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Chronic venous abnormalities in symptomatic and asymptomatic protein C deficiency

J. EMMERICH,* C. Y. VOSSSEN,† P. W. CALLAS,‡ C. DEMERS,§ S. NAUD,‡ G. L. LONG,‡ P. COUTURE,§ F. R. ROSENDAAL† and E. G. BOVILL‡

*University of Paris 5 and INSERM U428, Paris, France; †Leiden University Medical Center, Leiden, The Netherlands; ‡Department of Pathology, University of Vermont, Burlington, VT, USA; and §Laval University, Québec City, Québec, Canada

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Summary. *Background:* Thrombophilia is a frequent medical condition associated with symptomatic deep vein thrombosis (DVT). Unlike other clinical risk factors associated with DVT, such as surgery, thrombophilia has not been demonstrated to be associated with asymptomatic venous thrombotic events. Our aim was to search for asymptomatic sequelae of DVT in a protein C (PC)-deficient family. *Methods:* We studied 228 individuals from a large kindred with PC deficiency and performed a systematic ultrasound examination. *Results:* Among the 203 patients without a known history of venous thrombosis we found seven patients with abnormalities indicative of prior asymptomatic thrombosis: six (7.4%) in the PC-deficient group ($n = 81$) and only one (0.8%) in the non-deficient group ($n = 122$). The relative risk for these sequelae associated with PC deficiency was 9.0 (95% CI: 1.1–73.7). *Conclusions:* These data suggest that chronic venous abnormalities are frequently present and that thrombotic events in asymptomatic individuals with familial PC deficiency may be underestimated.

Keywords: deep vein thrombosis, protein C, ultrasound.

Introduction

Deep vein thrombosis (DVT) is a common disease among patients who undergo surgery or who are hospitalized for an acute medical problem [1]. Each year, about one in 1000 people in industrialized countries develop deep venous thrombosis (DVT) of the lower extremities [2,3]. Between 1% and 2% of these patients will die from pulmonary embolism (PE), and as many as 25% will suffer the chronic effects of the post-thrombotic syndrome at 20 years after DVT [4]. Systematic

screening studies by bilateral ascending phlebography of patients who had undergone orthopedic surgery or were hospitalized for medical conditions have demonstrated that asymptomatic DVT of the leg occurs at a rate of 40%–60% [5]. Furthermore, asymptomatic proximal DVT is found in approximately 70% of patients who present clinical symptoms of PE but no overt clinical symptom of DVT in the leg [6]. The post-thrombotic syndrome is frequently observed in the general population and has been widely assumed to be due to DVT [7,8]. Post-thrombotic syndrome is caused by venous hypertension, which most frequently occurs as a consequence of either recanalization of a previous major vein thrombus that led to valve destruction and reflux, or to persistent outflow obstruction. Thrombophilia is a medical condition, which is associated with an increased risk of symptomatic venous thrombotic events [9,10]. We hypothesized that familial protein C (PC) deficiency, a well-recognized cause of thrombophilia, might also be associated with an increased risk of asymptomatic venous thrombotic events. Review of the literature reveals that this has not been systematically assessed to date. We thus systematically assessed by ultrasound (US) a large kindred with PC deficiency to look for venous reflux and/or obstruction.

Methods

Population

We evaluated a large PC-deficient kindred of French Canadian origin [11–13]. The study included 228 individuals from northeastern USA and Québec, Canada, with or without a history of symptomatic DVT or PE, and with or without PC deficiency. All individuals belonging to a single large extended family with a high rate of venous thrombosis partially attributable to type I PC deficiency because of a 3363 InsC mutation in the PC gene. The pedigree spans six generations, and individuals aged 18 and older from the most recent four generations were all consecutively studied between July 2002 and March 2003. The University of Vermont, Burlington, Vermont, USA and Laval University Québec City, Québec, Canada Committees on Human Research approved the

Correspondence: Edwin G. Bovill, Department of Pathology, University of Vermont, Given Building, 89 Beaumont Avenue, VT 05405, USA.

Tel.: 1 802 656 0359; fax: 1 802 656 8892; e-mail: edwin.bovill@uvm.edu

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experimental protocol and all subjects provided informed consent. Symptomatic subjects included family members who had a history of venous thrombosis, confirmed through objective tests: phlebography or echography for DVT and pulmonary angiography, helical computed tomography (CT) scan or ventilation/perfusion scintigraphy for PE. Patients were defined as asymptomatic, according to the absence of a personal clinical history of venous thrombosis and the absence of objective anomaly in the lower limb according to the validated CEAP venous scoring system [14,15].

