Protein C Deficiency in a Controlled Series of Unselected Outpatients: An Infrequent But Clear Risk Factor for Venous Thrombosis (Leiden Thrombophilia Study)

By Ted Koster, Frits R. Rosendaal, Ernest Briët, Felix J.M. van der Meer, Louisa P. Colly, Paul H. Trienekens, Swibertus R. Poort, Pieter H. Reitsma, and Jan P. Vandenbroucke

A deficiency of protein C (PC), antithrombin, or protein S is strongly associated with deep-vein thrombosis in selected patients and their families. However, the strength of the association with venous thrombosis in the general population is unknown. This study was a population-based, patient-control study of 474 consecutive outpatients, aged less than 70 years, with a first, objectively diagnosed, episode of venous thrombosis and without an underlying malignant disease, and 474 healthy controls who matched for age and sex. Relative risks were estimated as matched odds ratios. Based on a single measurement, there were 22 (4.6%) patients with a PC deficiency (PC activity, less than 0.67 U/mL or PC antigen, less than 0.33 U/mL when using coumarins). Among the controls, the frequency was 1.5% (seven subjects). Thus, there is a threefold increase in risk of thrombosis in subjects with PC levels below 0.67 or 0.33 U/mL [matched odds ratio, 3.1; 95% confidence interval (CI), 1.4 to 7.0]. When a PC deficiency was based on two repeated measurements, the relative risk for thrombosis increased to 3.8 (95% CI, 1.3 to 10); when it was based on DNA-confirmation, the relative risk increased further to 6.5 (95% CI, 1.8 to 24). In addition, there was a gradient in thrombosis risk, according to PC levels. The results for antithrombin are similar to those for PC, although less pronounced (relative risk, 2.2; 95% CI, 1.0 to 4.7). We could not find an association between reduced total protein S (relative risk, 0.7; 95% CI, 0.3 to 1.8) or free protein S levels (relative risk, 1.6; 95% CI, 0.6 to 4.0) and thrombosis risk. Although not very frequent, PC and antithrombin deficiency are clearly associated with an increase in thrombosis risk.

PROTEIN C, PROTEIN S, and antithrombin are components of physiologically important anticoagulant systems.[1-3] A deficiency of these proteins can cause thrombosis due to shortage of coagulation inhibition.

To date, a number of investigators have provided estimates of the prevalence of deficiencies of these three major coagulation inhibitors in selected patient groups or in large populations of healthy individuals.[4-10] Obviously, the wide range in these observed prevalences is related to the selection of the subjects.

Heterozygosity for protein C deficiency, and also for protein S and antithrombin deficiency, has been associated with a tendency to venous thrombosis in selected patients and their families.[11,12] The total frequencies of these defects among thrombosis patients have been estimated at 3% to 8%.[4,5] This view was challenged by Miletich et al[8] in 1987, who, among 5,422 healthy blood donors, identified 79 subjects with low protein C levels, none of whom had a personal or family history of venous thrombotic disease.

The results of the studies in selected groups are probably overstated: in studies that investigated a selected group of patients with repeated thrombosis at a younger age or with a clear familial risk, the association between a protein deficiency and deep-vein thrombosis is likely to be higher than in the average thrombosis patient. In contrast, among healthy blood donors recruited on the basis of absence of previous disease, the relation between a protein deficiency and thrombosis is likely to be underestimated. More importantly, most studies were uncontrolled; ie, there was no reference group or index group, which is crucial to investigate the association between a deficiency and venous thrombosis. The study by Heijboer et al[7] was the first controlled study that included 277 patients and 138 control subjects. However, this study was not designed to estimate the association between the hereditary deficiency and venous thrombosis, but rather to assess the positive predictive value for the presence of an isolated protein deficiency in patients with recurrent, familial, or juvenile deep-vein thrombosis. In another controlled pedigree study by Allaart et al,[13] it was reported that truly heterozygous protein C-deficient individuals had a ninefold higher thrombosis risk than their nondeficient family members. Nondeficient family members are excellent controls to investigate the etiologic question of the association between a deficiency and thrombosis, as they closely resemble the index group. They are less appropriate, however, to estimate the size of the relative risk when these families are identified by a highly selective referral process. This may lead to an underestimate of the true risk, eg, when the presence of other, possibly yet unidentified, risk factors in the family contributed to their thrombophilia, or it may lead to an overestimate, when families with an abundance of thrombosis (ie, with several probands) are selectively diagnosed or referred.

