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PAI-1 4G/5G and MTHFR C677T polymorphisms increased the accuracy of two prediction scores for the risk of acute lower extremity deep vein thrombosis

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Abstract

Aim: This study investigates the accuracy of two scores in predicting the risk of acute lower extremity deep vein thrombosis. *Patients and Methods*: The study included 170 patients [85 (50%) women and 85 (50%) men] who were diagnosed with acute lower extremity deep vein thrombosis (DVT) with duplex ultrasonography. Median age was 62 (52.75; 72) years. The control group consisted of 166 subjects [96 (57.8%) women and 70 (42.2%) men], without DVT, matched for age (± one year) to those in the group with DVT. The patients and controls were selected from those admitted to the internal medicine, cardiology and geriatrics wards within the Municipal Hospital of Cluj-Napoca, Romania, between October 2009 and June 2011. Clinical, demographic and lab data were recorded for each patient. For each patient we calculated the prior risk of DVT using two prediction scores: Caprini and Padua. *Results*: According to the Padua score only 93 (54.7%) patients with DVT had been at high risk of developing DVT, while 48 (28.9%) of controls were at high risk of developing DVT. When Padua score included PAI-1 4G/5G and MTHFR C677T polymorphisms, the sensitivity increased at 71.7%. Using the Caprini score, we determined that 147 (86.4%) patients with DVT had been at high risk of developing DVT, while 103 (62%) controls were at high risk of developing DVT. A Caprini score higher than 5 was the strongest predictor of acute lower extremity DVT risk. *Conclusions*: The Caprini prediction score was more sensitive than the Padua score in assessing the high risk of DVT in medical patients. PAI-1 4G/5G and MTHFR C677T polymorphisms increased the sensitivity of Padua score.

Keywords: deep vein thrombosis, risk, Padua prediction score, Caprini prediction score.

Introduction

Venous thromboembolism [deep vein thrombosis (DVT) and pulmonary embolism (PE)] are the most common preventable causes of hospital-related death. DVT is a disease with high incidence, high morbidity and mortality, and significant socio-economic costs [1]. DVT is often asymptomatic, being confused with other diseases, and the lack of a routine necropsy highly underestimates the prevalence of this pathology. DVT occurs with an incidence of approximately one per 1000 adults [2]. In the United States, the annual prevalence of venous thromboembolism (VTE) is between 300 000 and 600 000 cases [3].

The etiology of DVT is extremely diverse. DVT occurs when coagulation homeostasis is characterized by an unbalanced procoagulation activity. This may be due to several factors working together. These factors can be grouped into: intrinsic (*e.g.*, thrombophilia), acquired (*e.g.*, obesity, cancer, prothrombotic medication) and external (*e.g.*, reduced mobility due to surgery, trips lasting more than four hours). Although these risk factors are known, DVT prophylaxis is still much underused. In some cases, preventing drugs are not used due to an increased risk of hemorrhage (uncontrolled hypertension, concomitant antiplatelet or anti-inflammatory medication, and so on). But, in most cases, high risk of DVT is ignored despite existing guidelines and recommendations [4].

DVT prophylaxis is effective when applied to cases that benefit the most from the substantially reduced thrombotic risk. There are several studies that have developed risk prediction scores. They combine anamnestic, clinical and laboratory data in an attempt to accurately identify cases that have high risks of developing DVT. Caprini *et al.* have created such a prediction algorithm based on 35 items [5]. This algorithm divides the risk of DVT into: low, moderate, high and very high. Another risk score is the Padua Prediction Score. It consists of 11 items and it classifies thrombotic risk into low and high [6]. The 9th edition of *ACCP* (*American College of Chest Physicians*) *Guidelines* for the prevention of DVT indicates the use of Padua score for assessing the risk of DVT within subjects hospitalized for medical illnesses, and the use of Caprini score for non-orthopedic surgical patients [7, 8].

This study investigates the accuracy of two scores in predicting acute lower extremity DVT. The study also

evaluated the additional effect of inherited thrombophilia on the accuracy of these scores.

Patients and Methods

This was an observational, analytical, transversal, case-control study.

