The Incidence of Venous Thromboembolism in Asymptomatic Carriers of a Deficiency of Antithrombin, Protein C, or Protein S: A Prospective Cohort Study

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Deficiencies of antithrombin, protein C, and protein S are associated with an increased risk of venous thromboembolism. The objective of this study was to prospectively assess the incidence of venous thromboembolism in nontreated asymptomatic subjects with such a deficiency. We conducted a prospective cohort study in asymptomatic family members of unselected patients who presented with a venous thromboembolic event and who were found to have a deficiency of antithrombin, protein C, or protein S. No anticoagulant prophylaxis was given to the study participants, except during risk periods for venous thromboembolism. All venous thromboembolic events were diagnosed by objective diagnostic tests. A total of 208 individuals with a mean age of 37 years (range, 15 to 79) were included in the study. A total of 611 patient observation years was obtained. Nine venous thromboembolic events occurred, resulting in an annual incidence of 1.5% (95% confidence interval [CI], 0.7 to 2.8) for the 3 deficiencies combined. Five of these events occurred spontaneously, resulting in an annual incidence of spontaneous venous thromboembolism of 0.8% (95% CI, 0.3 to 1.9). For antithrombin, protein C, and protein S deficiencies separately, this figure was 1.6%, 1.0%, and 0.4%, respectively. Thirty-four subjects experienced a total of 40 risk periods during which 4 venous thromboembolic events occurred (10% per risk period). We conclude that the use of continuous anticoagulant prophylaxis seems not warranted in asymptomatic individuals with a deficiency of antithrombin, protein C, or protein S. During risk periods for venous thromboembolism, adequate anticoagulant prophylaxis is necessary.

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THROMBOPHILIA is a term used to describe an inherited tendency toward venous thromboembolism. Deficiency of the anticoagulant antithrombin was the first inherited risk factor for venous thromboembolism discovered in 1965. After this, deficiencies of the naturally occurring anticoagulants protein C and protein S were described as causes of inherited thrombophilia. In recent years, several other thrombophilic risk factors have been discovered, the most common of which are the factor V Leiden and the prothrombin 20210A gene mutations.

There is ample evidence that deficiencies of antithrombin, protein C, and protein S are associated with an increased risk of venous thromboembolism. Over the years, many family studies have reported on the risk of venous thromboembolism in such subjects. However, the reported incidences in these studies are considered to be overestimations of the true incidence due to the fact that they may have been prone to selection and publication bias. A further disadvantage of previous studies is that the diagnosis of venous thromboembolic events was made primarily on clinical grounds, which is known to be highly unspecific, thus adding to the overreporting of venous thromboembolism.

Until recently, perhaps the most reliable figures on the incidence of venous thromboembolism in subjects with a deficiency of either antithrombin, protein C, or protein S were those reported in a review analysis of the available literature, which reported an incidence of a first thromboembolic event in asymptomatic subjects of approximately 3% per year for these 3 thrombophilic defects combined. An earlier smaller prospective study in 44 asymptomatic individuals with a deficiency of protein C or protein S reported an incidence of spontaneous venous thromboembolism of 1.4% per year. In a recent retrospective study in 181 previously asymptomatic deficient subjects, an incidence of spontaneous venous thromboembolism of only 0.4% per year was found. In that study, the incidence of a first venous thromboembolic event related to surgery, trauma, or immobilization was 8.1% per risk period, and the incidence during pregnancy and the postpartum period was 4.1% per pregnancy.

The screening of patients with a venous thromboembolic event for thrombophilic factors, and subsequently their family members, has led to the recognition of an increasing number of asymptomatic individuals with a thrombophilic defect. However, it has remained unclear what the appropriate clinical approach should be in these individuals. For clinical decisions regarding the use of anticoagulant prophylaxis in these asymptomatic individuals with antithrombin, protein C, or protein S deficiency, reliable incidence figures of venous thromboembolism in the various clinical situations are needed. The decision whether these individuals should receive anticoagulant prophylaxis...
laxis must be made by balancing the risk of developing a venous thromboembolic complication, either spontaneously or risk period–related, against the risk and severity of bleeding complications associated with the use of anticoagulant prophylaxis. This clinical dilemma has led to the design of the present study of which the objective was to prospectively assess the incidence of venous thromboembolism (both spontaneous and risk period–related) in nontreated asymptomatic subjects with a deficiency of antithrombin, protein C, or protein S.