At present, the impact of a deficiency of one of the major coagulation inhibitors as a cause of venous thrombosis in the general population is still unclear. Therefore, we have investigated 474 unselected and consecutive patients, aged less than 70 years, with a first, objectively confirmed episode of deep-vein thrombosis and without underlying malignant disease and compared the plasma levels of protein C, protein S, and antithrombin with those of 474 matched healthy con-

From the Department of Clinical Epidemiology and the Hemostasis and Thrombosis Research Center, University Hospital Leiden, Leiden; and the Anticoagulation Clinics of Leiden, Amsterdam, and Rotterdam, The Netherlands.

Submitted September 8, 1994; accepted January 4, 1995.

Supported by The Netherlands Heart Foundation (Grant No. 89.063).

Address reprint requests to Ted Koster, MD, Dept of Clinical Epidemiology, University Hospital Leiden, Bldg 1 CO-P-43, PO Box 9600, 2300 RC Leiden, The Netherlands.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. section 1734 solely to indicate this fact.

0006-4971/95/8510-0010$3.00/0

trol subjects. The study was part of a population-based, case-control study on hereditary venous thrombosis: the Leiden Thrombophilia Study (LETS).

PATIENTS AND METHODS

Study design. Patients were selected from the files of the Anticoagulation Clinics in Leiden, Amsterdam, and Rotterdam, The Netherlands. In The Netherlands, anticoagulation clinics monitor coumarin treatment in virtually all patients with venous thrombosis, with each clinic monitoring for patients in a well-defined geographic area.[14,15] As all of our patients originated from one of these areas, geography was the first criterion for eligibility. We included all 474 consecutive outpatients aged younger than 70 years who were referred for diagnosis or monitoring of anticoagulant treatment after a first, objectively confirmed episode of deep vein thrombosis that occurred between January 1, 1988, and December 31, 1992 (Leiden area, n = 271); January 1, 1989, and December 31, 1992 (Amsterdam area, n = 90); and January 1, 1990, and June 31, 1992 (Rotterdam area, n = 113). Patients with known malignant disorders were excluded. Information about the inclusion and exclusion criteria was obtained from general practitioners, from discharge records from the hospitals, and from the records of the anticoagulation clinics. Patients on short-term coumarin treatment were seen only after anticoagulant treatment was discontinued for at least 3 months. Forty-eight (10%) of the 474 included patients, however, were on long-term coumarin treatment and were not allowed to interrupt their medication for various reasons: of these 48 patients, 16 patients had recurrent thrombosis before the end of the study, 11 patients had additional cardiac indications (myocardial infarction, atrial fibrillation, coronary bypass grafting, congenital abnormalities), seven patients were already known to have a coagulation inhibitor deficiency, six patients had peripheral arterial disease, two patients had severe postthrombotic symptoms, and six patients had other, mainly prophylactic, indications. The median time between the occurrence of the deep-vein thrombosis and venepuncture for this study was 19 (range, 6 to 68) months. Of eligible patients, 90% were willing to participate in the study.

Each thrombosis patient was asked to find his own healthy control subject according to the following criteria: same sex, same age (±5 years), no biologic relationship, no history of venous thromboembolism, no use of coumarins for at least 3 months, and no known malignant disorders. Partners of patients were also invited to volunteer as control subjects. When a patient was unable to find a control subject, the first individual from the list of partners who matched for age and sex was asked to join the study; 225 (47%) matched control subjects were partners of other patients. The control subjects and cases were inhabitants of the same geographic areas.

All subjects completed a standard questionnaire that contained questions about hormone use at the time of the venepuncture and about the presence of acquired risk factors in the past, confined to a specific period before the index date (ie, date of the thrombotic event). As acquired risk factors, we considered surgery or hospitalizations in the year preceding the index date, postpartum periods (within 30 days of the index date); and pregnancy at the time of the index date.

