Possible predictors of hereditary thrombophilia in a group of patients with thrombosis of undetermined cause

Predictori posibili ai trombofiliei ereditare la un grup de pacienți cu tromboză de cauză nedeterminată

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Astract

Background: There are certain clinical circumstances in which laboratory thrombophilia screening is recommended. Aim: To determine the value of generally accepted clinical criteria in predicting hereditary thrombophilia (HT). Methods: 180 patients with thromboembolism (TE) of undetermined cause were tested for HT: protein S, protein C, and antithrombin deficiency, activated protein C resistance, elevated factor VIII level and hyperhomocysteinemia were assessed. Positive family history of TE, first thrombotic event at young age (under 45), TE at unusual sites and spontaneous TE were analyzed as predictive factors for positive HT test results. The characteristics that reached at least 0.1 as a level of significance were included in a logistic regression model. Results: 94 patients with at least one thrombophilic defect were found. A positive family history of TE had a statistically significant positive influence on the risk of HT (p=0.027; OR=0.053, 95% CI 0.989 – 3.492). First thrombotic event under 45 and spontaneous TE had a statistically significant negative influence on the risk of HT (p=0.008; OR=0.016, 95% CI 0.119 – 0.840 and p=0.001; OR=0.004, 95% CI 0.231 – 0.765, respectively). Conclusions: A positive family history of thromboembolism could be a predictor for hereditary thrombophilia in patients with thromboembolism. Independent criteria like young age, unusual site of thrombosis or spontaneous thrombotic events are not reliable tools to identify this pathology and they should be considered in connection to each other.

Keywords*: hereditary thrombophilia, predictive factors, thromboembolism*

Rezumat

Premise: Screening-ul de laborator al trombofiliilor este recomandat în anumite circumstanțe clinice. Scop: evaluarea unor criterii clinice general acceptate ca predictive pentru trombofiliile ereditare. Metode: Au fost testați pentru trombofilie ereditară 180 de pacienți cu tromboembolism de etiologie neprecizată: deficit de proteina C, proteina S sau antitrombina, rezistența la proteina C activată, nivel crescut de factor VIII şi hiperho-

mocisteinemie. Au fost analizaţi ca factori predictivi pentru diagnosticul pozitiv de trombofilie ereditară: istoricul familial de tromboembolism, vârsta tânără, sub 45 de ani, la primul eveniment trombotic, localizarea atipică a trombozelor şi absenţa factorilor precipitanţi. Caracteristicile semnificative statistic au fost incluse într-un model logistic regresional. Rezultate: Au fost depistaţi 94 de pacienţi cu cel puţin un defect trombofilic. Prezenţa istoricului familial de tromboembolism a avut o influenţă pozitivă, semnificativă statistic asupra riscului de trombofilie ereditară (p=0.027; OR=0.053, 95% CI 0.989 – 3.492). Vârsta tânără şi trombozele spontane au avut o influenţă negativă semnificativă statistic asupra riscului de trombofilie ereditară (p=0.008; OR=0.016, 95% CI 0.119 – 0.840, respectiv p=0.001; OR=0.004, 95% CI 0.231 – 0.765). Concluzii: La pacienţii cu tromboembolism, prezenţa unui istoric familial de tromboze poate constitui un factor predictiv pentru diagnosticul de trombofilie ereditară. Vârsta tânără, localizarea atipică sau lipsa factorilor precipitanţi pentru evenimentele trombotice, nu reprezintă criterii sigure de identificare a acestei patologii dacă sunt considerate individual, ele trebuind interpretate în ansamblu.

Cuvinte cheie: *factori predictivi, tromboembolism, trombofilie ereditară*

Introduction

Hereditary thrombophilia can be defined as a genetically determined tendency to thromboembolism. During the last decades, a number of genetic risk factors associated with hypercoagulability have been identified: deficiencies of antithrombin (AT), protein C (PC), or protein S (PS), activated protein C resistance (APCR) due to the factor V gene Leiden mutation (Arg506Gln), hyperhomocysteinemia, elevated factor VIII levels and the prothrombin gene G20210A variant. Their presence is used as a tool for stratifying the patients into groups at different levels of risk of thromboembolic events' recurrence, and accordingly adapting the duration of the anticoagulant therapy.

