Thromboembolic disease of the venous and the arterial system
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Document Version
Publisher's PDF, also known as Version of record

Publication date:
2010

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):
Mahmoodi, B. K. (2010). Thromboembolic disease of the venous and the arterial system: two different entities or two different sides of the same coin? Groningen: s.n.

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Chapter 6

A prospective cohort study on the absolute risks of venous thromboembolism and predictive value of screening asymptomatic relatives of patients with hereditary deficiencies of protein S, protein C or antithrombin

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Jan van der Meer

J Thromb Haemost. 2010;8:1193-200
ABSTRACT

**Background:** Absolute risks of venous thromboembolism (VTE) in protein S-, protein C-, or antithrombin-deficient subjects are mainly based on retrospective data. Screening asymptomatic relatives of these patients are disputed, though studies addressing this issue have yet to be conducted.

**Methods:** We prospectively followed 382 relatives of 84 probands. Participants were assessed for other thrombophilic defects and occurrence of exogenous risk factors (i.e., surgery, trauma, immobilization, malignancies, use of systemic estrogens, and pregnancy or puerperium). After screening, deficient subjects were advised to use thromboprophylaxis during exogenous risk factors; use of oral contraceptives was discouraged.

**Results:** Overall annual incidence of VTE was 1.53% (95%CI, 1.00-2.34) in deficient versus 0.29% (0.13-0.64) in non-deficient relatives; adjusted hazard ratio, 7.0 (95%CI, 2.7-18.0). Annual incidence of unprovoked VTE was 0.95% in deficient versus 0.05% in non-deficient subjects; age-adjusted hazard ratio, 22.3 (P=0.003). In contrast, annual incidence of provoked VTE was 0.58% versus 0.24%; age-adjusted hazard ratio, 2.8 (P=0.08). Fifty-five (37%) deficient and 80 (34%) non-deficient subjects experienced 91 and 143 exogenous risk factors, respectively, during which 6 versus 5 VTE (6.6% vs 3.5% per risk-period) occurred, despite the higher compliance with recommended thromboprophylaxis use in deficient (51%) versus non-deficient (22%) subjects. In deficient subjects all provoked VTE occurred when thromboprophylaxis was not used.

**Conclusions:** Protein S, protein C or antithrombin deficiencies confer high absolute risk of VTE. Screening and subsequent augmentation of thromboprophylaxis use may result in reduction of provoked VTE, whereas risk of unprovoked VTE could not be affected by screening.
INTRODUCTION

Several coagulation disorders are associated with an increased risk of venous thromboembolism (VTE). These thrombophilic disorders include hereditary deficiencies of protein S, protein C and antithrombin; factor V Leiden; the prothrombin G20210A mutation; high levels of clotting factor VIII; and antiphospholipid antibodies.\cite{1} Hereditary deficiencies of protein S, protein C or antithrombin are rare (0.1% to 0.4% each in the general population), but the strongest hereditary risk factors for VTE.\cite{2} In retrospective studies the incidence rate of VTE ranged from 0.5% to 3.1% per year.\cite{3-6} Only three prospective studies addressed this issue, which reported incidence rates ranging from 0.7% to 4.0% per year.\cite{7-9} The differences between studies may be explained by differences in study populations as these deficiencies interact with other genetic and acquired risk factors for VTE.\cite{2,10}

Since in the general population long-term oral anticoagulant treatment is associated with a major bleeding risk of about 2.8% per year,\cite{11} there is reluctance to advocate long-term primary prophylaxis in asymptomatic subjects with protein S, protein C or antithrombin deficiencies.\cite{12} As about 50% of VTE cases are provoked by exogenous risk factors,\cite{10} transient thromboprophylaxis at exposure to exogenous risk factors is nowadays highly recommended even in non-deficient subjects.\cite{13} Therefore, screening asymptomatic relatives of subjects with protein S, protein C or antithrombin deficiencies is a matter of debate,\cite{14,15} even though studies addressing this issue have yet to be conducted.