Laboratory studies. Blood was collected from the antecubital vein into Monovette tubes (Sarstedt, Nümbrecht, Germany) containing 0.106 mmol/L trisodium citrate. Plasma was prepared by centrifugation for 10 minutes at 2,000*g* at room temperature and stored at −70°C in 1.5 mL volumes until used. High-molecular-weight DNA was isolated from leukocytes and stored at 4°C.

Protein C activity and antithrombin activity were measured with Coamate (Chromogenix, Mölndal, Sweden); factor II activity, with a chromogenic method using S-2238 (Chromogenix) and Echis Carinatus[16] (Sigma Chemical Co, St Louis, MO) on an ACL 200 (Instrumentation Laboratory, Milan, Italy). Protein C antigen was assessed by electroimmunoassay as described elsewhere.[17] Total protein S was measured by polyclonal enzyme-linked immunosorbent assay (ELISA),[18] and free protein S was measured directly in plasma by ELISA using two monoclonal antibodies specific for free protein S (Asserachrom free Protein S, Stago Diagnostica, Asnieres-sur-Seine, France).[19,20] The results are expressed in units per milliliter, where 1 U refers to the activity or antigen present in 1 mL pooled normal plasma (so, 1 U/mL = 100%). The activated partial thromboplastin time (APTT), prothrombin time; and thrombin time were measured using Cephotest (Nycomed, Oslo, Norway), Thromborel S (Behringwerke AG, Marburg, Germany), and Thrombin Reagent (Baxter, Miami, FL), respectively, on an Electra 1000 (MLA, Pleasantville, NY).

For the identification of mutations in the protein C gene, we used polymerase chain reaction (PCR) amplification followed by direct sequencing.[21]

To exclude observer bias, the technicians were blinded to the status of the sample, ie, whether it was from a patient or a control subject.

The criteria for the diagnosis of protein deficiencies were plasma levels below the lower limit of normal, as used in our laboratory, combined with normal values of factor II and prothrombin time (to exclude a vitamin K deficiency or coumarin use). The lower limits of normal were 0.67 U/mL for protein C and total protein S and 0.80 U/mL for antithrombin. For the new free protein S assay, a reference range was derived from the results in the healthy control subjects. After logarithmic transformation of the data, the lower limit of normal was 0.57 U/mL (mean minus 1.96 SD; none of the subjects had values outside 3 SD of the mean). For those on stable long-term coumarin therapy, the lower limit of normal for protein C antigen (Laurell) and total protein S antigen was 0.33 U/mL. For the primary analysis, we only used the outcomes of the first measurements. In all subjects with a protein C level below 0.67 (or 0.33) U/mL, DNA analysis was performed. For secondary analysis, all subjects with protein C, total protein S, or antithrombin levels below the cut-off points were seen on a second occasion for re-evaluation.

Analysis and statistics. We calculated relative risks, as estimates of the crude matched odds ratios, for each of the protein deficiencies by simple crosstabulation. We used Miettinen's test-based 95% confidence limits.[22] The relative risk reflects the thrombosis risk when the plasma level is below a certain limit relative to the risk when it is within the normal range, adjusted for the matching factors of age and sex. As the plasma levels of the inhibitory proteins have a continuum of values instead of binary outcomes, we examined if there was a relation between the plasma levels and thrombosis risk (dose-response) by calculating relative risks over levels of the plasma factor (only for those not on oral anticoagulants).

RESULTS

The mean age for patients and controls was 47 years (range, 16 to 70 years for patients, 16 to 73 years for controls); and the male:female ratio among patients and controls alike was 3:4.

Table 1 lists the risk estimates for protein C. Based on a single measurement, the relative risk for thrombosis was 3.1 (95% confidence interval [CI], 1.4 to 7.0). In a secondary analysis, which included the re-evaluation in 28 subjects with initially low levels (one patient was not available), the thrombosis risk for persistently low results became almost 4. For all subjects with initial plasma levels below 0.67 U/mL, the protein C gene was investigated. In 13 patients and

Table 1. Comparison of Prevalence and Thrombosis Risk in 474 Patients and 474 Control Subjects for Several Definitions of a Protein C Deficiency

Definition of Protein C Deficiency (lower limit of normal for those on coumarin)	No. of Patients (%)	No. of Controls (%)	OR*	95% CI
First measurement				
<0.67 U/mL (0.33)	22 (4.6)	7 (1.5)	3.1	1.7-7.0
First and second measurement				
<0.67 U/mL (0.33)	15 (3.1)	4 (0.8)	3.8	1.3-10
First measurement				
<0.67 U/mL (0.33) and the presence of a mutation	13 (2.7)	2 (0.4)	6.5	1.8-24

Abbreviation: OR, age- and sex-matched odds ratio.