Routine screening for hereditary thrombophilia remains an expensive laboratory exercise. Therefore, testing for the above mentioned risk factors needs justifiable clinical criteria to orientate the practitioner towards ordering such investigations. In textbooks and scientific papers the clinical features presented as suggestive for an inherited thrombotic disorder are: thrombosis occurring at an early age, a family history of thrombotic disease, thrombosis occurring at unusual sites (e.g. mesenteric or cerebral venous thrombosis), recurrent thrombosis with or without apparent precipitating factors, recurrent thrombosis during adequate anticoagulant therapy, recurrent miscarriage, preeclampsia, HELLP syndrome, and/or warfarin-induced skin necrosis (1-3).

The aim of this study was to determine the predictive value of some of the generally accepted criteria in predicting hereditary thrombophilia: family history of thrombosis, early age at onset of the thrombotic event, unusual site of thrombosis, and the lack of apparent precipitating factors.

Patients and methods

The study included patients diagnosed with thromboembolism of undetermined cause who were referred to the Hematology Department of the Municipal Hospital of Timişoara between 2002 and 2008. They formed a group of "primary patients" suspected of hereditary thrombophilia and consisted of subjects who met at least one of the following criteria: (i) first thrombotic event before 45 years (age $\leq$ 45); (ii) unprovoked first thrombotic event; (iii) thrombotic event at unusual site; (iv) family history of thromboembolism; (v) recurrent miscarriage.

A thrombotic event was considered as unprovoked if no circumstantial risk factors could be identified in a period of 30 days before it had occurred. Circumstantial or precipitating risk factors included: prolonged orthostatism, chair sitting or driving more than five hours, train or airplane travel longer than eight hours, immobilization for more than 14 days, trauma, surgery, pregnancy, puerperium, and the use of oral contraceptives or hormonal replacement therapy. The family history was

considered as positive when at least one of the first- or second-degree relatives had had a provable thrombotic event.

Data from each patient were collected, using an adapted validated questionnaire (4), They included: date of birth, family history of thromboembolism, existing conditions for prolonged static performance, medication, history of thrombotic events (age, site, known circumstantial or precipitating factors), obstetrical events.

When possible, the previous thromboembolic events were documented, otherwise they were taken into consideration if an anticoagulant treatment was associated.

The study was approved by the Ethics Committee of the University of Medicine and Pharmacy "Victor Babeş" of Timişoara and the patients signed an informed consent.

All the primary patients were tested for: PC, PS and AT functional activity, APCR, factor VIII activity and homocysteine level (IL Test™ ProClot, IL Test™ Protein S, IL Test™ Antithrombin III, IL Test™ APC™ Resistance V, HemosIL Test™ Factor VIII deficient plasma – Instrumentation Laboratory kits, ACL2000 analyzer, IMx Homocysteine – Abbott kit, ABBOTT IMx® analyzer). Except for the factor VIII activity, the reference levels for each parameter were determined by testing a cohort of healthy individuals who matched, in age and sex, the patients.

Patients with at least one pathological result from the functional tests (PC, PS, AT and/or APCR) were retested in six months. Possibly acquired causes, i.e. infection, inflammation, neoplasia, liver failure, or lupus anticoagulants, were excluded. When the case, in the second determination, the chromogenic method for PC activity was used (IL Test™ Protein C – Instrumentation Laboratory). When the functional deficiency for PC or PS was confirmed, antigenic levels for PC, free PS and total PS, respectively, were determined by enzyme-linked immunosorbent assay (ELISA, Asserachrom, Stago).

Statistical analysis

Statistical analysis was performed using SPSS v. 15.0. The results for continuous variables were expressed as mean values, standard deviations, and ranges; for the categorical data, results were presented as counts and percentages.