We conducted a prospective follow-up study to assess the absolute risk of VTE in a large series of deficient versus non-deficient asymptomatic relatives of protein S-, protein C- or antithrombin-deficient patients. We also assessed the impact of screening, followed by preventative recommendations, on the VTE risk in deficient relatives of these patients.
METHODS

Study population and design
Details of the study protocol have been published elsewhere.[10,16] In brief, 1600 consecutive patients with VTE were screened over 12 years to identify 91 index subjects (probands) with either protein S, protein C or antithrombin deficiency. First-degree relatives, of these probands, older than 15 years of age were identified by pedigree analysis. As the number of antithrombin-deficient probands was small, second degree relatives from a deficient parent were also identified. For living relatives, response rates between 90% and 97% per cohort allowed us to identify 725 relatives. Subjects were enrolled after informed consent was obtained. Detailed information on previous episodes of VTE and anticoagulant treatment were collected, using a standardized questionnaire and reviewing medical records. Blood samples were taken after clinical data had been collected. Relatives were tested for other thrombophilic defects in addition to their index deficiencies, including deficiencies of protein S, protein C, and antithrombin; factor V Leiden; the prothrombin G20210A mutation; high levels of factor VIII; and lupus anticoagulant.

Asymptomatic relatives (i.e., without a history of VTE), irrespective of their deficiency status, were eligible for inclusion in the current prospective analysis (Figure 1). Subjects were excluded if they were on long-term (≥ 12 months) treatment with vitamin K antagonists. All subjects were instructed to seek medical attention when they encountered signs or symptoms of VTE. Both study subjects and their general physicians received written information concerning presence or absence of protein S, protein C or antithrombin deficiencies. In deficient subjects, additional information was provided concerning the implications of the observed deficiency and the advice to strongly consider anticoagulant thromboprophylaxis at exposition to exogenous risk factors (i.e., major surgery or trauma, immobilization for >7 days, pregnancy and puerperium). Nonetheless, the decision to use thromboprophylaxis was left to the discretion of the treating physician. Furthermore, use of oral contraceptives and hormonal replacement therapy were discouraged in deficient subjects. In nondeficient subjects no preventative recommendations were given and their treating physicians were expected to apply thromboprophylaxis in agreement with national guidelines that are based on the contemporary American College of Chest Physicians guidelines.[17]
Figure 1. Recruitment of the family cohorts with hereditary deficiencies of protein S, protein C, or antithrombin.
VKA denotes vitamin K antagonists; VTE, venous thromboembolism.

Data from the participants were collected in family groups, and were seen at our outpatient clinic at start of the study and during follow-up that took place about
Hereditary thrombophilia and VTE

once every 3 years by phone or by visits to our outpatient clinic. In each case a
standardized questionnaire was used to update information on the occurrence of
VTE, exposition to exogenous risk factors and the use of thromboprophylaxis.
Participants were last contacted in the period between September 2005 and
December 2007. The study protocol was approved by the institutional review board
of our hospital.

Diagnosis of venous thromboembolism
Only objectively verified symptomatic thromboembolic events were considered.
Events were independently adjudicated and were classified using the following
criteria: deep vein thrombosis had to be confirmed by compression ultrasound; and
pulmonary embolism by ventilation/perfusion lung scanning or spiral computed
tomography. Isolated calf vein thrombosis and superficial phlebitis were not
classified as VTE. VTE was considered provoked if it had occurred at or within 3
months after exposure to exogenous risk factors, including major surgery or
trauma, immobilization for >7 days, oral contraceptives, hormone replacement
therapy, pregnancy, or malignant disease. In the absence of these risk factors, VTE
was considered unprovoked.

Laboratory studies
Protein S and protein C antigen levels were measured by Enzyme Linked Immuno
Sorbent Assay (ELISA) (reagents obtained from DAKO, Glostrup, Denmark),
activity of protein C (Berichrom Protein C, Dade Behring, Liederbach, Germany),
and antithrombin (Coatest, Chromogenix, Mölndal, Sweden) by chromogenic
substrate assays. Normal ranges (mean±SD) were determined in 393 healthy blood
donors, who had no (family) history of thromboembolism, were not pregnant, and
had not used oral contraceptives for at least three months. Protein S deficiency type
I was defined by lowered total (<68 IU/dl) and free (<65 IU/dL) protein S levels.
Protein C deficiency type I and type II were defined by reduced levels of either
protein C antigen (<63 IU/dl) and/or activity (<64 IU/dl). Antithrombin deficiency
was defined by decreased levels of antithrombin activity (<74 IU/dl), using heparin
cr-co-factor assay which identifies both type I and type II antithrombin
deficiencies.[18] Deficiencies were considered inherited if they were confirmed by
measuring a second sample that was collected 3 months later and were found in at
least 2 family members, while acquired conditions were excluded. If there was a
discrepancy between the results of the 2 tests, a third sample was tested. A
deficiency was considered acquired, through use of oral contraceptives or
pregnancy, unless it was confirmed at least 3 months after withdrawal of oral contraceptives or delivery, respectively. Factor V Leiden and the prothrombin G20210A mutation were demonstrated by polymerase chain reactions.\textsuperscript{19,20} Factor VIII:C was measured by one-stage clotting assay and was considered increased at levels above 150 IU/dl.\textsuperscript{21} Lupus anticoagulant was defined by abnormal values of dilute Russell viper venom time, activated partial thromboplastin time and/or tissue thromboplastin inhibition, which normalized by adding phospholipids to the subject’s plasma.\textsuperscript{22}