* All discordant patient-control pairs.

Table 3. Thrombosis Risk for Categories of Protein C Activity Levels

Protein C Strata (U/mL)	No. of Patients	No. of Controls	OR*	95% CI
<0.55	4 (4)	1 (1)	4.0	0.5-36
0.55-0.65	10 (5)	3 (2)	3.7	1.0-13
0.65-0.75	16 (ND)	10 (ND)	1.8	0.8-4.2
0.75-0.85	54 (ND)	44 (ND)	1.3	0.9-2.0
≥0.85	342 (ND)	368 (ND)	1.0†	

Patient-control pairs (n = 426) without coumarin use (first measurements). Data in parentheses indicate number of subjects in whom a mutation was found.

Abbreviation: ND, not done.

* Test for trend, $P < .01$.

† Reference category.

two controls, a mutation was found (Table 2), which led to a thrombosis risk for abnormally low levels of protein C confirmed by DNA analysis of 6.5 (95% CI, 1.8 to 24). Table 1 also shows the drop in prevalence of a protein C deficiency among patients (from 4.6% to 2.7%) and controls (from 1.5% to 0.4%) when more stringent criteria were used to establish a diagnosis. This resulted in a clear increase of the relative risk for thrombosis (from 3.1 to 6.5).

Table 3 gives the odds ratios for the categories of protein C plasma levels, especially in the range where discrimination between true heterozygotes and normal individuals with low levels is difficult. It shows an inverse relation between the risk of thrombosis and the plasma level of protein C, for levels below the reference category (≥0.85 U/mL).

Table 4 shows that we found slightly more control subjects than patients with initial and persistently low total protein S levels, which ranged from 0.45 to 0.66 U/mL among the controls, and from 0.59 to 0.65 U/mL among the patients (0.26 to 0.27 U/mL for two patients on oral anticoagulant treatment). Thus, there was no association between low total protein S levels and thrombosis risk. The male:female ratio within individuals with low protein S levels was 2:17 (among those low at remeasurement, 2:9), and contrasted with the male:female ratio for protein C-deficient (13:16) and antithrombin-deficient (13:11) subjects. The two males with low total protein S levels were both from the patient group. There was no difference in frequency of oral contraceptive use between female patients (67%) and controls (73%) with low protein S levels.

Based on a single free protein S measurement in the 426 patient-control pairs not using oral anticoagulants, the relative risk for thrombosis was 1.6 (95% CI, 0.6 to 4.0). When we used both total and free protein S levels below the lower limit of normal as criteria for the diagnosis of a protein S deficiency, the relative risk became 1.7 (95% CI, 0.4 to 6.9).

Table 4 also gives the risk estimates for antithrombin. Based on one measurement, the relative risk for thrombosis

Table 2. Protein C Mutations in 13 Patients and Two Control Subjects

Exon	Nucleotide Position and Mutation	Amino Acid Change	No. of Patients	No. of Controls
	1515, G → A	None, near 5 donor splice junction of intron C		1
6	3439, C → T	132, Q → stop	1	
7	6219, G → A	169, R → Q	2	
9	8403, C → T	230, R → C	7	1
9	8505, Ins TGG	264, ins V; in frame insertion	1	
9	8506, Ins TGGC	Frameshift, stop at 331	1	
9	8631, C → T	306, R → stop	1	

Table 4. Comparison of Prevalence and Thrombosis Risk in Thrombosis Patients and Control Subjects for Protein S and Antithrombin Deficiency