Univariate analysis with estimated Odds Ratios for potentially predictive factors was applied. Factors with over 0.1 significance were included in a logistic regression model of multivariate analysis. In the regression model, the statistical significance of the predictive factors and the pseudo-R2 Nagelkerke coefficient of determination were calculated, followed by the analysis of the quality of risk prediction.

For the statistical tests and risk estimates, the 0.05 (i.e. 5%) two-tailed level of significance was considered, with a 0.95 (i.e. 95%) confidence interval (CI) around the incidence rates or the point estimates.

Results

Our study included 180 patients as "primary patients", 76 males and 104 females, aged between 16 and 69. Of the primary patients, 94 were found with at least one thrombophilic defect. The identified thrombophilic defects are presented in *Table 1*. The main characteristics, both for the whole group of primary patients and for the thrombophilic ones are summarized in *Table 2.*

In the group of primary patients, there was no case of obesity. Eight of the 104 enrolled females (7.7%) were using oral contraceptives. From these, seven (87.5%) were found with a thrombophilic defect.

Among the 75 thrombophilic patients with venous thrombosis, 33 (35%) were with deep vein thrombosis and 42 (45%) with superficial vein thrombosis of the lower-extremities. Eight patients with at least one thrombophilic defect experienced the first thrombotic event at an unusual site: retinal (two patients), portal,

Table 1. Defects found in thrombophilic patients (n=94)

Defect	Number of patients (*percentage*)
Protein C deficiency	17 (18%)
Protein S deficiency	12 (13%)
Activated protein C resistance	15 (16%)
High factor VIII	14 (15%)
Hyperhomocysteinemia	16 (17%)
Combined defects	20 (21%)*

* There were 20 patients (21%) with two or three combined defects; 17 patients with two defects: PC deficiency combined with PS deficiency, AT deficiency, high factor VIII level or hyperhomocysteinemia (1, 2, 2, and 3 patients respectively); PS deficiency combined with APCR, high factor VIII level or hyperhomocysteinemia (1, 1, and 3 patients respectively), APCR combined with hyperhomocysteinemia or high factor VIII level (3 and 1 patient,respectively). Three patients were found with three defects: PC deficiency combined with high factor VIII level and hyperhomocysteinemia, APCR combined with high factor VIII level and PS deficiency or hyperhomocysteinemia (1 patient for each association).

Table 2. The main characteristics of primary and thrombophilic patients. Except for the age, all the other characteristics are expressed as numbers of patients and percents calculated from the respective *n*

Characteristic	Primary patients	Thrombophilic patients
n	180	94
Males	76 (42%)	48 (51%)
Age in years: mean ± std dev (range)	37 ± 11 (16 – 69)	38 ± 12.35 (16 – 69)
Young age (≤ 45) at onset of first thrombotic event	156 (87%)	76 (81%)
Positive family history of thrombosis	61 (34%)*	38 (40%)
Venous thrombosis/pulmonary embolism	120/5 (67%/3%)**	75/4 (80%/4%)
Miscarriage	24 (13%)	3 (3%)
Cerebral stroke	14 (8%)	3 (3%)
Acute myocardial infarction	2 (1%)	none
Unusual sites of thrombosis	14 (8%)***	8 (8%)
Unprovoked first thrombotic event	93 (52%)	39 (41%)

* Positive family history of thrombosis included patients' first- or second-degree relatives with at least one of the following previous events: deep venous thrombosis for 31 patients (17%); cerebral stroke for 18 patients (10%); acute myocardial infarction for 8 patients (4%); miscarriage for 3 patients (< 2%); and portal thrombosis for 1 patient (0.6%).

** Patients with venous thrombosis experienced as their first thrombotic event a deep or a superficial vein thrombosis of the lower-extremities: the groups consisted of 56 and 64 patients, respectively (31% and 36%, respectively).

