT - Euglobulin cloth lysis time; t-PA - tissue plasminogen activator; PAI-1 - plasminogen activator inhibitor-1; TAFI - thrombin activable fibrinolytic inhibitor

The results of testing the global functionality of the fibrinolytic system shown in Figure 1 indicate that patients who have experienced venous thrombosis have a significantly longer euglobulin clot lysis time, that is, suppressed fibrinolytic functionality compared to healthy controls (204.34±51.24 min. vs. 185.62±42.30 min; p=0.011).

Along with examining the global functionality of the fibrinolytic system, its components were analyzed. No differences in plasminogen concentrations were noted between patients and healthy subjects (123.90 ± 27.37 % vs. 117.09 ± 24.49 %; p=0.126). A statistically significant difference was not obtained when comparing the concentration of t-PA between patients and controls (18.65 ± 9.86 ng/ml vs. 16.78 ± 8.08 ng/ml; p=0.110), nor when comparing the concentration of PAI-1 between these two groups (5.23 ± 2.76 ng/ml vs. 5.42 ± 2.73 ng/ml; p=0.329). When comparing the concentration of TAFI, we observed that patients who have experienced a venous thrombotic

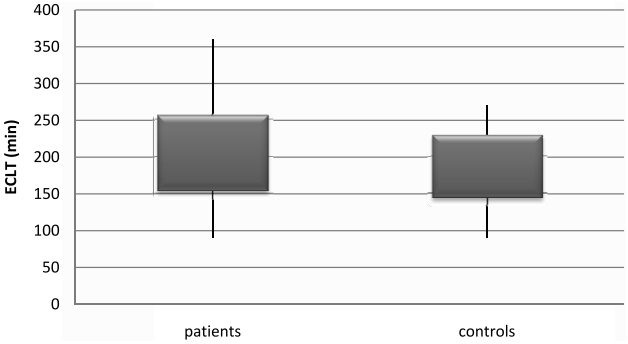

Figure 1. Comparison of the global functionality of the fibrinolytic mechanism between patients and controls.

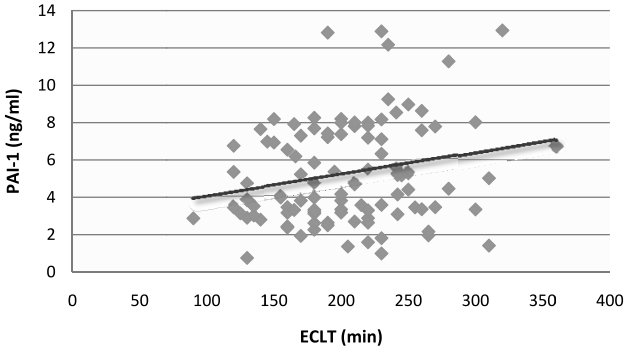

Figure 2. Analysis of the relationship between EVLK and PAI-1 in the patient group.

incident have a significantly higher concentration of this inhibitor of the fibrinolysis mechanism compared to healthy individuals (19.70 ± 5.17 ng/ml vs. 17.13 ± 4.25 ng/ml; p=0.001) (Table 2).

The results of the analysis of the connection between individual parameters of the fibrinolysis mechanism and its global functionality shown in Table 3, reveal that there is a significant relationship between plasminogen and euglobulin clot lysis time (EVLK), as a measure of global fibrinolytic activity if we look at all subjects (r=0.17;

Table 4. Assessment of the risk of venous thrombosis concerning the global functionality of the fibrinolysis mechanism and its individual components