**MATERIALS AND METHODS**

**Study subjects.** All patients presenting to the participating centers with a documented period of venous thromboembolism were screened for deficiencies of antithrombin, protein C, and protein S. The presence of a deficiency was confirmed by testing on two separate occasions. Patients with a deficiency of antithrombin (excluding heparin binding defect cases), protein C, or protein S served as index patients for the study. The patients were not tested for other prothrombotic states such as the factor V Leiden mutation, the prothrombin mutation, or hyperhomocysteinemia, as these were not known at the time of the start of the study. All participating centers are referral hospitals for the local community for the diagnostics of venous thromboembolism. The patient populations seen in the centers are therefore representative of typical patients with venous thromboembolism.

Family members of the index patients above the age of 15 years were contacted and were asked to participate. In first instance, only first-degree relatives were contacted. If these were not available, second-degree relatives were asked to participate. A detailed medical history was obtained from these family members with specific attention being paid to the occurrence of prior venous thromboembolic events. Hereafter, the deficiency status of these family members was determined. Those subjects who were found to be deficient on 2 consecutive determinations with a minimum interval of 1 month and who reported no prior venous thromboembolism in their medical history were eligible for inclusion in the study. A prior venous thromboembolic event was considered to have occurred if it had been confirmed by objective diagnostic methods or if anticoagulant therapy had been instituted for a minimum of 3 months after a clinical diagnosis. Exclusion criteria were the presence of a combined inhibitor deficiency and the known presence of an active malignancy. Informed consent was obtained from all study participants. Inclusion for the study was performed between April 1993 and July 1997; follow-up was completed in October 1997.

**Study design.** In this prospective cohort study, no continuous anticoagulant prophylaxis was given to the included subjects. During a risk period for the occurrence of venous thromboembolism, the use of anticoagulant prophylaxis was encouraged; however, the decision to do so and the regimen used was left to the discretion of the treating physician. The definition of a risk period was surgery, trauma, prolonged immobilization (>7 days), pregnancy, and the postpartum period. The use of oral contraceptives was discouraged, but not prohibited. Its use was recorded. Follow-up was performed every 6 months, either by a visit to the study center or by telephone contact. At this time, attention was paid to the occurrence of signs or symptoms possibly indicating the presence of a venous thromboembolic event. The occurrence of risk periods in the past period was also recorded.

At the start of the study, all subjects were instructed to present with any symptoms, which could indicate the presence of a venous thromboembolism (eg, swelling, pain, or redness of the leg; acute shortness of breath, chest pain, hemoptysis). In such a condition, a clinical assessment was made and diagnostic tests were performed when deemed appropriate. Both the study subjects and their general practitioners received written information concerning the implications of the observed deficiency and the advice to strongly consider anticoagulant prophylaxis during a risk period. The study protocol was approved by the medical ethical committees in the participating centers.

**Laboratory assays.** Blood samples were collected into plastic syringes containing 3.8% (vol/vol) sodium citrate in a ratio of 0.1:1.9 (vol/vol) anticoagulant to blood. Platelet poor plasma was obtained by centrifugation at 2,000 g for 20 minutes and stored at −80°C until it was analyzed.

The same materials and methods for the diagnosis of antithrombin, protein C, and protein S defects were used in all centers. Antithrombin antigen concentrations were measured using the Asseraplate Antithrombin III Kit (Boehringer Mannheim, Mannheim, Germany), and antithrombin activity was measured using Berichrom ATIII (Behringwerke, Marburg, Germany). Protein C antigen concentrations were measured with enzyme-linked immunosorbent assays (ELISA) using rabbit antiprotein C polyclonal antibody (DAKO, Glostrup, Denmark) as catching antibody. Rabbit antiprotein C polyclonal horseradish peroxidase (HRP)-conjugated antibody (DAKO) was used as the second antibody diluted 1:1,000 in the ELISA buffer. Protein C activity was measured using the Protein C Reagent Kit (Behringwerke). Concentrations of total and free protein S were measured by ELISA using rabbit antiprotein S polyclonal antibody (DAKO) and the 15C4 antiprotein S monoclonal antibody (Serbio, Gennevilliers, France) as catching antibodies, respectively. Rabbit antiprotein S polyclonal HRP-conjugated antibody (DAKO), diluted 1:1,000, was used as the second antibody. The 15C4 antiprotein S monoclonal antibody recognized only free protein S antigen, whereas PS-C4bp complexes were not detected. A calibration curve for free protein S antigen was obtained by dilution of pooled normal plasma, which contained by definition 100% of free protein S antigen. Therefore, the level of free protein S antigen in patients’ plasma was expressed as the percentage of the free instead of the total protein S antigen present in pooled normal plasma. Protein S activity was measured using the Protein S IL-Kit (Instrumentation Laboratories, Milan, Italy).

