Level of antithrombin III, protein C, protein S and other selected parameters of coagulation and fibrinolysis in the blood of the patients with recurrent deep venous thrombosis

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Summary

Background: Thrombophilia is caused mainly by disturbances of hemostasis involving excessive coagulation system activation, reduction of anticoagulation system (antithrombin III, protein C, protein S, RAPC) or fibrinolytic activity.

Material/Methods: In 34 young patients (aged <40 years) with recurrent deep venous thrombosis (>2 incidents) the activity of antithrombin III, protein C, S, platelet count, adhesion and aggregation, APTT, stipven-kephalin, prothrombin time and INR were investigated. Fibrinogen, factor XIII, ELT, FDP, Ag t-PA levels, antigen concentration and PAI-1 activity were determined. Patients with idiopathic DVT, after elimination of most important thromboembolism risk factors, were qualified for the study. DVT was confirmed in all patients by phlebography, plethysmography and ultrasonography. Results were compared with a group of 54 healthy controls.

Results: In almost 50% of patients with recurrent DVT (15/34) decrease of at least one plasma coagulation inhibitor (AT III, PC, PS) level was observed. In the patient group (with AT III and/or PC and/or PS decrease) statistically significant reduction of kaolin-kephalin time in comparison with controls was observed ($\alpha<0.01$). Analysis of fibrinolysis system demonstrated significant factor XIII level decrease (to 58.3%), fibrinogen level increase, ELT prolongation, and fibrinogen and fibrin degradation product increase in comparison with controls. The patients demonstrated 3-fold higher t-PA antigen level (13.1 ng/ml, $\alpha<0.0001$) and over 3-fold higher PAI-1 activity (26.7 AU/ml, $\alpha<0.001$) than healthy controls.

Conclusion: Reduced antithrombin III, protein C, protein S activity and excessive activation of the coagulation system with secondary fibrinolytic activity increase were found in patients with recurrent DVT.

key words: deep venous thrombosis • AT III • protein C • protein S • thrombophilia
BACKGROUND

Deep venous thrombosis develops as a result of coincidence of many factors. Thrombosis appearing in patients below 40 years of age with no known risk factors is defined as congenital thrombophilia [1,2]. Disturbances of hemostasis involving excessive coagulation system activation, or reduction of anticoagulation system activity (antithrombin III, protein C, protein S, RAPC) or fibrinolytic activity are the main causes of thrombophilia.

Reduction of AT III, PC, PS were the most common abnormalities observed before 1994 and together accounted for 9–21% of genetically determined thrombophilia cases [3,4]. Clinically, they are reflected in 70% by deep venous thrombosis of the lower extremities [5,6].

The aim of the study was to assess the levels of individual plasma coagulation inhibitors in patients with recurrent DVT, as well as coagulation and fibrinolysis system activity.

MATERIAL AND METHODS

The study was carried out in a group of 88 subjects, 36 females and 52 males aged 18–60, consisting of (F/M 22/12) patients with recurrent DVT and 54 healthy subjects, constituting the reference group (F/M 14/40). The patient group was selected from among several hundred patients treated for DVT in the Regional Outpatient Department of Vascular Diseases. In all 34 patients, the onset of the disease took place before they reached 40 years of age (at the age of 38 at the average) and it recurred at least twice. None of the patients had received antithrombotic agents (heparin, oral anticoagulants) during 6 months preceding inclusion in the study. The patients with most common thrombosis risk factors, such as obesity with obesity index exceeding 16, immobilization, recent surgical procedures, patients with symptoms of any acute and chronic diseases, e.g. acute infections, heart, liver, kidney diseases and diabetes, were excluded from the study. Also those with abnormalities in accessory investigations such as WBC, transaminase levels, BUN, creatinine, seromucoid, glucose, proteinogram were excluded.

In all the patients, the history of idiopathic DVT was confirmed by ascending phlebography, resistance plethysmography and real time ultrasonography using an MVL-MODULAB Life Sciences, Inc. and a SONOLINE SL-1 Siemens sonograph (tab. 1).

Blood samples were collected at least 6 months after the last thrombotic incident. None of the patients was taking any antithrombotic drugs at that time. The analyses were carried out in blood collected during morning hours. Blood was collected from the ulnar vein into a plastic tube containing 3.2% sodium citrate at 9:1 ratio, and immediately mixed and centrifuged at room temperature and 2500 rpm. For 10 min. plasma obtained in this way was used for determinations of kaolin-kephalin time after maximum factor XI and XII activation by kaolin in the presence of constant phospholipid concentration. Kephalin and calcium chloride were added to plasma prepared in this way and incubated for 10 min at 37°C. [7]. Stipven-kephalin time was determined using a proteolytic enzyme contained in Vipera Russeli venom [8], prothrombin time according to Quick [9], and euglobulin clot fibrinolysis time [10].