Variable	Patients n (%)	Controls n (%)	Odds ratio* (95% CI)	Odds ratio† (95% CI)	Odds ratio‡ (95% CI)	Odds ratio§ (95% CI)	Odds ratio¶ (95% CI)	Odds ratio□ (95% CI)	Odds ratio** (95% CI)
Venous thrombosis									
Normal fibrinolytic activity	75 (75)	89 (89)	1.0 (Reference)	1.0 (Reference)	1.0 (Reference)	1.0 (Reference)	1.0 (Reference)	1.0 (Reference)	1.0 (Reference)
Decreased fibrinolytic activity	25 (25)	11 (11)	2.70 (1.22-5.98)	2.49 (1.12-5.56)	2.47 (1.10-5.55)	2.72 (1.19-6.20)	2.67 (1.16-6.14)	2.85 (1.22-6.61)	3.02 (1.26-7.22)
Normal PAI-1	62 (62)	67(67)	1.0 (Reference)	1.0 (Reference)	1.0 (Reference)	1.0 (Reference)	1.0 (Reference)	1.0 (Reference)	1.0 (Reference)
Increased PAI-1	38 (38)	33 (33)	0.88 (0.66-1.16)	0.83 (0.61-1.13)	0.83 (0.60-1.15)	0.83 (0.59-1.17)	0.84 (0.59-1.19)	0.85 (0.60-1.21)	0.86 (0.59-1.25)
Normal t-PA	34 (34)	39(39)	1.0 (Reference)	1.0 (Reference)	1.0 (Reference)	1.0 (Reference)	1.0 (Reference)	1.0 (Reference)	1.0 (Reference)
Increased t-PA	66 (66)	61 (61)	1.30 (0.72-2.33)	1.40 (0.77-2.53)	1.48 (0.81-2.72)	1.42 (0.77-2.64)	1.43 (0.77-2.67)	1.40 (0.75-2.62)	1.53 (0.79-2.94)
Normal TAFI	58 (58)	75(75)	1.0 (Reference)	1.0 (Reference)	1.0 (Reference)	1.0 (Reference)	1.0 (Reference)	1.0 (Reference)	1.0 (Reference)
Increased TAFI	42 (42)	25 (25)	2.18 (1.19-3.99)	2.16 (1.17-3.98)	2.21 (1.19-4.11)	2.37 (1.26-4.46)	2.30 (1.22-4.35)	2.24 (1.18-4.25)	2.25 (1.16-4.35)

*adjusted for age and sex †adjusted for age, sex, BMI and smoking ‡adjusted for age, sex, BMI, smoking, hyperlipoproteinemia, and hyperLp(a)lipoproteinemia §Adjusted for age, sex, BMI, smoking, hyperlipoproteinemia, hyperLp(a)lipoproteinemia, and diabetes Adjusted for age, sex, BMI, smoking, hyperlipoproteinemia, hyperLp(a)lipoproteinemia, diabetes, CRP, and fibrinogen Adjusted for age, sex, BMI, smoking, hyperlipoproteinemia, hyperLp(a)lipoproteinemia, diabetes, CRP, fibrinogen, and hypertension **Adjusted for age, sex, BMI, smoking, hyperlipoproteinemia, hyperLp(a)lipoproteinemia, diabetes, CRP, fibrinogen, hypertension, and use of statins and hormones

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p=0.018). As far as the relationship between PAI-1 and euglobulin clot lysis time is concerned, there is no significant relationship when looking at all subjects (r=0.18; p=0.012), but a weak relationship between these two parameters is observed in the group of patients (r= 0.22; p=0.030) (Figure 2).

In further analysis, the assessment of the risk of venous thrombosis concerning individual components of the fibrinolysis mechanism was performed. Relative risk analysis showed a three-fold increase in the venous thrombosis risk accompanied by suppressed fibrinolytic activity. The crude OR was 2.70 (95% CI 1.22-5.98) and adjustments for sex, age, BMI, smoking, hypertension, hyperlipoproteinemia, hyper-Lp(a)-lipoproteinemia, diabetes, fibrinogen, CRP, use of statins, and use of hormone therapy yielded an OR of 3.02 (95% CI 1.26-7.22). An increase in t-PA or PAI-1 concentrations doesn't influence the risk of venous thrombosis as fully adjusted ORs were 1.53 (95% CI 0.79-2.94) and 0.86 (95% CI 0.59-1.25), respectively. Only TAFI concentration showed influence on venous thrombosis risk, as crude OR was 2.18 (95% CI 1.19-3.99), and fully adjusted one was in the same range (OR 2.25; 95% CI 1.16-4.35) indicating a two-fold increase in venous thrombotic risk associated with increased TAFI concentrations (Table 4).

DISCUSSION

In recent years interest in the role of fibrinolysis in the pathophysiology mechanism responsible for the occurrence of both arterial and venous thrombosis is significantly growing, representing at the same time a research topic with many unexplored areas (21).