The study included 170 patients [85 (50%) women and 85 (50%) men] who were diagnosed with acute lower extremity DVT with duplex ultrasonography. These patients represent the DVT group. Median age was 62 (52.75; 72) years. The control group consisted of 166 subjects [96 (57.8%) women and 70 (42.2%) men] close in age (± one year) to those in the group with DVT. The diagnosis of acute lower extremity DVT was excluded in controls by duplex ultrasonography. Patients and controls were selected from those admitted to internal medicine, cardiology and geriatric units of Municipal Hospital Cluj-Napoca, Romania between October 2009–June 2011.

Subjects were included in the study after signing the consent form for participation in the study and genetic determinations. The study protocol was approved by the Ethics Committee of "Iuliu Haţieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania.

Patients aged over 18 years were enrolled in the study after signing the informed consent form and after being diagnosed with acute lower extremity DVT. The diagnosis of acute DVT was established by Doppler ultrasonography, using an Aloka SSD 4000 unit, with a transducer with variable frequency of 7–10 MHz.

Patients receiving oral or injectable anticoagulant therapy at the time of recruitment were not included in the study.

General (age, gender, area of origin), clinical and laboratory data were recorded for each patient. Medical history and clinical examination provided data regarding the presence of comorbidities or different circumstances that could increase the risk for acute DVT: chronic obstructive pulmonary disease, hypertension, heart failure, cancer, cerebrovascular accident, history of DVT or PE, bed rest for more than three days, major surgery in the previous month, broken leg or pelvic fracture in the previous month, autoimmune or infectious diseases, plane or car journeys longer than four hours, varicose veins of the legs and local trauma. The presence or absence of concomitant chemotherapy was also noted. Body mass index (BMI) was calculated for each patient. For each subject, we calculated the Padua and Caprini scores.

A total of 3 mL of venous blood was collected from each patient into a Vacutainer containing EDTA. DNA was obtained from the blood samples using a DNA extraction kit (Wizard Genomic DNA Purification Kit, Promega, Madison, WI, USA). This procedure was performed within the Department of Genetics of "Iuliu Haţieganu" University of Medicine and Pharmacy, Cluj-Napoca.

A PCR-RFLP based protocol, originally described by Bertina *et al.* in 1994, was adapted for factor V Leiden mutation (g.1691 G>A, p.Arg506Gln) genotyping [9]. A PCR-RFLP based protocol, originally described by Ferraresi *et al.* in 1997, was adapted for prothrombin G20210A mutation genotyping [10].

PAI-1 polymorphism (plasminogen activator inhibitor-1) 4G/5G genotyping was performed by adapting a PCR-RFLP protocol, originally described by Brown *et al.* in 2001 [11].

MTHFR polymorphism (methylene tetrahydrofolate reductase) C677T genotyping was performed by adapting a PCR-RFLP protocol, originally described by A *et al.* in 2007 [12]. An ARMS-PCR protocol (Amplification Refractory Mutations System – Polymerase Chain Reaction) was used for MTHFR A1298C polymorphism genotyping, based on selective amplification of the mutant allele and of the normal allele in two different reactions, using two allele-specific primers (normal and mutant), and a common primer for both types of alleles, as previously described by Wu *et al.* [13].

A simplex PCR protocol was adapted for genotyping of CBS polymorphism (Cystathionine beta synthase) 844ins68, originally described by Boyles *et al.* in 2006 [14].

Statistical analysis was performed using the MedCalc Software, version 12.6.0.0. Data were labeled as nominal or continuous variables. Nominal variables were described using frequencies and continuous variables were characterized by median and percentiles (25–75%). The continuous variables were compared using Mann–Whitney test. A chi-square test was used for comparison of the frequencies of nominal variables. The sensibility, specificity, positive predictive value and negative predictive value of the two scores in predicting the onset of DVT were calculated. Deviations of allelic frequencies from Hardy–Weinberg equilibrium were calculated using a chi-square test. The agreement between the scores was assessed by calculating the Cohen's kappa coefficient.

The diagnostic value of the combinations of different scores was assessed using binary logistic regression and ROC analysis.

The level of statistical significance was set at $p<0.05$.