Criterion of a Deficiency	No. of Patients (%)	No. of Controls (%)	OR	95% CI
Total protein S (lower limit of normal for those on coumarin)				
First measurement				
<0.67 U/mL (0.33)	8 (1.7)	11 (2.3)	0.7*	0.3-1.8
First and second measurement				
<0.67 U/mL (0.33)	5 (1.1)	6 (1.3)	0.8†	0.2-3.0
Free protein S‡				
First measurement				
<0.57 U/mL	13 (3.1)	9 (2.1)	1.6§	0.6-4.0
Total and free protein S‡				
Both below lower limits of normal	5 (1.2)	3 (0.7)	1.7§	0.4-6.9
Antithrombin				
First measurement				
<0.80 U/mL	20 (4.2)	9 (1.9)	2.2§	1.0-4.7
First and second measurement				
<0.80 U/mL	5 (1.1)	1 (0.2)	5.0§	0.7-34

* Seventeen discordant patient-control pairs; in seven of the pairs, the patient had a lowered protein S level and the control subject did not.

† Nine discordant patient-control pairs; in four of the pairs, the patient had a lowered protein S level and the control subject did not.

‡ Patient-control pairs (n = 426) not using oral anticoagulants.

§ All discordant patient-control pairs.

Table 5. Frequency of Acquired Risk Factors in Patients With and Without a Coagulation Inhibitor Deficiency and in Healthy Controls

	Presence of Acquired Risk Factor* (%)	
	Yes	No
Thrombosis patients		
With abnormal PC/PS/AT tests†	31	69
With normal PC/PS/AT tests†	34	66
Control subjects	6	94

Abbreviations: PC, protein C; PS, protein S; AT, antithrombin.

* Considered were surgery, hospitalization, pregnancy, postpartum bedrest, and prolonged immobilization.

† The proportions did not alter when only PC and AT were considered.

was 2.2 (95% CI, 1.0 to 4.7) and became 5.0 (95% CI, 0.7 to 34) when it was based on two persistently low measurements. There was insufficient variation in plasma levels to perform a dose-response analysis (27 of the 29 subjects with initially lowered levels had values between 0.70 and 0.80 U/mL).

The number of subjects in whom at least one acquired risk factor was present in the period before the thrombotic event (or before the index date for the control subjects) is presented in Table 5. It shows that acquired risk factors were equally frequent among thrombosis patients with normal and with abnormal test outcomes for one of the coagulation inhibitors (difference, 3%; 95% CI, −17% to 12%), showing that spontaneous thrombotic episodes are not at all limited to the group with protein deficiencies. Among the healthy controls, acquired risk factors were also frequently present (6%), demonstrating that these acquired states in themselves are insufficient to cause thrombosis.

DISCUSSION

Among 474 consecutive patients aged less than 70 years with a first, objectively diagnosed episode of venous thrombosis and without an underlying malignancy, functional protein C deficiency was the most frequent coagulation inhibitor deficiency. Protein C deficiency also conferred the highest relative risk of thrombosis. Additionally, there was a gradient of thrombosis risk according to protein C levels. When stringent diagnostic criteria were applied, the relative risk became 6.5, which is likely due to less misclassification and, therefore, is the most reliable estimate of the relative risk of thrombosis associated with hereditary protein C deficiency. The results for antithrombin are approximately similar to those for protein C, although somewhat less pronounced. Low protein S levels were not associated with an increased thrombosis risk.

Depending on the applied criteria, the prevalence of protein C deficiency among the controls varied from 1.5% to 0.4%. These figures (1:70 to 1:250) are strikingly similar to those reported by Miletich et al,[8] who found 79 (1.5%) of 5,422 healthy blood donors with antigen levels below 65% of normal. Of these 79 subjects, 62 to 71 were considered likely to be part of the continuous distribution of normal, which led to an extrapolated estimated prevalence of 1 in 200 to 1 in 300. This shows that the study by Miletich et al[8] gave reproducible prevalence figures for a healthy population but did not clarify the risk of thrombosis.

The prevalence of a protein C deficiency among our unselected outpatients was 2.7% to 4.6%, again depending on the criteria, which is similar to the 3.2% (95% CI, 1.5% to 6.2%) prevalence reported by Heijboer et al[7] but is lower than reported in studies that investigated patients with a history of unusual thrombosis at an early age.[4,5] Obviously, this relates largely to the method of patient selection in the different groups.