FDP determination by Merskey’s method was carried out in the serum obtained from whole bood collected on a clot and incubated for 3 h at 37°C. The method makes use of a phenomenon of agglutination caused by anti-sibrinogen antibodies. Euglobulin clot fibrinolysis time was determined by measuring the time of liquefaction of an euglobulin clot. Euglobulin precipitate is obtained by acidification of plasma with acetic acid solution to pH 5.3. The precipitate obtained in this way is dissolved in 0.5 ml borate buffer and coagulated with calcium chloride [11]. Fibrinogen concentration was determined by measuring tyrosine content in coagulated protein using Folina-Cicolteau method [12]. Activity and level of t-PA antigen, as well as PAI-1 activity were determined using Biopool kits.

AT III and PC were determined using kits manufactured by Behring and Kabi-Vitrum, and PS activity – using immunoenzymatic Diagnostica Stago kit with specific anti-PS antibodies.

The results were compared between the patient and control groups, and also within the patient group according to plasma coagulation inhibitor levels.

RESULTS

In the group of selected 34 patients with the history of recurrent DVT, decreased levels of at least one plasma coagulation inhibitor were found in 15 cases (Tab. 2).
Considering all the patients with the history of DVT, a statistically significant reduction of APTT (p<0.001) and decrease of factor XIII activity (to 64.1%) was observed in comparison with healthy controls (p<0.0001). The patient group exhibited also significantly higher fibrinogen levels and prolonged ELT (p<0.001), as well as a significant increase of FDP - to 11.7 mg/ml (p<0.0001). DVT patients were also found to have 4-fold higher levels of t-PA antigen, amounting to 14.5 ng/ml (p<0.0001) and 2-fold higher PAI-1 – 19.5 AU/ml (p<0.0001) than the reference group (tab. 3 and 4).

Selected coagulation and fibrinolysis system parameters in patients with recurrent deep venous thrombosis demonstrating a decrease of plasma fibrinolysis inhibitors in comparison with those found in healthy controls are presented in tables 5 and 6.

In the group of patients (with AT III and/or PC and/or PS) decrease, a statistically significant reduction of kaolin-kephalin time was observed in comparison with healthy subjects (α<0.01). Analysis of the fibrinolysis system revealed a significant decrease of factor XIII level (to 58.3%), increase of fibrinogen and fibrin level, prolonged ELT and increase of fibrinogen and fibrin degradation products in comparison with the control group. The patients were also characterized by 3-fold higher level of t-PA antigen (13.1 ng/ml, α<0.0001), as well as over 3-fold increase of PAI-1 activity (26.7 AU/ml, α<0.0001) in comparison with healthy subjects (tab. 6).

In 1 patient coincident decrease of AT III and PS, and in 2 – of PS and PC was observed (fig. 1).

Mutual correlations between the values of coagulation system inhibitors were also analyzed. As it follows from
no significant correlations were found between AT III and PC levels. It was observed, however, that there is a correlation between protein C and S levels, but the correlation did not reach statistical significance.

The correlation between coagulation system parameters was found in the group of patients on analysis of PAI-1 and t-PA antigens. The values have been presented in table 1. Fig. 2 presents in graphic form the correlation between these parameters.

Positive familial history was found in 15 patients with decreased coagulation parameters.

Familial history of recurrent DVT was obtained from 71% of patients with decreased AT III, 75% of patients with decreased PC and 66% of patients with decreased PS levels.

**DISCUSSION**

The group of so-called idiopathic venous thromboses (IVT) or thrombophilias includes patients in whom venous thrombosis occurred below the age of 40–45 without any triggering factors. Such patients usually have positive familial history and are at risk of recurrent episodes of the disease. Studies initiated in the 1960's allowed to isolate a group of thromboses determined by genetic factors, which may account for 10.0–37.5% IVT [1,4]. The presented study investigated a group of patients with the history of DVT of unknown origin. All the patients complied with the definition of idiopathic thrombosis. [2,13].