Results

In order to calculate probability risk scores for DVT, we recorded the following clinical and demographic variables (Table 1).

Table 1 – ***Clinical characteristics and comorbidities of patients in the DVT group and in the control group***

Variable	Patients with DVT	Controls
Age (median; percentile 25–75%) [years]	62 (52.75; 72)	63 (53.75; 72)
Men (N; %)	85 (50%)	70 (42.8%)
Women (N; %)	85 (50%)	96 (57.8%)
Rural area (N; %)	61 (31.8%)	75 (45.1%)
Urban area (N; %)	109 (64.1%)	91 (54.8%)
Cancer (N; %)	19 (11.1%)	6 (3.6%)
Chemotherapy (N; %)	8 (4.7%)	0
COPD GOLD III/IV (N; %)	7 (4.1%)	14 (8.4%)
Heart failure NYHA III/IV (N; %)	11 (6.4%)	30 (18%)
History of DVT (N; %)	76 (44.7%)	27 (16.2%)
Obesity (N; %)	44 (25.8%)	48 (28.9%)
Immobilization >3 days (N; %)	5 (2.9%)	0
Bed rest (N; %)	18 (10.5%)	9 (5.4%)
Travel >4 hours (N; %)	4 (2.3%)	0

Variable	Patients with DVT	Controls
Major surgery in the last month (N; %)	10 (5.8%)	0
Varicose veins (N; %)	87 (51.1%)	50 (30.1%)
Infections (N; %)	22 (12.9%)	15 (9%)
Autoimmune disease (N; %)	4 (2.3%)	1 (0.6%)
Bone fracture (N; %)	5 (2.9%)	0
Local trauma (N; %)	7 (4.1%)	0

For the DVT group, Padua score was calculated based only on clinical and demographic factors. The low score was 0, the high 10, the median 3 (1; 4.25). For the control group, Padua score was calculated based only on clinical and demographic factors. The low score was 0, the high 8, the median 1 (0; 3). Padua prediction score for DVT was of higher statistical significance in the DVT group than in the control group (p<0.001).

According to the cut-off value of 4 indicated by the authors of Padua prediction score, only 74 (43.5%) patients with DVT had presented an increased risk of developing DVT, while 31 (18.6%) of controls were at high risk of developing DVT. For Padua score, we calculated the following parameters: sensitivity of 43.5% (CI 95%, 35.9–51.3%), specificity of 81.3% (CI 95%, 74.5–86.9%), positive predictive value of 70.4% (CI 95%, 60.7–78.9%) and negative predictive value of 58.4% (CI 95%, 51.8–64.8%).

For the DVT group, Caprini score was calculated based only on clinical and demographic factors. The low score was 0, the high 10, the median 4.5 (3; 6). For the control group, Caprini score was calculated based only on clinical and demographic factors. The low score was 0, the high 10, the median 3 (2; 4). Caprini prediction score was of higher statistical significance in the DVT group than in the control group (p<0.001).

According to the cut-off value of 3 indicated by the authors of Caprini prediction score, 140 (82.3%) patients with DVT had been at high risk of developing DVT, while 94 (56.6%) of controls were at high risk of developing DVT. For Caprini score, we calculated the following parameters: sensitivity of 82.3% (CI 95%, 75.7–87.7%), specificity of 43.3% (CI 95%, 35.7–51.2%), positive predictive value of 59.8% (CI 95%, 52.3–66.1%) and negative predictive value of 70.5% (CI 95%, 60.7–79.2%).

The frequency of the thrombophilic risk factors in both groups can be observed in Table 2.