Seven distinct mutations were shared by the 15 subjects in whom a protein C gene abnormality was identified (Table 2). The position-230, R → C replacement was most common and was present in seven patients and one control. The position-169, R → Q substitution recurred twice, whereas the remaining five mutations all occurred once. Three of the mutations resulted in premature stop codons. This leaves no room for doubt regarding the causative relationship between low protein C levels and mutation. The relationship between half-normal functional levels of protein C is also obvious for the position-169, R → Q replacement, which involves the arginine in the cleavage site for thrombin. The position-230, R → C substitution is believed to be the direct cause of the protein C deficiency, as this defect is the most common abnormality in Dutch protein C-deficient subjects.[21] The mutation that leads to the insertion of a valine after amino acid position 264 is novel, and, presently, there are no additional data that support the causative nature of this mutation. Comparison of the homologous amino acid sequences of protein C, factor IX, factor VII, factor X, and prothrombin shows that the mutation is in a stretch of two nonconserved amino acids that are flanked by two invariable amino acids at positions 263 and 266. From this it seems reasonable to hypothesize that the mutation disrupts the correct folding of the protein C molecule. More problematic is the interpretation of the position-1515, G → A transition. This nucleotide replacement occurs 15 nucleotides into intron C. It does not seem to create a new consensus sequence for splicing. Therefore, it is possible that the mutation represents a rare sequence variant without functional consequences. One should also note that we do not know whether the nucleotide change is in the allele that putatively cosegregates with the protein C deficiency. This doubt about the certainty of a protein C deficiency in one of the two control subjects may lead to a twofold error in our estimate of the relative risk (6.5 *v* 13); ie, the true risk might be twice as high as reported in Table 1.

To date, studies among healthy blood donors and among highly selected patient groups have provided estimates of the prevalence within particular populations. However, such prevalences are insufficient to quantify the relation between a deficiency and thrombosis risk. To answer this question, a comparison between unselected patients and population-based thrombosis-free controls is necessary. The controls in the study of Heijboer et al[7] were individuals suspected of deep-vein thrombosis that could not be confirmed by impedance plethysmography. These individuals do not constitute

a satisfactory sample of the population of thrombosis-free controls, as impedance plethysmography is insensitive in detecting calf-vein thrombosis.[23] It is, therefore, likely that some of these individuals actually had venous thrombosis of the calf veins, which might have been caused by a protein C deficiency. The result of a 1.4% prevalence of a protein C deficiency among the controls, defined as at least two measurements below the lower limit of normal, might, therefore, have been an overestimate. This is supported by the present results; we found a twofold lower prevalence (0.8%) with persistent lowered protein C levels among the controls (two measurements on fresh blood samples).

To our surprise, we found that lowered total protein S levels were not associated with venous thrombosis. By using lower cut-off points, we consistently found slightly more control subjects than patients with lower values (data not shown). Even among those who repeatedly had low levels, controls were overrepresented. The male:female (2:17) ratio was also unexpected for an autosomal inherited disorder. Therefore, we investigated, by multiple linear regression analysis, the influence of oral contraceptives on the protein S level; among the female controls, the age-adjusted reduction in protein S levels was on average 0.09 U/mL for pill users. However, this difference in protein S levels does not explain our result, as the number of women with low protein S levels who were using oral contraceptives was approximately similar for patients and controls. It does show that different cut-off points for protein S antigen levels are required for males and females, if not also for pill users and non-pill users. Nevertheless, even with a separate analysis for men and women and the strictest cut-off point for nonanticoagulated subjects, no clear thrombosis risk emerged (females, 0 cases and two controls $\leq$0.55 U/mL; no males with levels $\leq$0.55 U/mL). Our result is in disagreement with many reports of protein S-deficient kindreds, in which venous thrombosis appears highly prevalent and is clearly related to the deficiency.[24-26] One possibility might be that protein S deficiency is so rare that we found only a few hereditary deficiencies. This explanation seems unsatisfactory, as we found two control subjects who had persistent levels at 0.50 U/mL: ie, were very likely heterozygous. This renders an extremely low prevalence of the deficiency in the general population unlikely. We, therefore, believe that the possibility that the previous findings in families are the result of cosegregating additional genetic defects should be seriously considered. The possibility that protein S is not a clear risk factor for deep-vein thrombosis in unselected consecutive patients is further supported by the finding that, in systems of purified proteins, protein S has little cofactor activity to activated protein C.[27,28] The data on the free protein S assay showed also no clear relation between a deficiency and thrombosis, although a positive effect cannot be excluded.