The studied group with thrombophilia demonstrated in 23% of cases decreased AT III levels, in 31% – protein S and in 11% – protein C levels. According to other authors, the incidences of the above abnormalities amount to 3–36% for AT III, 1–18% for PS and 5–32% for PC [6,14–16]. The wide range of results obtained by different authors results primarily from the lack of uniform selection criteria. Other abnormalities, e.g. resistance to APC reported by Antignani, may mimic low PS activity, and the degree of cell saturation with thrombomodulin or the amount of active factor V may obscure PC deficiency. Therefore, it is difficult to determine the actual incidence of DVT in retrospective studies carried out in large groups of patients with coagulation inhibitor deficiency [14,17,18]. A relatively high proportion of patients with deficient inhibitors in the studied group results from strict selection criteria, including e.g. lack of immediate triggering factor and absence of concurrent diseases increasing the risk of thrombosis. No family histories, or data concerning recurrences and early onset of the disease were taken in the literature from 33% of patients with deficient plasma coagulation inhibitors. On the other hand 30% of patients with the above three elements demonstrate the deficiency of one or more inhibitors [19]. According to Liu, positive family histories are found in 18–60% of patients with confirmed congenital deficiency [15]. In our material, 53% of patients with positive histories concerning recurrence, early onset and familial occurrence of the disease were found to have decreased levels of plasma coagulation inhibitors, including two cases of coincident PS and PC and 1 of PS and PC deficiency. On the other hand 30% of patients with the above three elements demonstrate the deficiency of one or more inhibitors [19]. According to Liu, positive family histories are found in 18–60% of patients with confirmed congenital deficiency [15]. In our material, 53% of patients with positive histories concerning recurrence, early onset and familial occurrence of the disease were found to have decreased levels of plasma coagulation inhibitors, including two cases of coincident PS and PC and 1 of PS and PC deficiency. On the other hand 30% of patients with the above three elements demonstrate the deficiency of one or more inhibitors [19]. According to Liu, positive family histories are found in 18–60% of patients with confirmed congenital deficiency [15]. In our material, 53% of patients with positive histories concerning recurrence, early onset and familial occurrence of the disease were found to have decreased levels of plasma coagulation inhibitors, including two cases of coincident PS and PC and 1 of PS and PC deficiency. On the other hand 30% of patients with the above three elements demonstrate the deficiency of one or more inhibitors [19]. According to Liu, positive family histories are found in 18–60% of patients with confirmed congenital deficiency [15]. In our material, 53% of patients with positive histories concerning recurrence, early onset and familial occurrence of the disease were found to have decreased levels of plasma coagulation inhibitors, including two cases of coincident PS and PC and 1 of PS and PC deficiency. On the other hand 30% of patients with the above three elements demonstrate the deficiency of one or more inhibitors [19].

**Table 7.** Mutual correlations of plasma coagulation inhibitors in the studied group of patients with recurrent deep venous thrombosis.

<table>
<thead>
<tr>
<th>Correlated pairs</th>
<th>Correlation coefficient k</th>
<th>Regression coefficient r</th>
<th>Student-t test</th>
<th>Significance level α</th>
</tr>
</thead>
<tbody>
<tr>
<td>antithrombin III /protein C</td>
<td>0.105</td>
<td>-0.08</td>
<td>-0.49</td>
<td>ns</td>
</tr>
<tr>
<td>antithrombin III /protein S</td>
<td>0.029</td>
<td>-0.02</td>
<td>-0.13</td>
<td>ns</td>
</tr>
<tr>
<td>protein C/protein S</td>
<td>0.2</td>
<td>0.17</td>
<td>1.0</td>
<td>ns</td>
</tr>
</tbody>
</table>

**Table 8.** Correlation between the levels of t-PA and PAI-1 antigen in the studied group of patients with recurrent deep venous thrombosis.

<table>
<thead>
<tr>
<th>Correlated pairs</th>
<th>Correlation coefficient k</th>
<th>Regression coefficient r</th>
<th>Significance level α</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ag t–PA/Ag PAI–1</td>
<td>0.77</td>
<td>1.62</td>
<td>0.001</td>
</tr>
</tbody>
</table>

**Figure 2.** Correlation between the levels of t-PA and PAI-1 antigen in the studied group of patients with recurrent deep venous thrombosis.
The mean age of onset of the first DVT incident in the studied group was 38 and fell within the range reported by other authors (20–40 years of age) [6,18,21]. Most authors emphasize the occurrence of the first incidence of thrombosis in subjects with deficient inhibitors before 45 years of age [1,20,22].

Among 31 patients who had AT III levels determined, their decrease below 70% was observed in 7. Other authors report the values of 43.8–5.4% [14, 23]. AT III deficiency may have favored thrombosis in the studied group. Undoubtedly, it is difficult to answer whether the decreased AT III levels observed in the studied group were primary or secondary. The lack of evident triggering factor, recurrent character and early onset of thrombosis (below 40) suggest a congenital, primary character of the deficiency [24, 25].