The following reference values were used: antithrombin antigen concentration, 80% to 120%; antithrombin activity 80% to 120%; protein C antigen concentration, 70% to 130%; protein C activity, 70% to 130%; total protein S concentration, 70% to 120%; free protein S concentration, 70% to 120%; and protein S activity, 70% to 130%. The criteria used for the classification of antithrombin, protein C, and protein S defects were in accordance with those reported in the current literature.\(^\text{13}\)

DNA analysis for the factor V Leiden mutation and the 20210A prothrombin variant were performed in those patients who became symptomatic during follow-up using previously described methods.\(^\text{14,15}\)

In the same group of patients, levels of homocysteine, as well as the presence of antiphospholipid antibodies, were determined according to previously reported methods.\(^\text{16,17}\)

**Outcomes.** The primary outcome was the occurrence of an objectively documented venous thromboembolic event. This was defined as any of the following: (1) deep vein thrombosis of the leg or of the arm, documented by either contrast venography or compression ultrasonography; (2) pulmonary embolism documented either by a high-probability ventilation-perfusion scan or by pulmonary angiography; and (3) objectively documented thrombosis of deep veins elsewhere in the body. A venous thromboembolic event was categorized as being either spontaneous or secondary to a risk period. A spontaneous venous thromboembolism was defined as a thrombotic event occurring without a predisposing risk period. A secondary venous thromboembolism was defined as an event occurring during or within a 3-month period after a risk period.

**Statistical analysis.** The annual incidence of spontaneous venous thromboembolism and its 95% confidence interval (CI) were calculated for the total study population and for the individual deficiencies separately. The incidence of risk period–related venous thromboembolism and its 95% CI were calculated. The occurrence of risk period–
were obtained, 125 years in the antithrombin-deficient group, 37 years (range, 15 to 79) at the time of their inclusion in the study. Ninety-five of these subjects were male (47/45) and 70 a protein S deficiency (type I or II), and 208 were found to be deficient and served as index patients in this study. From these 94 families, 841 family members were identified as potentially eligible for participation in the study. In total, 106 subjects were not interviewed or tested for their deficiency status due to refusal in 49 cases and nonavailability of the subject in 57 cases (eg, living abroad, contact not able to be established). The remaining 735 subjects (mean, 8 per index patient; range, 0 to 18) were interviewed and their blood was tested for the presence of the thrombophilic defect. Eighty-seven (11.8%; mean, 1 per index patient; range, 0 to 3) of these subjects reported a prior episode of venous thromboembolism and were excluded from the study. Twenty of these subjects came from families with an antithrombin deficiency, 27 from families with a protein C deficiency, and 40 from families with a protein S deficiency. Of the remaining 648 subjects, 208 were found to be deficient and were included in the study. Forty-five subjects had an antithrombin deficiency (type I or II), 93 a protein C deficiency (type I or II), and 70 a protein S deficiency (type I or III). None exhibited protein S variants (type II defects). Ninety-five of these subjects were male and 113 were female. The mean age of the subjects at the time of their inclusion in the study was 37 (range, 15 to 79) years (Table 1).

Observation years. A total of 611 patient observation years were obtained, 125 years in the antithrombin-deficient group, 204 years in the protein C–deficient group, and 282 years in the protein S–deficient group. The average duration of observation per subject was 3 years (range, 0.3 to 4.5 years). Nine venous thromboembolic events occurred during the course of the study, 5 in the antithrombin-deficient group and 2 in both the protein C– and protein S–deficient groups. The overall annual incidence of venous thromboembolism was 1.5% (95% CI, 0.7 to 2.8).

Spontaneous events. A total of 5 spontaneous venous thromboembolic events occurred. This results in an annual incidence of 0.8% per year (95% CI, 0.3 to 1.9) when the 3 deficiency groups are combined. Two of these events occurred in the antithrombin-deficient group (1.6% per year; 95% CI, 0.2 to 5.8); 2 in the protein C–deficient group (1.0% per year; 95% CI, 0.1 to 3.5); and 1 in the protein S–deficient group (0.4% per year; 95% CI, 0.3 to 1.9). All of these patients were female, none of whom were taking oral contraceptives or hormone replacement therapy at the time of their thrombosis. The average age of the subjects at the time of the thromboembolic event was 60 years (range, 42 to 69 years). Four patients had proximal deep vein thrombosis, while the fifth presented with a nonfatal pulmonary embolism (Table 2). Two patients presented with minimal complaints during a routine follow-up visit and were found to have deep vein thrombosis on ultrasonography.