The prevalences of low levels of antithrombin are in agreement with the recent literature. Heijboer et al[7] reported a strikingly similar prevalence for repeatedly low levels of antithrombin of 1.1% among unselected patients with thrombosis. A relative risk could not be recalculated from that report, as all 138 control subjects had normal antithrombin levels. In the present study, the relative risk varied from 2.2 to 5.0, depending on the applied criteria. The prevalence of antithrombin deficiency among the controls was 1.9% (one measurement) or 0.2% (two repeated measurements), which is close to the recently reported prevalences in healthy blood donors.[9,29] These findings indicate that antithrombin deficiency is a very rare disease, and this probably explains why our relative risk estimate of 5.0 could not reach statistical significance.

The control subjects were selected by the patients. We do not believe that this practice may have biased our findings on these hereditary abnormalities in the coagulation system, as it is difficult to imagine that patients choose their friends or partners on the basis of unknown plasma levels of anticoagulant proteins.

This population-based, case-control study confirms that, among unselected patients, the prevalence of a (hereditary) protein C deficiency and of lowered protein S and antithrombin plasma levels is relatively low compared with the 20% prevalence of activated protein C-resistance (factor V Leiden) among unselected patients.[30,31] Although the clinical burden is, therefore, limited, protein C deficiency clearly confers a threefold to almost sevenfold increase in risk of thrombosis.

ACKNOWLEDGMENT

We thank T. Visser for laboratory assistance, A. van Beek for secretarial and administrative support, and Prof R.M. Bertina for his advice.

REFERENCES

1. Esmon CT: The regulation of natural anticoagulant pathways. Science 235:1348, 1987

2. Clouse LH, Comp PC: The regulation of hemostasis: The protein C system. N Engl J Med 314:1298, 1986

3. Preston FE, Briët E: Familial thrombophilia, in Brenner MK, Hoffbrand AV (eds): Recent Advances in Haematology. London, UK, Churchill Livingstone, 1994, p 217

4. Gladson CL, Scharrer I, Hack V, Beck KH, Griffin JH: The frequency of type I heterozygous protein S and protein C deficiency in 141 unrelated young patients with venous thrombosis. Thromb Haemost 59:18, 1988

5. Ben-Tal O, Zivelin A, Seligsohn U: The relative frequency of hereditary thrombotic disorders among 107 patients with thrombophilia in Israel. Thromb Haemost 61:50, 1989

6. Tabernero MD, Tomas JF, Alberca I, Orfao A, Lopez Borrasca A, Vicente V: Incidence and clinical characteristics of hereditary disorders associated with venous thrombosis. Am J Hematol 36:249, 1991

7. Heijboer H, Brandjes DPM, Büller HR, Sturk A, ten Cate JW: Deficiencies of coagulation-inhibiting and fibrinolytic proteins in outpatients with deep-vein thrombosis. N Engl J Med 323:1512, 1990

8. Miletich J, Sherman L, Broze G: Absence of thrombosis in subjects with heterozygotes protein C deficiency. N Engl J Med 317:991, 1987

9. Tait RC, Walker ID, Perry DJ, Islam SIAM, Daly ME, McCall F, Conkie JA, Carrell RW: Prevalence of antithrombin deficiency in the healthy population. Br J Haematol 87:106, 1994

10. Tait RC, Walker ID, Islam SIA: Prevalence of protein C deficiency in the general population. Br J Haematol 81S:4, 1992 (abstr)

11. Broekmans AW, Veltkamp JJ, Bertina RM: Congenital pro-

tein C deficiency and venous thromboembolism: A study in three Dutch families. N Engl J Med 309:340, 1983

12. Horellou MH, Conard J, Bertina RM, Samama M: Congenital protein C deficiency and thrombotic disease in nine French families. Br Med J 289:1285, 1984

13. Allaart CF, Poort SR, Rosendaal FR, Reitsma PH, Bertina RM, Briët E: Increased risk of venous thrombosis in carriers of hereditary protein C deficiency defect. Lancet 341:134, 1993