**Statistical analysis**

We calculated absolute risks of VTE in subjects with protein S, protein C or antithrombin deficiency, separately, and in the pooled cohorts of deficient and non-deficient subjects. Annual incidences were calculated by dividing the number of symptomatic subjects by the total number of follow-up years. Follow-up time was defined as the period from testing for the index deficiency until first VTE, death or end of study, whichever occurred first. The 95 percent confidence intervals (95% CI) around the annual incidences were assessed with the Poisson distribution assumption. Annual incidences in current prospective analysis were compared to our previously published retrospective analysis\textsuperscript{10} by calculating incidence rate ratios with the corresponding 95% CI and P-values by using the immediate method in STATA software, version 10.1 (StataCorp LP, College Station, Texas, USA).\textsuperscript{23} Annual incidences in previous retrospective analysis were calculated as the numbers of subjects with VTE that have occurred prior to screening divided by the total observation years. Observation period in the retrospective analysis was defined as the period from the age of 15 years until VTE, death or screening for the index deficiencies, whichever came first.\textsuperscript{10}

In a multivariate Cox-model, hazard ratios conferred by these deficiencies were adjusted for age, sex, concomitant thrombophilic defects (i.e., factor V Leiden, the prothrombin G20210A mutation or factor VIII:C >150 IU/dl) and for clustering of VTE within families that used the robust sandwich method in STATA software. Results were expressed as hazard ratios, with 95% CIs and P-values.

Continuous variables were expressed as median values with the interquartile range (IQR) and categorical data as counts with frequencies. Differences between groups were evaluated by the Student’s t test or Mann-Whitney U test, depending on the normality of data for continuous data, and by Fisher exact test for categorical data.
Statistical significance was considered as a 2-tailed probability <0.05. All statistical analyses were performed using STATA software, version 10.1 (StataCorp LP, College Station, Texas, USA).

RESULTS

Subjects
Ninety-one subjects with objectively verified VTE and either protein S, protein C or antithrombin deficiency served as index patients in this study (Figure 1). In 7 families, inheritance of the index deficiency was not established in at least two relatives, therefore, these families were excluded from analysis (19 relatives). After exclusion due to various reasons depicted in Figure 1, the current analysis was performed on 382 relatives. These subjects belonged to 84 different kindreds with protein S (n=34), protein C (n=38) or antithrombin (n=12) deficiency.

Table 1 shows the clinical characteristics of the pooled and separate study cohorts. Overall, 39% of subjects were deficient for either protein S, protein C or antithrombin. Deficiencies were equally distributed between men and women. Deficient subjects were on average 10 years younger than non-deficient subjects at testing for index deficiencies ($P<0.001$). If subjects with prior VTE and/or subjects using long-term vitamin K antagonists were not excluded from the analysis, the mean age at testing for these deficiencies in deficient subjects was 39 years versus 41 years in non-deficient relatives. Concomitance of other thrombophilic defects (i.e., factor VIII:C >150 IU/dl, prothrombin G20210A and factor V Leiden mutations) were comparable between deficient and non-deficient subjects.

<table>
<thead>
<tr>
<th>Table 1. Baseline characteristics of the study population</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variable</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Subjects, n</td>
</tr>
<tr>
<td>Women, n (%)</td>
</tr>
<tr>
<td>Median age at testing for index deficiency (IQR), yr</td>
</tr>
<tr>
<td>Concomitance of, n (%)*</td>
</tr>
<tr>
<td>PT G20210A</td>
</tr>
<tr>
<td>Factor V Leiden</td>
</tr>
</tbody>
</table>

IQR denotes interquartile range: FVIII:C, factor VIII:C; PT G20210A, the prothrombin G20210A mutation.