Risk period–related events. Thirty-four of the 208 subjects experienced a total of 40 risk periods. Eleven of these risk periods were surgery, 11 were a trauma, 4 were immobilization, 9 were pregnancies, and 5 were other types of risk periods. Anticoagulant prophylaxis was administered by the treating physician during 22 of these risk periods. Fifteen subjects received low-dose unfractionated heparin, 2 low-dose, and 3 high-dose low-molecular-weight heparin, and 3 subjects received oral vitamin K antagonists (international normalized ratio [INR], 2 to 3) as prophylaxis during the risk period. Eight women used oral contraceptives for variable times during the study for a total duration of 18 years. These women had chosen to continue the use of oral contraceptives despite being informed of the potential risk of venous thromboembolism. None of them developed a venous thromboembolism.

Four venous thromboembolic events occurred during or within 3 months of a risk period (Table 3). This results in an overall incidence of 10.0% per risk period (95% CI, 2.8% to 23.7%). One event occurred in a protein S–deficient woman 1 day postpartum while receiving low-dose unfractionated heparin and whether anticoagulant prophylaxis had been used.

### Table 1. Baseline Characteristics of the Asymptomatic Deficient Subjects Included in the Study

<table>
<thead>
<tr>
<th>Deficiency</th>
<th>Average Age (range), Years</th>
<th>Antigen %, Mean ± SD</th>
<th>Activity %, Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antithrombin</td>
<td>37 (15-71)</td>
<td>64.8 ± 14.3</td>
<td>54.6 ± 11.4</td>
</tr>
<tr>
<td>Protein C</td>
<td>36 (15-79)</td>
<td>56.1 ± 14.4</td>
<td>46.7 ± 7.8</td>
</tr>
<tr>
<td>Protein S</td>
<td>39 (15-74)</td>
<td>55.5 ± 18.8</td>
<td>40.1 ± 14.2</td>
</tr>
<tr>
<td>Total</td>
<td>37 (15-79)</td>
<td>49.9 ± 6.5</td>
<td></td>
</tr>
</tbody>
</table>

*PS total antigen.
†PS free antigen.
The remaining 3 events occurred in antithrombin-deficient subjects who received no anticoagulant prophylaxis for their risk period. During the 22 risk periods in which anticoagulant prophylaxis was administered 1 event occurred, resulting in an incidence of 4.5% per risk period, while 3 events (16.7%) occurred during the 18 risk periods in which no form of anticoagulant prophylaxis was given. The average age of the subjects at the time of the risk period–related venous thromboembolic event was 41 years (range, 24 to 66) (Table 2).

All subjects who developed a venous thromboembolic event during the study were evaluated for the presence of multiple genetic risk factors. None were found to be carriers of the factor V Leiden mutation or the prothrombin 20210A gene mutation, or have hyperhomocysteinemia, antiphospholipid antibodies, or dysfibrinogenemia.

**DISCUSSION**

This large prospective cohort study was designed to assess the incidence of both spontaneous and risk period–related venous thromboembolism in asymptomatic deficient family members of patients who have presented with a venous thromboembolism and a deficiency of antithrombin, protein C, or protein S. The observed incidence of spontaneous venous thromboembolism in the total group was 0.8% per year (95% CI, 0.3% to 1.9%). The incidence of spontaneous venous thromboembolism in the 3 deficiency groups separately shows a relatively large variation (antithrombin, 1.6% per year; protein C, 1.0% per year; and protein S, 0.4% per year). Therefore, it could be questioned whether it is correct to group these 3 thrombophilic defects into 1 category. It must be noted, however, that the incidence found in all 3 groups falls within each other’s 95% confidence limits and that similar odds ratios for the occurrence of venous thromboembolism have been reported for these 3 thrombophilic defects. This study has been limited to the deficiencies of antithrombin, protein C, and protein S, as these were the only known inherited risk factors at the start of this study. Furthermore, these deficiencies carry a significantly greater risk for venous thromboembolism than the factor V Leiden mutation.