Genetically determined causes of DVT may also be supported by positive family history. 71% of patients with AT III deficiency reported in anamnesis the occurrence of similar disorders in the family (mother, father, sister, brother, grandparents), whereas among 11 subjects with normal levels of all plasma coagulation inhibitors only 18% confirmed positive family history. Thomas et al. claim that inhibitor deficiency and positive family history allow to establish a diagnosis of congenital disorder [26]. However, the genetic character of the observed abnormalities can be confirmed only by investigation of all family members of the patients with inhibitor deficiency or investigations of DNA structure [4]. Familial thrombosis is, however, unlikely in view of lack of thrombotic changes in atypical sites such as the retina, kidneys or brain [27].

Laboratory tests performed before qualification of the patients for the study excluded the most common disorders leading to reduced synthesis or loss of plasma coagulation inhibitors through the kidneys. Some diseases, which might have been present in laten form, e.g. neoplasms, could not be excluded completely [28, 29]. The 6-month period elapsed from discontinuation of antithrombotic medication was meant to eliminate the influence of these drugs on the hemostasis system or to detect chronic diseases, which might be manifested during that time.

AT III deficiency leads to enhanced thrombin generation and hypercoagulability. This is supported by increased aggregation, reduced kaolin-kephalin time and elevated fibrinogen. Studies by Elque demonstrated that even a moderate decrease of plasma AT III leads to enhanced thrombin generation and shortens the clotting time [30]. This is consistent with the observations made by other authors, who confirm it by assessing more specific markers, such as F 1+2 fragments or Fibrynopeptide A and TAT complex [31–34]. Marked deficiency of factor XIII in the group of DVT patients as compared with the controls (p<0.0001) was probably associated with its utilization during continuously stimulated coagulation process [35,36]. Decreased levels of coagulation inhibitors in the group with deficiency of any plasma coagulation inhibitors enhanced the activity of the coagulation system, which was reflected in reduced kaolin-kephalin time and higher utilization of factor XIII in comparison to the group with normal inhibitor levels.

Patients with recurrent DVT were found to have decreased t-PA and PAI-1 levels, which was particularly marked in the patients with deficiency of any plasma coagulation inhibitors. Decreased levels of inhibitors caused probably activation of the intrinsic coagulation pathway [19,37]. Increased fibrinolytic activity accompanied increased coagulability in the whole group of patients with recurrent DVT. Consequently, an increase of t-PA led to enhanced PAI-1 release, which was confirmed by Levin et al. [38].

Positive correlation between t-PA and PAI-1 observed in our study has been confirmed by other authors [39]. It cannot be excluded that high PAI-1 level in the studied group was not a risk factor present before the onset of the disease [21,26,40]. The studies by Cogo, Elías and their co-workers describe persistent hypercoagulability condition in subjects with the history of DVT and confirm our findings [33,41]. The observed fibrinogen level increase reflects quite well the increased coagulation potential in the studied group and may be at least partially secondary to a chronic intravascular coagulation process and partially to stimulation by fibrinogen and fibrin degradation products, and their D fragment in particular [42]. The data concerning fibrinogen levels and recurrent DVT are contradictory. Lowe finds no correlation between the level of fibrinogen, blood viscosity and history of DVT [43]. High fibrinogen level may be a factor predisposing for hypercoagulability by increase of viscosity and formation of platelet aggregates [37,39,44,45].

Increased FDP concentrations in the patient group can be explained by the predominance of active t-PA, protected in a way by continuously formed fibrin deposits inside venous blood vessels, over PAI-1 [46,47]. It is inconsistent with the results obtained by Korninger and Arandy and their co-workers, who did not observe decreased t-PA release in patients with DVT [48,49]. The above findings allow to state that reduction of APTT, decrease of factor XIII, AT III, PC and PS levels, as well as that of fibrinogen, indicate the hypercoagulability condition in patients with DVT. On the other hand, despite the decrease of PAI-1 and prolongation of ELT, marked increases of t-PA and FDP indicate also a slight decrease of fibrinolytic activity in that group of patients.

**Conclusions**

1. Decreased antithrombin III, protein C and protein S activity was observed in the group of patients with recurrent deep vein thrombosis.

2. The finding of decreased level of plasma coagulation inhibitors is an important diagnostic element in the search for the causes of recurrent deep venous thrombosis.
3. The group of patients with recurrent deep venous thrombosis demonstrated excessive activation of the coagulation system and secondary increase of fibrinolytic activity.

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