* Of total study cohort 17%, 13% and 10% of subjects were not tested for FVIII:C, the prothrombin G20210A mutation and factor V Leiden, respectively.

None of these subjects were positive for lupus anticoagulant.
The total follow-up time was 1,375 years in deficient subjects (mean±SD, 9.2±5.9) and 2,097 years in non-deficient subjects (mean±SD, 9.0±5.9). Participating families were contacted on average once every 3 years. During follow-up, five (3.4%) deficient subjects and seven (3.0%) non-deficient subjects died; according to death certificates, none of the death causes were related to VTE.

**Risk of venous thromboembolism**

Twenty-one (14.1%) deficient and six (2.6%) non-deficient subjects developed VTE during follow-up, corresponding to an annual incidence of 1.53% (95%CI, 1.00 – 2.34) in deficient and 0.29% (95%CI, 0.13 – 0.64) in non-deficient subjects, respectively (Table 2). Nineteen (70%) of the 27 VTE were classified as deep-vein thrombosis in the leg and 8 (30%) as pulmonary embolism either alone or in combination with deep-vein thrombosis. There was no difference in types of VTE (i.e., deep-vein thrombosis and pulmonary embolism) between deficient and non-deficient subjects (P=0.82). Compared to the pooled cohort of non-deficient subjects, subjects with antithrombin deficiency had the highest risk for VTE, followed by protein S and protein C deficiencies. In a multivariate Cox-model that was adjusted for age, sex, concomitant thrombophilic defects (i.e., factor V Leiden, the prothrombin G20210A mutation or factor VIII:C >150 IU/dl) and clustering, any (i.e., protein S, protein C or antithrombin) deficiency conferred a hazard ratio of 7.0 (95% CI, 2.7 – 18.0; P<0.001), as compared to non-deficient relatives. Adjusted hazard ratios for separate deficiencies were 9.6 (95% CI, 3.0 – 30.1), 4.1 (95% CI, 1.2 – 13.9) and 10.2 (95% CI, 3.3 – 31.6) in subjects with protein S, protein C and antithrombin deficiencies, respectively.