The observed incidence of 0.8% per year of spontaneous venous thromboembolism is lower than was calculated based on the published family studies. These earlier estimates of the incidence are likely to be inflated because these family studies were prone to selection bias and relied primarily on clinical diagnoses of venous thromboembolism. In this study, selection bias was prevented by including index patients independent of their family history. Patients with a previous episode of venous thromboembolism were excluded. However, a broad range of subjects remained in the study with a relatively low average age. It could be argued that exclusion of the 87 patients with a history of thrombosis would bias the current analysis toward a lower incidence of first thrombotic events. However, in the retrospective analysis of a subset of the same families, in which these cases were the outcome event, even a lower incidence was observed. The on average relatively high age of the patients at the time of the thrombotic events in this study, which is still lower than in large prospective cohort studies of unsolicited patients with venous thromboembolism, can be attributed to its prospective design. Retrospective studies, as previously performed, naturally tend toward collection of many observations and thus events in the younger age groups. In this study, all venous thromboembolic events were diagnosed by means of objective diagnostic methods. The determination of deficiency status was performed, as in most laboratories, by measurement of functional plasma concentrations and not by genetic determination.

The observed incidence of spontaneous venous thromboembolism is also slightly lower than that reported in a small prospective study of asymptomatic protein C– and protein S–deficient subjects by Fabinger et al (1.4% per year), but as mentioned, is slightly higher than that which we found in the previous retrospective study (0.4% per year). A possible explanation for this difference in the observed incidence is that because proper information was provided systematically to (potential) participants, the subjects were made aware of the signs and symptoms of venous thromboembolism. Moreover, we performed objective diagnostic testing when complaints occurred. Therefore, it is possible that thrombotic events were diagnosed, which would otherwise have passed unnoticed. However, the observed incidences in this study and our retrospective study fall within each other’s 95% confidence limits.

Clinical conditions such as surgery, immobilization, trauma, pregnancy, and the postpartum period, as well as the use of oral contraceptives, are well-recognized risk periods for the occurrence of venous thromboembolism. Patients with a deficiency of antithrombin, protein C, or protein S, and 1 of these clinical conditions are at an increased risk in comparison to the normal population. In the present study, the incidence of risk period–related venous thromboembolism in the subjects who received anticoagulant prophylaxis was 4.5% as compared with 16.7% in the group who did not receive prophylaxis. Limitations in the observations concerning risk period–related venous thromboembolism in this prospective study are the relatively small number of risk periods during the observation period and the nonuniform approach concerning anticoagulant prophylaxis. However, because it is likely that anticoagulant prophylaxis was not given in the presumed lower risk situations, the incidence of thrombosis without prophylaxis is probably an underestimation of the true incidence and that likewise the effect of prophylaxis is underestimated.

However, on the basis of the high incidence of venous thromboembolism related to risk periods found in this study (10%), in which both patients and physicians were aware of the
implications of the presence of a deficiency, it is warranted to stress the importance of prophylactic strategies in asymptomatic-deficient individuals during risk periods. It remains unknown whether during such risk periods higher dosages of anticoagulation are required, whether the prophylaxis should be continued for a longer period of time, and whether there should be a lower threshold for the administration of anticoagulant prophylaxis in comparison to patients without a thrombophilic defect.

What are the implications of this study for decisions concerning the continuous use of anticoagulant prophylaxis in asymptomatic carriers of a deficiency of antithrombin, protein C, or protein S? When considering the use of long-term prophylactic anticoagulation, in particular vitamin K antagonists, the benefits of the use of these agents must be carefully weighed against the risk of bleeding complications associated with their use. The annual incidence of serious bleeding during the use of therapeutic dosages of vitamin K antagonists is approximately 2%, while that of fatal bleeding complications is approximately 0.25% per year.\(^{19,20}\) These incidences clearly increase with age. We have found an annual incidence of a first spontaneous thrombotic event in the 3 deficiencies combined of 0.8%. In this study, no fatal venous thromboembolic events occurred; however, it has been estimated that 5% of venous thromboembolic events may be fatal.\(^{21}\) Taking these various factors into consideration, the annual incidence of spontaneous fatal venous thromboembolism would be 0.04% in asymptomatic-deficient individuals. If these assumptions are used on the upper limit of the 95% confidence interval of the incidence (1.9%), we find an annual incidence of spontaneous fatal venous thromboembolic events of 0.10%. Based on these assumptions, the use of continuous anticoagulant prophylaxis seems not warranted in asymptomatic individuals with a deficiency of antithrombin, protein C, or protein S.

**REFERENCES**

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