*** Unusual sites of first thrombotic event included: retinal thrombosis (4 patients), thrombosis of the upper-extremity (3 patients), portal thrombosis (2 patients), cava, jugular, suprahepatic, and renal thrombosis (1 patient with each of these sites), and mesenteric infarction (1 patient). One patient was included due to positive thrombophilic family history only, without personal thromboembolic history.

cava, jugular, renal, mesenteric, and upper-extremity. For less than half (41%) of these patients with at least one thrombophilic defect, the first thrombotic event happened without any evident precipitating factors.

The univariate analysis of the considered possible predictors for hereditary thrombophilia is synthesized in *Table 3.*

The characteristics which reached at least 0.1 as a level of significance were included

Table 3. The univariate analysis of potential risk factors for hereditary thrombophilia

Characteristic	**Thrombophilic patients**		Statistical significance (Pearson Chi-square, df=1)
	+	-	**OR** [95% CI]
Young age (≤ 45) at onset of first thrombotic event	76	80	0.016*
absent	18	6	0.317 [0.119 - 0.840]
Family history of thrombosis	38	23	0.053[ms]
absent	56	63	1.859 [0.989 - 3.492]
Unusual sites of thrombosis	8	6	0.7
absent	86	80	0.420 [0.412 - 3.731]
Unprovoked first thrombotic event	39	54	0.004**
absent	55	32	0.420 [0.231 - 0.765]

* for significant results; ** for very significant results; [ms] for marginally significant results.

Table 4. The logistic regression model for hereditary thrombophilia

Characteristic (considered risk factors)	**Coefficient of determination *pseudo-R²* *Nagelkerke:* 0.147 Sensitivity = 70.2; Specificity = 58.1**				
	B	S.E.	Wald score	df	p
Young age (≤ 45) at onset of first thrombotic event	-1.385	.522	7.044	1	.008**
Unprovoked first thrombotic event	-1.050	.324	10.491	1	.001**
Positive family history of thrombosis	.757	.341	4.923	1	.027*
Constant	.548	.502	1.189	1	.275

* for significant results; ** for very significant results.

in a logistic regression model, with the presence of the thrombophilic defect as the dependent variable. The results are presented in *Table 4.*

As we can see, a positive family history of trombosis had a small, but positive influence on the risk of hereditary thrombophilia, statistically significant at the same time (p=0.027). Contrary to initial expectation at the beginning of the study, both the young age at onset of the first thrombotic event and the unprovoked first thrombotic event proved to have a statistically significant negative influence on the risk of thrombophilic defect.

At the same time, one can notice the low value of the coefficient of determination, indicating that this model can explain less than 15% of the risk in the hereditary thrombophilia. Although the sensitivity of the predictive model is over 70%, the specificity is less than 60%. This indicates that, based on this model, we can correctly indicate an increased risk for hereditary thrombophilic defect in 70% of the positive cases, and its absence in only about 60% of the negative cases. These values are not surprising, as the coefficient of determination clearly shows that other risk factors should be taken into consideration (in addition to these three included in the logistic regression) when estimating the probability of hereditary thrombophilic defect.

Discussion

In our study, the prevailing single thrombophilic defect was PC deficiency. Data reported in literature indicate APCR as the

most frequent hereditary defect encountered in patients with recurrent thrombosis, with an incidence of 20-50 %, while PC, PS and AT deficiencies have a lower incidence of 2-10% each [5]. In our study, when each defect was independently considered, we identified 26 cases with PC deficiency (27.7 %), 22 with APCR (23.4%), 19 with PS (20.2%), and two with AT (2.1%, both of them combined with PC deficiency – see Table 1). This incidence of PS and PC deficiency could be partially explained by the restrictive selection of the patients included in the study. Therefore, the interpretation of these results is limited.

Use of oral contraceptives was encountered in seven thrombophilic women, but the low incidence did not allow any statistical analysis for this group.

Our results showed that a young age at the onset of first thrombotic event, unusual sites of thrombosis or unprovoked thrombotic events could not be considered as predictors for a positive diagnosis of hereditary thrombophilia when considered alone. Although a positive family history could be considered a predictive factor for inherited thrombophilia, its predictive value is still poor when considered alone.