A total of 13 deficient and only one non-deficient subject developed unprovoked VTE, corresponding to an annual incidence of 0.95% (95%CI, 0.55 – 1.63) in deficient versus 0.05% (95%CI, 0.01 – 0.34) in non-deficient subjects; age adjusted hazard ratio 22.3 (95% CI, 2.9 – 172.7) (Table 2). The risk of provoked VTE in deficient subjects was not significantly higher (P=0.08). Fifty-five (37%) deficient and 80 (34%) non-deficient subjects experienced a total of 91 and 143 high-risk periods, respectively (Table 3). At time of high-risk periods, thromboprophylaxis was used more often in deficient (51%) subjects as compared to non-deficient subjects (22%). The incidence of risk-period related VTE (provoked VTE) per risk-period was almost two-fold higher in deficient subjects (6.6% vs. 3.5%), despite more often used thromboprophylaxis. Furthermore, whereas no VTE were
encountered during 166 pill-years in non-deficient subjects, 2 VTE occurred during only 54 pill-years in deficient subjects.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total Follow-up, yrs</th>
<th>First VTE, n</th>
<th>Annual incidence, % (95% CI)</th>
<th>Hazard ratio (95% CI)*</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any VTE</td>
<td>3,472</td>
<td>27</td>
<td>0.78 (0.53 – 1.13)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No deficiency</td>
<td>2,097</td>
<td>6</td>
<td>0.29 (0.13 – 0.64)</td>
<td>1.0, reference</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Any deficiency</td>
<td>1,375</td>
<td>21</td>
<td>1.53 (1.00 – 2.34)</td>
<td>7.0 (2.7 – 18.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PSD</td>
<td>453</td>
<td>7</td>
<td>1.55 (0.74 – 3.24)</td>
<td>9.6 (3.0 – 30.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PCD</td>
<td>528</td>
<td>5</td>
<td>0.95 (0.39 – 2.27)</td>
<td>4.1 (1.2 – 13.9)</td>
<td>0.02</td>
</tr>
<tr>
<td>ATD</td>
<td>394</td>
<td>9</td>
<td>2.29 (1.19 – 4.39)</td>
<td>10.2 (3.3 – 31.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Unprovoked VTE</td>
<td>3,472</td>
<td>14</td>
<td>0.40 (0.24 – 0.68)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No deficiency</td>
<td>2,097</td>
<td>1</td>
<td>0.05 (0.01 – 0.34)</td>
<td>1.0, reference</td>
<td></td>
</tr>
<tr>
<td>Any deficiency</td>
<td>1,375</td>
<td>13</td>
<td>0.95 (0.55 – 1.63)</td>
<td>22.3 (2.9 – 172.7)</td>
<td>0.003</td>
</tr>
<tr>
<td>PSD</td>
<td>453</td>
<td>5</td>
<td>1.10 (0.46 – 2.65)</td>
<td>25.5 (2.9 – 221.3)</td>
<td>0.003</td>
</tr>
<tr>
<td>PCD</td>
<td>528</td>
<td>1</td>
<td>0.19 (0.03 – 1.34)</td>
<td>4.4 (0.3 – 71.7)</td>
<td>0.29</td>
</tr>
<tr>
<td>ATD</td>
<td>394</td>
<td>7</td>
<td>1.78 (0.85 – 3.73)</td>
<td>42.7 (5.2 – 350.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Provoked VTE</td>
<td>3,472</td>
<td>13</td>
<td>0.37 (0.22 – 0.64)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No deficiency</td>
<td>2,097</td>
<td>5</td>
<td>0.24 (0.10 – 0.57)</td>
<td>1.0, reference</td>
<td></td>
</tr>
<tr>
<td>Any deficiency</td>
<td>1,375</td>
<td>8</td>
<td>0.58 (0.29 – 1.16)</td>
<td>2.8 (0.9 – 8.6)</td>
<td>0.08</td>
</tr>
<tr>
<td>PSD</td>
<td>453</td>
<td>2</td>
<td>0.44 (0.11 – 1.77)</td>
<td>2.0 (0.4 – 10.7)</td>
<td>0.40</td>
</tr>
<tr>
<td>PCD</td>
<td>528</td>
<td>4</td>
<td>0.76 (0.28 – 2.02)</td>
<td>3.6 (0.9 – 13.8)</td>
<td>0.06</td>
</tr>
<tr>
<td>ATD</td>
<td>394</td>
<td>2</td>
<td>0.51 (0.13 – 2.03)</td>
<td>2.5 (0.5 – 12.9)</td>
<td>0.28</td>
</tr>
</tbody>
</table>

VTE denotes venous thromboembolism; PSD, protein S deficiency; PCD, protein C deficiency and ATD, antithrombin deficiency.

* Hazard ratios for any VTE are adjusted for age, sex, concomitant thrombophilic defects (i.e., Factor V Leiden, prothrombin G20210A mutation or factor VIII levels >150 IU/dl) and clustering. Hazard ratios for unprovoked and provoked VTE were only adjusted for age due to the low numbers of events.
We compared the incidence rates of VTE in the current prospective analysis to our previously published retrospective analysis in these same thrombophilic families (Figure 2).\[^{10}\] In deficient subjects, the overall annual incidence of VTE during the prospective time period was comparable to annual incidence of VTE during the retrospective period (1.53% vs 1.65%; P=0.77). Compared to our retrospective