Ultrasound examination

Patients were assessed clinically using the CEAP scoring system [14] and then an echo-Duplex US examination was systematically performed, as recently described [13]. Briefly, a venous US was performed on each patient with the SonoSite™ 180PLUS (SonoSite, Inc., Bothell, WA, USA) a lightweight, portable system. Transverse compressions were performed from the common femoral vein just below the inguinal ligament down to the popliteal and sural veins, including the superficial veins. The US criteria for identification of venous sequelae of the lower limbs were defined before patient enrollment. Reflux was defined as abnormal valve closure times that produced reversal of venous flow of >0.5 s [7,13]. One individual (J.E.) performed all clinical examinations and venous US examinations and was blinded with respect to the subjects' medical histories of venous thrombosis and their PC deficiency status.

Protein C genotype

Patients from Quebec were genotyped at the University of Laval, Quebec City, Quebec, Canada and patients from the USA were genotyped at the University of Vermont College of Medicine, Burlington, VT [16,17].

Statistical analysis

Relative risks were calculated by taking the ratio of the proportions of venous sequelae among individuals with or without PC deficiency. We used stratification to adjust for age and sex by computing adjusted Mantel-Haenszel relative risks.

Results

The characteristics of the 228 patients studied are depicted in Table 1. The 3363 insertion mutation in the PC gene was found in 102 subjects (45%). A history of DVT or PE was reported by, respectively, 21% and 3% of the PC-deficient and normal subjects. Considering the entire group (with or without a history of venous thrombosis), 23 venous abnormalities (10%) were found by US examination (obstruction or reflux); 19 in the 102 subjects (19%) who carried the PC mutation, and four in the 126 (3%) who did not (adjusted relative risk: 3.8; 95% CI: 1.6–9.3). Among the 25 patients with a known history of venous thrombosis, one had experienced an isolated PE and one had a history of DVT of the arm. In the 23 remaining patients with a history of DVT in the lower limb, 16 had DVT sequelae on US examination (eight partial obstruction associated with reflux and eight reflux without obstruction). Among these 16 patients (Table 2), no difference was observed in the incidence of venous abnormalities between patients with or without PC deficiency (adjusted relative risk: 1.2; 95% CI: 0.7–1.9). Among the 203 patients with no known history of venous thrombosis we found seven patients with significant abnormal popliteal valve closure times: six (7.4%) in the PC-deficient group and only one (0.8%) in the non-deficient group. Three of these seven patients had also partial obstruction of the vein. The relative risk for the finding of deep vein reflux associated with PC deficiency was 9.0 (95% CI: 1.1–73.7). Adjustment for age and sex did not change the relative risk (8.3; 95% CI: 1.0–70.6). Clinical finding among these patients with venous abnormalities and no history of VTE were analyzed according to the CEAP classification. Five patients were asymptomatic

Table 1 Characteristics of the population

| | Total | PC-deficient | Non-PC-deficient |
|------------------------------------|------------|--------------|------------------|
| Number | 228 | 102 | 126 |
| Mean age (range) | 43 (15–80) | 46 (18–80) | 41 (15–78) |
| Men, <i>n</i> (%) | 102 (45) | 39 (38) | 63 (50) |
| History of DVT or PE, <i>n</i> (%) | 25 (11) | 21 (21) | 4 (3) |
| History of DVT, <i>n</i> (%) | 23 (10) | 19 (19) | 4 (3) |

PC, protein C; DVT, deep vein thrombosis; PE, pulmonary embolism.

Table 2 Frequency of DVT sequelae assessed by functional ultrasound and relative risk according to PC status

| | PC-deficient (<i>N</i> = 102) | Non-PC-deficient (<i>N</i> = 126) | RR (95% CI) |
|--|--------------------------------|------------------------------------|-----------------------------------|
| Number of subjects with a history of DVT/PE | 21 | 4 | |
| Number of DVT sequelae | 13 (62%) | 3 (75%) | 0.8 (0.4–1.6) 1.2 (0.7–1.9)* |
| Number of subjects with no history of DVT/PE | 81 | 122 | |
| Number of DVT sequelae | 6 (7.4%) | 1 (0.8%) | 9.0 (1.1–73.7) 8.3 (1.0–70.6)* |

PC, protein C; DVT, deep vein thrombosis; PE, pulmonary embolism; RR, relative risk; CI, confidence interval.

*Relative risk adjusted for age and sex via stratification.

and two report intermittent pain in their leg. Varicose veins were found in two patients (CEAP 2S and 2A, respectively) and telangiectases in one other patient (CEAP 1A). Two patients without aching had mild edema on clinical examination (CEAP 3A) and another one had skin pigmentation attributed to venous disease (CEAP 4A). Interestingly, the only patient with venous abnormalities and no PC deficiency had a history of severe traumatic injury with a tibial fracture of the affected leg and a 2-week stay in the intensive care unit. Superficial venous thrombosis sequelae were found in three and five of the PC-deficient and non-deficient subjects respectively.