Epidemiological studies have identified numerous potential causative factors for the development of venous thrombosis belonging to the fibrinolytic system, but the results lack consistency and have numerous methodological obstacles (22).

Our study aimed to examine the influence of t-PA, PAI-1, and TAFI, as the most significant factors of the fibrinolytic mechanism, on the risk for the occurrence of the first venous thrombosis incident. During the creation of the study, special attention was paid to exclude any possibility for the introduction of bias in the study. It is known that there are countless directions from which an error can creep into an epidemiological study, but three are key: selection of participants, collection of information, and "confounding". In our study, the controls were selected by randomization from the population from which the group of patients originates, regardless of the examined risk factors, in line with the basic epidemiological postulate of a properly created case-control study that each subject in the patient group corresponds to the subject in the control group before he experienced venous thrombosis, as well as that every subject from the control group could become a subject from a group of patients if they experience venous thrombosis. Errors that would result from the collection of information were eliminated with the creation of a strictly formulated questionnaire, while data were collected by only one person for all participants. Finally, numerous potential confounding factors were recognized, and risk analyses were adjusted for each of them. We believe that by designing the study in this way, we have significantly contributed to its quality and the validity of the results.

The results of the examination of the fibrinolysis mechanism related to the examination of its global functionality showed that patients who have experienced deep vein thrombosis have significantly longer euglobulin clot lysis time, i.e. suppressed functionality compared with healthy subjects (204.34±51.24 vs. 185.62±42.30; p=0.011). Risk analyses showed that suppressed functionality of the fibrinolytic mechanism triples the risk of occurrence of deep vein thrombosis (OR 3.02; 95% CI 1.26-7.22). The first review article that dealt with the relationship between reduced fibrinolytic activity and venous thrombosis was published 33 years ago (23) but the authors concluded that the evidence supporting the existence of this connection is inconclusive. Yet, they also highlighted the fact that although it does not seem that reduced fibrinolytic activity can be used as a predictor of total venous thromboembolism, there seems to be a relationship between suppressed fibrinolysis and postoperative vein thrombosis. In the last two decades, a relatively small number of studies dealing with this topic was published, and worth mentioning is certainly the prospective, cohort study by Crowther and associates, where authors did not show that the euglobulin clot lysis time has a predictive value concerning the recurrence of venous thrombosis (24).

There is also the MEGA study (Multiple Environmental and Genetic Assessment of risk factors for venous thrombosis), a large, population-based case-control study that included almost 2,500 patients with venous thrombosis and 3,000 controls, confirming the unequivocal existence of a link between suppressed fibrinolysis and venous thrombosis (25).

After analyzing the results obtained by examining the global functionality of the fibrinolytic mechanism, we analyzed the results obtained by testing individual components of the fibrinolysis mechanism. According to our results, there is no significant difference in concentration of plasminogen between patients who experienced venous thrombosis and healthy subjects (123.90 ± 27.37 % vs. 117.09 ± 24.49 %; p=0.126), which follows most existing literature data. A large, population-based, case-control study by Okamoto et al. showed that the prevalence of plasminogen deficiency is similar in

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patients with venous thrombosis and healthy controls (26). A study by Meltzer et al. (27) opened completely new perspectives regarding the connection between plasminogen and venous thrombosis. Namely, the results of this large study show an unexpected positive correlation between levels of plasminogen and the risk of deep vein thrombosis. It is worth mentioning that some authors of the population studies concluded that an elevated level of plasminogen is associated with an increased risk from the occurrence of arterial thrombosis (28, 29), which they explained by an inflammatory process (30), whereas the relationship disappeared after adjustment for inflammatory markers (31). The Meltzer study, adjusting for fibrinogen and FVIII coagulation, as acute phase proteins, attenuated but not completely reversed the association between plasminogen and venous thrombosis, suggesting that plasminogen is a marker of inflammation, but also a possible risk factor for venous thrombosis occurrence by alternative pathophysiological mechanisms.