In literature, the predictive value of a positive family history of thrombosis is controversial in orientating the clinicians towards hereditary thrombophilia testing. Lijfering et al (6), conducted a retrospective study with 877 probants and 5202 relatives, concluding that a strong positive family history of thrombosis (i.e. > 20% of relatives) could be used to identify patients with antithrombin, protein C or protein S deficiencies, as strong thrombophilic defects. Briéut et al (7) concluded, in their study, that the family history was a useful diagnostic test for inherited thrombophilia when used in a "critical way". On the other side, Amberger et al (8) demonstrated (on 56 patients) that a negative family history of venous thrombotic events was not sufficient to exclude thrombophilia. Van Sluis et al (9) designed a study considering three methods of characterizing the family history: positive family history, strongly positive family history, and family history score. They demonstrated that a simple positive family history did not accurately separate the two classes of thrombotic patients (i.e. with and without inherited thrombophilia). In our study, the criterion used for defining a positive family history, i.e. at least one relative with thromboembolism (without taking into account the number of relatives and their ages), matched the weakest method in van Sluis' article (10). Nevertheless, our results sustain the idea that a positive family history of thromboembolism should be taken into consideration when decision for thrombophilia testing is made.

In the present study, the young age at the onset of first thrombotic event failed as a predictive criterion for identifying the patients with hereditary thrombophilic defects. Our findings did not confirm those in Lijfering's study [6], where the young age could be used to identify patients with strong thrombophilic defects. This might be due to the fact that the included patients were already selected, having been referred to the Hematology Department of the Municipal Hospital of Timişoara for thromboembolism of undetermined cause. Most of those who entered the study were young, with 87% being under 45 years of age at the first thrombotic event. We should also take into account the fact that the mild thrombophilic defects need additional risk factors to trigger a thrombotic event. Therefore, many thrombophilic patients experience their first venous thrombotic event later in life. Nevertheless, considering the limitations of this study, our results should be seen only as hypotheses until more patients are investigated.

Cases of thrombosis at unusual sites are rare. In our study, the low number of such cases made the results inconclusive. In a retrospective case-control study (10), patients with thrombosis at unusual sites had a significantly

higher prevalence of thrombophilic defects compared to healthy controls, so the authors concluded that screening for hereditary thrombophilia was warranted in all patients with thrombosis at unusual sites except in those with retinal vein occlusion.

Thrombosis without triggering factors had no predictive value for hereditary thrombophilia in our study, even when strong thrombophilic defects were present.

The logistic regression model, which included family history, young age and unprovoked thrombotic events, confirmed our findings. However, the logistic regression explained only 14.7% of the risk of the hereditary thrombophilia, the only significant condition being the presence of positive family history of thrombosis. The relatively high sensitivity of 70% but low specificity (58%) of the model suggest that the classical criteria of suspecting a thrombophilic defect should be considered in connection to each other, while other criteria should be considered, as well. They could raise the suspicion of hereditary thrombophilia but cannot predict a positive or a negative accurate diagnosis.

Our study had some limitations, which introduced a bias in the results. A first one was the absence of prothrombin level determination. This thrombophilic risk factor, together with the activated protein C resistance is considered to be frequent in Caucasians (11, 12) and its absence from the considered risk factors could have influenced our results. A second limitation consisted of the entering criteria used for selecting the patients in the study and its effect on the results was already mentioned. Both limitations were due to the financial constraints we faced, for we tried to limit the possible negative results in the laboratory testing.

In conclusion, a positive family history of thromboembolism could be a predictor for hereditary thrombophilia in patients with venous thromboembolism. Independent criteria like a young age at the onset of first thrombotic event, unusual site of thrombosis or unprovoked thrombotic episodes are not reliable tools for identifying this pathology when considered alone, so they should be considered in connection to each other.

Abbreviations

APCR = activated protein C resistance;
AT – antithrombin;
CI – confidence interval
OR – odds ratio;
PC – protein C;
PS – protein S,

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Disclosure of Conflict of Interests

The authors declare that they have no conflict of interest.

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