14. van der Meer FJM, Rosendaal FR, Vandenbroucke JP, Briët E: Bleeding complications in oral anticoagulant therapy. An analysis of risk factors. Arch Intern Med 153:1557, 1993

15. Loeliger EA, van Dyk-Wierda CA, van den Besselaar AMHP, Broekmans AW, Roos J: Anticoagulant control and the risk of bleeding; organizational infrastructure, in Meade TW (ed): Anticoagulants and Myocardial Infarction: A Reappraisal. Chichester, UK, Wiley, 1984, p 157

16. Bertina RM, van der Marel-van Nieuwkoop W, Loeliger EA: Spectrophotometric assays of prothrombin in plasma of patients using oral anticoagulants. Thromb Haemost 42:1296, 1979

17. Bertina RM, Broekmans AW, van der Linden IK, Mertens K: Protein C deficiency in a Dutch family with thrombotic disease. Thromb Haemost 48:1, 1982

18. Deutz-Terlouw PP, Ballering L, van Wijngaarden A, Bertina RM: Two ELISAs for measurement of protein S, and their use in the laboratory diagnosis of protein S deficiency. Clin Chim Acta 186:321, 1989

19. Amiral J, Grosley B, Boyer-Neumann C, Marfaing-Koka A, Peynaud-Debayle E, Wolf M, Meyer D: New direct assay of free protein S antigen using two distinct monoclonal antibodies specific for the free form. Blood Coagul Fibrinolysis 5:179, 1994

20. Wolf M, Boyer-Neumann C, Peynaud-Debayle E, Marfaing-Koka A, Amiral J, Meyer D: Clinical applications of a direct assay of free protein S antigen using monoclonal antibodies. A study of 59 cases. Blood Coagul Fibrinolysis 5:187, 1994

21. Reitsma PH, Poort SR, Allaart CF, Briët E, Bertina RM: The spectrum of genetic defects in a panel of 40 Dutch families with symptomatic protein C deficiency type I: Heterogeneity and founder effects. Blood 78:890, 1991

22. Miettinen OS: Estimatibility and estimation in case-referent studies. Am J Epidemiol 103:226, 1976

23. Heijboer H, ten Cate JW, Büller HR: Diagnosis of venous thrombosis. Semin Thromb Haemost 17:259, 1991

24. Comp PC, Esmon CT: Recurrent venous thromboembolism in patients with a partial deficiency of protein S. N Engl J Med 311:1525, 1984

25. Broekmans AW, Bertina RM, Reinalda-Poot J, Engesser L, Muller HP, Leeuw JA, Michiels JJ, Brommer EJP, Briët E: Hereditary protein S deficiency and venous thrombo-embolism. A study in three Dutch families. Thromb Haemost 53:273, 1985

26. Engesser L, Broekmans AW, Briët E, Brommer EJP, Bertina RM: Hereditary protein S deficiency: Clinical manifestations. Ann Intern Med 106:677, 1987

27. Bakker HM, Tans G, Janssens-Claessen T, Thomassen MCLGD, Hemker HC, Griffin JH, Rosing J: The effects of phospholipids, calcium ions and protein S on rate constants of human factor Va inactivation by activated human protein C. Eur J Biochem 208:171, 1992

28. Koedam JA, Meijers JCM, Sixma JJ, Bouma BN: Inactivation of human factor VIII by activated protein C. Cofactor activity of protein S and protective effect of von Willebrand factor. J Clin Invest 82:1236, 1988

29. Wells PS, Blajchman MA, Henderson P, Wells MJ, Demers C, Bourque R, McAvoy A: Prevalence of antithrombin deficiency in healthy blood donors: A cross-sectional study. Am J Hematol 45:321, 1994

30. Koster T, Rosendaal FR, Ronde de H, Briët E, Vandenbroucke JP, Bertina RM: Venous thrombosis due to poor anticoagulant response to activated protein C: Leiden Thrombophilia Study. Lancet 342:1503, 1993

31. Bertina RM, Koeleman BPC, Koster T, Rosendaal FR, Dirven RJ, de Ronde H, van der Velden PA, Reitsma PH: Mutation in blood coagulation factor V associated with resistance to activated protein C. Nature 369:64, 1994