Thrombosis Research 134 (2014) 320–325

Contents lists available at ScienceDirect

Thrombosis Research

journal homepage: www.elsevier.com/locate/thromres

Regular Article

D-dimer, FVIII and thrombotic burden in the acute phase of deep vein thrombosis in relation to the risk of post-thrombotic syndrome

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ARTICLE INFO

Article history:
Received 16 February 2014
Received in revised form 4 May 2014
Accepted 30 May 2014
Available online 8 June 2014

Keywords:
D-dimer
FVIII
Thrombotic score
Deep vein thrombosis
Post-thrombotic syndrome
Residual venous obstruction

ABSTRACT

Background: Post-thrombotic syndrome (PTS) is the most common complication of deep vein thrombosis (DVT), but few data are available on the risk factors for PTS.
Aims: To assess whether the time-course of D-dimer, FVIII, and thrombotic burden are related to PTS development.
Methods: Patients (n = 59) with proximal DVT of the lower limbs (age 64; range:20-88 years; male 56%) were enrolled on the day of diagnosis (D0) and all received heparin for 5-7 days, overlapped and followed by vitamin K antagonists (VKA) for 3 months. Whole-leg compression ultrasound examination was conducted on D0 and 7 (D7), 30 (D30), and 90 (D90) days afterwards, when blood samples were also taken for D-dimer (STA Liatest) and FVIII (chromogenic assay) testing. Thrombotic burden was defined at each time point according to a score, which considered thrombosis extent and occlusion degree. Villalta score was evaluated at D30, D90, and D180.
Results: At D90, 12 patients developed PTS (Villalta score ≥5) and the median Villalta score was 1 (IQR 0.3-3.0) and was not correlated with either D-dimer or FVIIII time course. At D180, 13 patients had PTS and they had similar thrombotic score at D0, D30 to those without PTS, but higher at D90 (7.6 ± 5.1 vs. 3.2 ± 3.6; p = 0.011). Thrombotic score at D90 was correlated with Villalta score at D90 (rho = 0.374, p = 0.009) and at D180 (rho = 0.436, p = 0.006).
Conclusions: Thrombotic burden after 90 days of VKA is correlated with PTS.

Introduction

The post-thrombotic syndrome (PTS) is a common chronic complication of deep venous thrombosis (DVT) that develops in 20%-50% of patients after DVT, even in spite of optimal anticoagulant therapy for DVT [1]. Although it is generally believed that PTS develops because of venous hypertension, due to either persistent venous obstruction or venous reflux [2], few studies have properly addressed this issue [2–4]. In fact, the risk factors for PTS are only partly understood. The only identified risk factors for PTS so far are an increased body mass index (BMI) and recurrent ipsilateral DVT [5]. Age, gender and duration of anticoagulant therapy did not appear to be associated with the risk of developing PTS [5]. Recently, some studies reported that elevated levels of biomarkers such as D-dimer and FVIII [6–8] might be associated with PTS development. However, results are conflicting, and most studies were characterized by a single biomarker measurement, whereas D-dimer and FVIII are not stable during acute phase of DVT [9,10] and their time-course during antithrombotic therapy is little known [9].

The main aim of our study was to ascertain whether the time-course of D-dimer, FVIII, and thrombotic burden during the acute phase of proximal DVT of the lower limbs is related to PTS development. The secondary aim was to assess whether PTS signs and symptoms are correlated with the degree of residual venous obstruction after three months of vitamin K antagonist (VKA) treatment for DVT.

Methods

Study Population

The study was performed in a tertiary care teaching hospital (S. Orsola-Malpighi University Hospital, Bologna, Italy). Symptomatic outpatients with suspected acute DVT of the lower limbs were eligible for the study. They were referred by general practitioners or emergency department physicians to our vascular emergency room. Patients were excluded if younger than 18 years, if they were receiving vitamin K antagonists, or low-molecular -weight heparin or fondaparinux for more than 24 h, pregnant or in puerperium, or clinical suspicion of either pulmonary embolism or acute superficial vein thrombosis. Patients with a history of prior DVT or intravenous drug use were excluded, along with those unable to return for follow-up or unwilling to consent. The physician in charge, who also performed a physical examination, elicited

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http://dx.doi.org/10.1016/j.thromres.2014.05.043
0049-3848/

a personal and family history from each patient. The DVT was considered as idiopathic when none of the following venous thrombo-embolism risk factors were present: cancer, surgery, bed confinement, trauma at symptomatic leg, history of vein thrombosis, and use of oestrogen-containing therapy.

Since obesity and recurrent ipsilateral DVT are the most important risk factors for PTS, patients with obesity (BMI $\geq$ 30 Kg/m^2) and patients with previous DVT were excluded. Patients with the presence of varicose veins and/or a history of venous insufficiency in the legs (CEAP class > C1) were excluded from the study to ensure a correct diagnosis of PTS during follow-up.

Study Design

A prospective study was conducted in which participants were enrolled on the day of diagnosis and treated with enoxaparin 1 mg/kg subcutaneously twice a day and concurrent warfarin (vitamin K antagonist, VKA) with a target International Normalized Ratio (INR) of 2.5. Patients with chronic renal failure were initially managed with infusional unfractionated heparin with a target Activated Partial Thromboplastin Ratio of 1.7 to 2.5. Enoxaparin or heparin was continued for at least 5 days and until the INR was above 2.0. All patients were prescribed European class II graduated compression stockings (GCS) with ankle compression 30-40 mmHg.