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**Table 3. Number of risk periods and correlated venous thromboembolic events.**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total cohort</th>
<th>Non-def (n=233)</th>
<th>Deficient (n=149)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Any def (n=149)</td>
<td>PS def (n=50)</td>
</tr>
<tr>
<td>Surgery, trauma or immobilization, n</td>
<td></td>
<td>116</td>
<td>60</td>
</tr>
<tr>
<td>With Prophylaxis, n (%)</td>
<td></td>
<td>24 (21)</td>
<td>27 (45)</td>
</tr>
<tr>
<td>VTE, n</td>
<td>5</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Associated incidence of VTE/risk period, %</td>
<td>4.3</td>
<td>8.3</td>
<td>4.5</td>
</tr>
<tr>
<td>Pregnancy or puerperium, n</td>
<td>27</td>
<td>31</td>
<td>8</td>
</tr>
<tr>
<td>With Prophylaxis, n (%)</td>
<td>7 (26)</td>
<td>19 (61)</td>
<td>4 (50)</td>
</tr>
<tr>
<td>VTE, n</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Associated incidence of VTE/risk period, %</td>
<td>0.0</td>
<td>3.2</td>
<td>0.0</td>
</tr>
<tr>
<td>Any risk moment, n*</td>
<td>143</td>
<td>91</td>
<td>30</td>
</tr>
<tr>
<td>With Prophylaxis, n (%)</td>
<td>31 (22)</td>
<td>46 (51)</td>
<td>15 (50)</td>
</tr>
<tr>
<td>VTE, n</td>
<td>5</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>Associated incidence of VTE/risk period, %</td>
<td>3.5</td>
<td>6.6</td>
<td>3.3</td>
</tr>
<tr>
<td>Oral contraceptive use, pill-yrs</td>
<td>166</td>
<td>54†</td>
<td>24</td>
</tr>
<tr>
<td>VTE, n</td>
<td>0</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Associated incidence of VTE/pill-yrs, %</td>
<td>0.0</td>
<td>3.7</td>
<td>4.2</td>
</tr>
</tbody>
</table>

* Two subjects in non-deficient group and 3 subjects in deficient group were known with malignant disease. One subject with protein C deficiency and malignant disease developed VTE. Any risk moment included surgery, trauma, immobilization, pregnancy, puerperium and malignant disease.

† Fifty-four pill years were obtained in 6 deficient women.
analysis,[10] the slight elevation in annual incidence of unprovoked VTE (0.77% vs 0.95%; P=0.58) and a decrease in the annual incidence of provoked VTE (0.84% vs 0.58%; P=0.32) were statistically not significant. In contrast, in the 46 of the 91 risk moments in which thromboprophylaxis was used, no VTE have occurred; underlining the effectiveness of primary thromboprophylaxis in VTE prevention in deficient subjects.

**Figure 2. Incidence rates of VTE prior to screening versus after screening in the same thrombophilic families.**

Histogram represents annual incidence of VTE in previously published retrospective (white)[10] versus current prospective analysis (gray), with the corresponding 95% CIs represented by the error-bars. IRR denotes incidence rate ratio.

* All provoked VTE during prospective follow-up have occurred when thromboprophylaxis was not used.
DISCUSSION

This prospective analysis confirmed the high risk of VTE in subjects with hereditary deficiencies of protein S, protein C or antithrombin. As compared to the pooled cohort of nondeficient subjects, antithrombin deficient subjects had the highest risk for VTE followed by protein S and protein C deficient subjects. Whereas the risk of unprovoked VTE was about 22-fold higher in subjects with any deficiency (i.e., protein S, protein C or antithrombin), the risk of provoked VTE was only 2 to 3-fold elevated, as compared to non-deficient subjects. The incidence of exogenous risk-periods was similar between deficient and non-deficient subjects, but primary thromboprophylaxis was used more frequently in deficient subjects, especially in subjects with antithrombin deficiency. Although the overall annual incidence of VTE in the current prospective analysis was comparable to our retrospective analysis, the probability to capture a VTE is higher in a prospective than in a retrospective analysis. Therefore the two studies are not comparable.

Our results on the absolute risks are in line with previous prospective studies. In a comparable family cohort study by Sanson et al, the overall annual incidence of VTE was 0.7% (protein S), 1.0% (protein C) and 4.0% (antithrombin), in contrast to 1.6% (protein S), 1.0% (protein C) and 2.3% (antithrombin) in our study. The higher risk of VTE in protein S deficient subjects in our study could be attributed to the selection of severely affected subjects in our study (only type I protein S deficiency), whereas in the study of Sanson et al both type I and type III protein S deficient subjects were enrolled. Though the risk of overall VTE in antithrombin deficient subjects was lower in our study, the risk of unprovoked VTE in our study was comparable to the study by Sanson et al (1.8% vs 1.6%). Of note, the risk estimates in the study by Sanson et al were based on only 9 cases of VTE in 209 deficient subjects, reflecting a substantially shorter follow-up, as compared to our study. In another study by Vossen et al, annual incidences of 0.7% for each protein S and protein C deficiency, and 1.7% for antithrombin deficiency were reported. The observed differences might be attributed to difference in subjects’ selection as we enrolled subjects from families with familial thrombophilia with proven inheritance, which was established if at least two relatives were tested positive for the index deficiency, whereas in the study of Vossen et al only one relative with the index deficiency was required. Furthermore, both type I and type III protein S deficient subjects were included in
that study. Finally, in a prospective study by Pabinger et al annual incidences of VTE were much higher than in our study, in both protein S (3.5% vs. 1.6%) and protein C deficient subjects (2.5% vs. 1.0%). However, annual incidences in that study were based on only 3 VTE in each protein S (n=24) and protein C (n=20) deficient subjects with total follow-up of only 93 and 119 person-years, respectively. Given that the risk estimates in previous prospective studies were based on very low number of VTE and/or small cohort with short follow-up, we presume that our estimates reflect the most accurate assessment of the absolute VTE risk conferred by these deficiencies.