Among individuals without a history of venous thrombosis, individuals with sequelae ($n = 7$) compared to those without sequelae ($n = 196$) showed a higher body mass index (mean BMI 30.9 vs. 28.0 kg m⁻² respectively) and a higher number of major surgeries in the past (e.g. hernia operations, hysterectomies; 71% vs. 51% respectively). Among individuals with a history of venous thrombosis, BMI was slightly higher in individuals with sequelae ($n = 16$) compared to those without sequelae ($n = 9$; mean BMI 28.4 and 26.8 kg m⁻² respectively) as well as the number of major surgeries (69% vs. 56% respectively).

Discussion

In this large kindred of genotyped thrombophilic subjects, we found a 7.4% incidence of deep venous valvular incompetence among those with PC deficiency and no history of venous thrombosis, using an objective US examination study performed blinded with respect to the personal medical history and the PC status. In a large epidemiologic study, including 2211 men and women in San Diego, Criqui *et al.* demonstrated a strong association of deep thrombotic events with deep valvular insufficiency, evaluated by reflux duration or Valsalva reflux of more than 0.5 s [18]. The present study is the first to demonstrate that deep venous valvular incompetence, which mainly reflects DVT sequelae, is a frequent event in PC-deficient patients with no history of DVT. This observation thus demonstrates a higher incidence of DVT in PC-deficient patients than the incidence depicted by clinical history alone. It is possible that the frequency of 7.4% is also an underestimate, as the sensitivity of US to detect sequelae in our population with a known history of lower limb DVT was only 70% in our study. Nevertheless, the results of a systemic review of US in the diagnosis of DVT in asymptomatic patients demonstrate that it is an accurate means to detect proximal DVT, with a mean sensitivity estimated to be 80% [19]. There is no gold standard test for the diagnosis of post-thrombotic syndrome or DVT sequelae. The detection of venous valvular incompetence by Doppler US, as performed in our protocol, is a validated method to objectively diagnose venous reflux and obstruction [4,13].

The clinical significance of asymptomatic DVT is debated in both thrombophilic and postsurgical patients [20]. After surgery, systematic screening for DVT revealed that around half of asymptomatic DVT resolve spontaneously and only

one-sixth extend to the proximal vein with a risk of PE [21]. Furthermore, whether asymptomatic DVT, detected by routine screening, leads to post-thrombotic syndrome remains controversial [4]. For these reasons, although we demonstrated a ninefold increased risk of asymptomatic DVT among PC-deficient individuals, these findings do not allow us to draw clinical conclusions about thromboprophylaxis or treatment of patients with PC deficiency or other forms of thrombophilia. Furthermore, as discussed above, we cannot exclude that some instances of deep valvular reflux are not related to previous DVT. Nevertheless, our study clearly demonstrates that asymptomatic events, detected by a functional US examination, are frequent in patients with PC deficiency. The clinical significance of this finding remains to be demonstrated in future studies of thrombophilic populations. The risk of recurrent thrombosis is increased in patients with residual venous thrombosis after a symptomatic proximal DVT compared to patients with complete recanalization [22]. In the study by Prandoni *et al.*, this observation applied to patients with idiopathic DVT, with thrombosis secondary to transient risk factors, as well as patients with thrombophilic abnormalities. Thus, despite the fact that our study was not designed to demonstrate an increased risk of DVT in the subgroup of patients with DVT sequelae, it seems likely that these individuals would be at increased risk for DVT.

Finally, our study opens a new field in the evaluation of thrombophilia. To date only two phenotypes have been described in clinical studies related to DVT and thrombophilia: symptomatic and asymptomatic patients. Our findings demonstrate the misclassification of a substantial number of asymptomatic patients who should be reclassified as asymptomatic with sequelae of DVT. Although the clinical utility of this reclassification remains to be determined, the increased refinement of phenotype definition should be useful in the search for gene-gene interaction in this polygenic disease [12].

Contribution

J. Emmerich performed the US examination, interpreted the data and prepared the manuscript. C. Y. Vossen organized the study, made the statistical analysis and prepared the manuscript. P. W. Callas, C. Demers, S. Naud, and P. Couture organized the study, organized the visits and contributed to the revision of the manuscript. G. L. Long organized and performed the biochemical and DNA analysis. F. R. Rosendaal co-organized the study and contributed to the statistical analysis and revision of the manuscript. E. G. Bovill designed and coordinated the study, assessed the data, and prepared the manuscript.

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