Table 2 – ***The frequency of thrombophilia in the DVT group and in the control group***

Variable		DVT group	Control group
Factor V Leiden mutation	Absent	130 (77.8%)	148 (90.7%)
	Present	37 (22.1%)	15 (9.2%)
Prothrombin G20210A mutation	Absent	158 (93.4%)	154 (93.3%)
	Present	11 (6.5%)	11 (6.6%)
PAI-1 polymorphism 4G/5G	4G/4G	51 (30.3%)	38 (23.4%)
	4G/5G	71 (42.2%)	95 (58.6%)
	5G/5G	46 (27.3%)	29 (17.9%)
CBS polymorphism 844ins68	ww	150 (89.2%)	147 (89%)
	wm	18 (10.7%)	18 (10.9%)
MTHFR polymorphism C677T	ww	63 (39.3%)	80 (50.9%)
	wm	71 (44.3%)	55 (35%)
	mm	26 (16.2%)	22 (14%)
MTHFR polymorphism	ww	72 (39.3%)	72 (45%)
	wm	77 (44.3%)	66 (41.2%)
	mm	13 (16.2%)	22 (13.7%)

For the DVT group, we calculated the Padua score also including the thrombophilic risk factors specified by the authors (factor V Leiden and prothrombin G20210A mutations). The low score was 0, the high score 13, the median 4 (1.75; 6). For the control group, we also calculated the Padua score including the thrombophilic risk factors specified by the authors (factor V Leiden and prothrombin G20210A mutations). The low score was 0, the high score 9, the median 1 (0; 4). Padua prediction score for DVT was of higher statistical significance in the DVT group than in the control group (p<0.001).

According to the cut-off value of 4 indicated by the authors of Padua score, only 93 (54.7%) patients with DVT had been at high risk of developing DVT, while 48 (28.9%) of controls were at high risk of developing DVT. For Padua score, we calculated the following parameters: sensitivity of 54.7% (CI 95%, 46.9–62.3%), specificity of 71% (CI 95%, 63.5–77.8%), positive predictive value of 65.9% (CI 95%, 57.5–73.2%) and negative predictive value of 60.5% (CI 95%, 53–67.4%).

When Padua score included PAI-1 4G/5G and MTHFR C677T polymorphisms, 122 (71.7%) patients with DVT had been correctly classified as having a high risk of developing DVT, while 73 (43.9%) subjects in the control group had been classified as being at risk of developing DVT. We calculated the following parameters: sensitivity of 71.7% (CI 95%, 64.3–78.3%), specificity of 56% (CI 95%, 48.1–63.7%), positive predictive value of 62.5% (CI 95%, 55.3–69.3%) and negative predictive value of 65.9% (CI 95%, 57.5–73.7%).

For the DVT group, we calculated the Caprini score including the thrombophilic risk factors specified by the authors. The low score was 0, the high 12, the median 5 (3; 7). For the control group, we calculated the Caprini score including thrombophilia. The low score was 0, the high 11, the median 3 (2; 5). Caprini score for high risk of thrombosis was of higher statistical significance in the DVT group than in the control group (p<0.001).

According to the cut-off value of 3 indicated by Caprini score (the threshold value for high risk of DVT), 147 (86.4%) patients with DVT had been at high risk of developing DVT, while 103 (62%) controls were at risk of developing DVT. We calculated the following parameters: sensitivity of 86.4% (CI 95%, 80.3–91.2%), specificity of 37.9% (CI 95%, 30.5–45.8%), positive predictive value of 58.8% (CI 95%, 52.4–64.9%) and negative predictive value of 73.2% (CI 95%, 62.6–82.2%).

We determined a moderate concordance between Padua and Caprini scores in estimating the high risk of developing acute lower extremity DVT (Cohen's kappa coefficient, kappa=0.387, p<0.001). When Padua score also included other thrombophilic risk factors besides factor V Leiden and prothrombin mutations, there was a slight increase in concordance (kappa=0.477, p<0.001).

Using a binary logistic regression, we combined Padua score and other thrombophilic risk factors with the Caprini

score and we calculated an AUC of 0.693 for the accurate estimation of the risk for DVT.

We calculated the risk of developing acute DVT for each score variant (Table 3). Thus, a Caprini score higher than 5 was the strongest predictor of acute lower extremity DVT risk.