On the day of diagnosis (D0), at 7 (D7), 30 (D30), 90 (D90) days thereafter the following were performed: -a standardized clinical examination, -b complete Compression Ultrasound (C-CUS) reported on a standardized form, -c venous blood sampling for: INR, FVIII, and D-dimer. The Villalta score was evaluated at D30, D90 and after 6 months from the enrollment (D180).

The study was approved by the Ethical Board of our Institution, and all patients gave their written informed consent for study participation.

D-Dimer and FVIII

Blood samples for D-dimer and FVIII testing were taken before ultrasonography investigation. At D0 blood samples were collected before the first dose of LMWH and before VKA treatment. Blood was drawn by clean venipuncture from an antecubital vein with a 19-gauge butterfly needle and collected into 4 ml plastic tubes containing 0.4 ml 0.106 M trisodium citrate. Whole blood was centrifuged at 2000 ×g for 20 min at 20 °C. Technicians performing D-dimer and FVIII testing were unaware of the symptoms of the patients. FVIII was measured by a chromogenic method using a commercial assay (Coamate Factor VIII, Chromogenix by Instrumentation Laboratory, Milan, Italy) as previously described [11]. The STA Liatest® D-Dimer (Diagnostica Stago, Asnières, France) was used for D-dimer assay. The STA Liatest® D-dimer was performed on the STA Compact® coagulation analyzer as previously described [12]. The results were expressed in ng/mL (expressed in fibrinogen equivalent unit). As previously described, the cut-off value for DVT exclusion was 500 ng/mL [12].

Whole-Leg Ultrasonography Investigation and Quantification of Thrombotic Durden

Patients underwent a comprehensive real-time B-mode and color Doppler compression ultrasonography examination of both legs by a vascular medicine physician as previously described [12]. Ultrasonography investigation was carried out with an EnVisor C HD instrument (Philips Medical System S.p.A, Monza, Italy), with a high-resolution broadband width linear array transducer L 5–10 MHz, according to the method of Schellong [13]. The proximal deep veins and the calf veins were evaluated as described elsewhere [12]. DVT diagnosis was confirmed if there was lack of compression of the vein, combined with the absence of venous flow with distal compression. The thrombotic burden was defined according to a new score, which considered the number of veins with thrombosis and occlusion degree (Table 1). Thrombosis presence was considered in the following segments (1 point each): -Common femoral, -Great saphenous vein (within 5 cm from the saphenous-femoral cross), -Superficial femoral (proximal), -Superficial femoral (medium), -Superficial femoral (distal), -Popliteal, -Small saphenous (within 5 cm from the saphenous-popliteal cross), -Posterior tibial, -Fibular, -Gastrocnemius, and -Soleal veins. The degree of occlusion was measured as diameter of the clot during maximal compression, giving 1 point when the diameter was 2-3 mm, 2 points for 4-5 mm, 3 points when $\geq$6 mm. Points were added to obtain the final score. For each patient, thrombotic score was calculated at D0, D7, D30, and D90.

Assessment of PTS

The Villalta score [14] was measured at D30, D90 and D180. The use of the Villalta score for defining the presence and severity of PTS has been recommended by the ISTH since it has been shown to be valid, reliable and sensitive to change over time [15]. In brief, patients are asked to rate five symptoms (pain, heaviness, cramps, paraesthesia and pruritus) on a four-point scale from 0 (none) to 3 (severe). Six clinician-graded signs (oedema, redness, hyperpigmentation, skin induration, venous ectasia and pain on calf compression) are evaluated using the same scale. The scores are then added, and PTS was considered to be present for a score $\geq$5. Classification of severity was: 5–9 mild, 10–14 moderate and >14 or presence of severe ulceration. In this study, a single investigator undertook all assessments and a score $\geq$5 on two consecutive visits (D90 and D180) indicated PTS. The investigators conducting whole-leg ultrasonography and Villalta score were blinded for each other.

Statistical Analysis

Analysis was carried out using the SPSS software package (version 15.0; SPSS Inc. Chicago, Illinois, USA). Categorical variables were expressed as frequency and percentage; continuous variables were expressed as mean ± SD when normally distributed and median with inter- quartile range (IQR) for data deviating for the normal distribution. Relationships between variables were assessed using Pearson correlation for continuous variables and chi-square or Fisher exact test for categorical variables. Student T-test for independent samples and for paired samples and multivariate analysis of variance with Bonferroni's correction for multiple comparisons was used to compare means among groups for normally distributed variables. Odds ratios were calculated with a logistic regression analysis: the association between outcome and risk factors for outcome was tested with univariable analyses followed by multivariable analysis. A parsimony model with predictors

Table 1
Ultrasound quantification of thrombosis.

Venous segments (1 point each):
-Common femoral
-Great saphenous vein (within 5 cm from the saphenous-femoral cross)
-Superficial femoral (proximal)
-Superficial femoral (medium)
-Superficial femoral (distal)
-Popliteal
-Small saphenous (within 5 cm from the saphenous-popliteal cross)
-Posterior tibial
-Fibular
-Gastrocnemius
-Soleal
Degree of occlusion for each venous segment (mm of residual thrombus):
• 2-3 mm : 1 point
• 4-5 mm : 2 points
• ≥6 mm : 3 points