When comparing the concentration of t-PA between patients and controls, a statistically significant difference was not obtained (18.65 ± 9.86 ng/ml vs. 16.78 ± 8.08 ng/ml; p=0.110), which follows most literature references. The Physicians' Health Study cohort, a clustered prospective case-control study that included 55 patients and controls followed up for 5 years concluded that the circulating level of t-PA is not a predictor of venous thrombosis (32). The results of another prospective cohort, including 303 people who experienced venous thrombosis, provide evidence that the level of t-PA has no predictive significance for the occurrence of venous recurrence thrombotic diseases (24).

According to the results of our study, patients with venous thrombosis do not differ from healthy subjects concerning PAI-1 concentration (5.23 ± 2.76 ng/ml vs. 5.42 ± 2.73 ng/ml; p=0.329). According to the results of the Physicians' Health Study (32), no difference in PAI-1 levels was found at the beginning of the study between subjects who later experienced venous thrombosis and those who did not suffer from it. These results were also confirmed by a case-control study derived from the LITE cohort (33) which included 308 patients and 640 controls, as well as by a cohort study of Crowther et al, which did not establish the existing association between PAI-1 activity or its antigenic level and venous recurrence thromboembolism (24). On the other hand, several studies have established the existence of higher levels of PAI-1 antigen or activity in patients with venous thrombosis recurrence compared to healthy controls (33-35). A more significant connection was established according to the results of the retrospective rather than prospective studies. Patients with deep vein thrombosis often have prolonged inflammatory response, which certainly contributes to elevated PAI-1 levels in plasma. Retrospective studies therefore provide limited information and is difficult to evaluate the question of the cause and the effect. One of the possible reasons why the results of our study do not indicate a higher level of PAI-1 in patients with deep vein thrombosis compared to healthy controls is the fact that the patients were included in the study often for several years after the occurrence of venous thrombosis, and the possible impact of even prolonged inflammatory response was eliminated.

By analyzing the concentration of TAFI, we observed that patients who experienced venous thrombosis have significantly higher concentrations of this inhibitor of the fibrinolysis in comparison to individuals who never had venous thrombosis (19.70 ± 5.17 ng/ml vs. 17.13 ± 4.25 ng/ml; p=0.001). Risk analyses confirmed that elevated TAFI level doubles the risk of this disease (OR 2.25; 95%CI 1.16-4-35). Other authors also believe that the level of TAFI is directly associated with venous thrombosis. Thus, according to the results of the LETS study, almost a two-fold increase in the risk for the first episode of deep vein thrombosis in persons with a TAFI level above the 90th percentile compared to people below this limit was established (OR 1.7; 95% CI 1.1–2.7) (36). The results of a case-control study by Verdu et al. (37) performed on 60 patients and 62 controls, showed a fourfold increase in the risk of occurrence of deep vein thrombosis in subjects with TAFI levels above the 90th percentile while the results of a large, prospective cohort (38) that included 600 patients with venous thrombosis, showed that TAFI levels above the 75th percentile of healthy controls double the risk for recurrence. A possible explanation for the relationship between elevated TAFI concentrations and venous thrombosis is that the level of TAFI increases with age, especially in women, as well as that it is increased in the usage of oral contraceptives (39). Still, the relationship between TAFI and venous thrombosis is much more complicated. The evidence supported by studies conducted in recent years makes clear that TAFI levels are partially genetically determined and that certain single-nucleotide polymorphisms in the promoter region of the TAFI gene are in direct relation with the antigenic level of TAFI in plasma (40) as well as with the increased risk of venous thrombosis (41). Finally, we should not forget the possibility that TAFI contributes to the risk not only through the inhibitory role of fibrinolysis but also through the influence on the inflammatory response (42).

Based on the gained results we can conclude that suppressed functionality of the fibrinolytic mechanism triples the risk of occurrence of deep vein thrombosis while elevated TAFI level doubles the risk of this disease. There is no influence of plasminogen concentrations, t-PA, and PAI-1 levels on venous thrombosis risk.

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ABBREVIATION LIST

CI – confidence interval

DVT – deep vein thrombosis

ECLT – euglobulin clot lysis time

FV – factor V

FVIII – factor VIII

OR – odds ratio

PAI-1 – plasminogen activator inhibitor-1

PAI-2 – plasminogen activator inhibitor-2

PE – pulmonary embolism

TAFI – thrombin activable fibrinolytic inhibitor

t-PA – tissue plasminogen activator

u-PA - urokinase plasminogen activator

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