Table 3 – ***Risk factors for DVT***

Variable		OR	CI 95%	*P*
Padua score (absolute value)		1.32	1.2–1.45	<0.001
Padua score + other thrombophilic risk factors (absolute value)		1.29	1.17–1.41	<0.001
Padua score >4 (high risk of DVT)		2.9	1.89–4.66	<0.001
Padua score + other thrombophilic risk factors >4		3.2	2–5	<0.001
Caprini score (absolute value)		1.3	1.19–1.43	<0.001
Caprini score >3 (high risk of DVT)		3.8	2.21-6.54	<0.001
Caprini score	2	1.38	0.52–3.63	0.5
	3–4	2.8	1.23–6.37	0.01
	>5	6	2.76–13.22	<0.001

Discussion

DVT is a frequent pathology in both medical and surgical wards. Incidence of venous thromboembolism in patients hospitalized for acute medical conditions is between 10% and 30% [15]. In the U.S., pulmonary embolism causes about 296 000 deaths annually, of which 30% are sudden deaths [16]. According to a study by Wakefield *et al.*, approximately 20% of subjects who survived a first episode of pulmonary embolism will die in the next three months [17]. Cost management of DVT cases in the U.S. annually amounts to about 40 billion dollars [18]. Non-fatal complications caused by DVT include post-thrombotic syndrome, patient treatment generating social and economic costs: 200 million dollars a year in the U.S. [19]. Avoiding these fatal and non-fatal complications as well as the high costs of treatment can be done through effective prevention. This requires precise knowledge of the individual risk factors for DVT.

This study analyzed two scores developed to estimate the risk of developing acute lower extremity DVT. We also tried to establish an opportunity to improve the sensitivity of these scores in predicting DVT by adding additional risk factors.

The thrombosis risk score proposed by Caprini has been validated by several trials that included both inpatients and surgical patients [20–23]. However, regarding the prevention of DVT, the 9th edition of *ACCP Guide* recommended Caprini score for predicting the risk of developing DVT only in patients who undergo non-orthopedic surgeries. None of the previous studies had performed measurements of thrombophilia, and one of them had no control group, so they could not determine the specificity of Caprini score.

In our study, whether calculated using only clinical and anamnestic data, or using the presence of thrombophilic risk factors as well, Caprini score had a high sensitivity for predicting the high risk of developing acute DVT (82.3% and 86.4%). Zakai *et al.* have determined similar values: 70% of patients with DVT and 56% of controls were identified as having high risk of developing DVT [19]. Our study achieved a better sensitivity due to the inclusion of a larger number of patients and by determining thrombophilic risk factors. The Caprini score had a low specificity for the increased risk of DVT (37.9%). The purpose of a prediction score is first to identify cases where the disease is present. Since the diagnosis of DVT is mainly assessed by the use of non-invasive methods (duplex ultrasound), low specificity of Caprini score should not prevent its application to DVT risk assessment.

Padua prediction score for DVT was developed in 2010 by Barbar *et al.* Padua score is based on a pre-existing model (Kucher) where authors have added additional variables [6, 24]. Padua score was studied and validated in patients admitted to medical wards [23, 24]. In the study conducted by Zhou *et al.*, Padua score estimated a less accurate risk for DVT than Caprini score (sensitivity 23.3% and 78.4%, respectively). Vardi *et al.* demonstrated that Padua score was not sensitive enough to estimate the risk for DVT in patients with sepsis admitted to medical wards [24].

In this study, we calculated a sensitivity of 43.5% and a specificity of 81.3% for Padua score, when we included only anamnestic and clinical data. After adjusting for thrombophilic risk factors indicated by authors (factor V Leiden and prothrombin mutations), sensitivity increased slightly to 54.7%, while specificity decreased to 71%. After adding additional thrombophilic risk factors (PAI-1 4G/5G and MTHFR C677T polymorphisms), sensitivity improved notably to 71.7% and specificity dropped to 56%. The higher sensitivity of Padua score in our study compared with studies in the literature is due to the adjustment for the presence of thrombophilia.

Some limitations of our study include the impossibility of following-up patients after discharge. This would have allowed the assessment of the accuracy of the prediction scores. Another is the fact that the study was conducted in a single hospital.

Conclusions

The Caprini prediction score was more sensitive than the Padua score in assessing the high risk of DVT in medical patients. PAI-1 4G/5G and MTHFR C677T polymorphisms increased the sensitivity of Padua score.

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The authors declare that they have no conflict of interest.

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