In previous prospective studies as well as in our study, annual incidences in the three types of deficiencies varied widely, with antithrombin deficiency conferring the highest absolute risk for VTE. It could be speculated that antithrombin deficiency is a stronger risk factor for VTE than are protein S or protein C deficiencies. Therefore, it could be questioned whether it is correct to pool these deficiencies. Nevertheless, annual incidences in protein S, protein C or antithrombin deficiencies fell within each other’s 95% confidence intervals. Furthermore, in retrospective studies, differences in annual incidences among the three types of deficiencies were less evident and similar relative risks were reported.

Though VTE risk estimates in current prospective analysis were nearly the same as the risk estimates prior to screening in these families (i.e., 1.53% vs 1.61%; Figure 2), these results may not give further credence against screening. This is especially important as the lack of overall VTE risk reduction, after screening and subsequent preventative recommendations, could be attributed to low compliance (51% of high risk-periods in deficient subjects). Moreover, all provoked VTE occurred when thromboprophylaxis was not used, underlining the effectiveness of thromboprophylaxis in these deficient subjects. Finally, two out of six deficient women using oral contraceptives developed VTE. Therefore, the relevance of discouraging oral contraceptive use in deficient women could be considered beneficial, but was hampered by small numbers. Taken together, since screening could be only considered beneficial for avoiding provoked VTE, it will be especially valuable in subjects with frequent external risk factors. Especially young women are more often exposed to external risk factors due to oral contraceptive use and pregnancy and may therefore benefit the most from screening.
For the first time we evaluated the impact of screening followed by preventative recommendations on VTE risk in asymptomatic deficient relatives of patients with protein S, protein C or antithrombin deficiency. Further strengths of the current study are its prospective nature, its relatively large size and long follow-up, and carefully established inheritance of protein S, protein C or antithrombin deficiencies. Moreover, in our study the control group consisted of non-deficient relatives, whereas in previous prospective studies controls were not available or consisted of unrelated subjects. Selecting unrelated subjects as controls for subjects with these hereditary deficiencies may result in overestimated relative risk of VTE, as other thrombophilic defects also aggregate in these families.

This study has some potential limitations. Subjects were contacted on average once every 3 years. This may have resulted in underreporting of exposure to exogenous risk factors and/or thromboprophylaxis use as this information was self-reported rather than additionally validated from medical records. However, it is likely that potential underreporting would have been similar in deficient versus non-deficient subjects, because data from the participants were collected in family groups, thereby avoiding bias in follow-up time between deficient versus non-deficient relatives. Moreover, the reasons for the low compliance in current study were not registered, nevertheless, the low compliance was in line with previous reports. Selection bias seems less likely as consecutive subjects with VTE and either protein S, protein C or antithrombin deficiency served as probands. To limit ascertainment bias, we considered only symptomatic VTE. An appropriate comparison between the prospective and our previous retrospective analysis could be hampered by differences in study design, as it is possible that awareness of the deficiency status could have resulted in higher capturing of VTE incidence in the prospective analysis. Even though this is the largest prospective study on this issue, the results should be handled with caution as numbers were small, while use of thromboprophylaxis was not specifically evaluated in this study.

In conclusion, we confirmed the high absolute risk of VTE in subjects with hereditary protein S, protein C or antithrombin deficiencies in a large well-defined prospective cohort with long follow-up. As far as screening of asymptomatic carriers of these deficiencies is concerned, it is important to differentiate between provoked and unprovoked VTE cases. For the prevention of unprovoked VTE, the value of testing is obviously limited. However, most cases of provoked VTE will
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be avoidable if appropriate thromboprophylaxis is applied, underlining the value of screening.

REFERENCES


