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Increased risk of venous thrombosis in carriers of natural anticoagulant deficiencies. Results of the family studies of the Spanish Multicenter Study on Thrombophilia (EMET study).

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Increased risk of venous thrombosis in carriers of natural anticoagulant deficiencies. Results of the family studies of the Spanish Multicenter Study on Thrombophilia (EMET study)

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Several studies have demonstrated a higher risk of thrombosis in carriers of anticoagulant deficiencies than in non-deficient individuals from families with thrombophilia. The prevalences in Spain were established in a multicenter study (the EMET study) and all the deficient individuals were invited to recruit all available family members to be screened for the same deficiency in order to establish the risk of thrombosis in deficient individuals. Five-hundred-and-eighty-three individuals from 114 families with natural anticoagulant deficiencies were analysed. Propositi and relatives with a history of thrombosis were asked about the localization and the age at the first episode and whether or not it was spontaneous. Three families with antithrombin deficiency, 35 with protein C, 60 with protein S, four with plasminogen, four with heparin cofactor II, seven with combined deficiencies and one family with dysfibrinogenemia were included in the analysis. The risk of thrombosis was increased for individuals deficient in antithrombin (adjusted odds ratio 21.23; 95% confidence interval 5.71–78.94), protein C (adjusted odds ratio 12.62; 95% confidence interval 4.75–33.51), protein S type I (adjusted odds ratio 19.95; 95% confidence interval 7.40–53.82), protein S type III (adjusted odds ratio 8.11; 95% confidence interval 2.66–21.99) or in protein C plus protein S (adjusted odds ratio 8.99; 95% confidence interval 2.79–28.93), but not for those deficient in plasminogen or heparin cofactor II. The thrombosis-free survival was shortened for deficient individuals in antithrombin (median 30 years), protein C (median 46 years), protein S type-I (median 48 years), protein S type III (median 61 years) and combined protein C and S (median 40 years). In conclusion, individuals carrying anticoagulant deficiencies have an increased risk of thrombosis, especially those with antithrombin, protein C or type I protein S deficiencies. *Blood Coag Fibrinol* 9:71–78 © 1998 Rapid Science Ltd.

Keywords: venous thrombosis, thrombophilia, familial thrombosis, thrombosis risk, natural anticoagulant deficiency

Introduction

One of the most common causes of morbidity, disability and mortality today is thromboembolic disease. Its incidence in the general population may be as high as one per 1000 individuals/year [1]. In recent years, abnormalities of the natural anticoagulant pathways in patients and families suffering from thrombophilia have been detected.

Hereditary deficiencies of antithrombin [2,3], protein C and protein S [4–12], and the newly-recognized factor V Leiden [13–15] are associated

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with a tendency to thrombosis, i.e. thrombophilia. Other abnormalities such as heparin cofactor II or plasminogen deficiencies and dysfibrinogenemia have been associated with thrombophilia, though their role is uncertain [16]. Several studies have demonstrated a higher risk of thrombosis in carriers of anticoagulant deficiencies versus non-deficient individuals, usually members of families with thrombophilia [3,4,6,7,11]. These studies have demonstrated a higher risk of thrombosis in deficient versus non-deficient individuals, all of them from families with thrombophilia. There are substantial discrepancies between the prevalence of these protein deficiencies in some studies carried out in different countries [17–25]. For this reason, the prevalences in Spain were established in a multicenter study [25], and all the deficient individuals were invited to recruit all available family members to be screened for the same deficiency. The results of the screening and the risk of thrombosis for each deficiency are discussed in this work.

Materials and methods

Families

From March 1989 to June 1992, 583 individuals from 114 families were studied. The propositi were patients from the Spanish Multicenter Study on Thrombophilia (the EMET study) [25]. In this study, 2132 consecutive unselected patients with proven venous thromboembolism were screened for biological abnormalities causing thrombophilia (the participant hospitals are listed in the appendix). Once a patient with a deficiency was identified, family members were screened for the same protein deficiency. Propositi and relatives with previous venous thrombosis were asked whether they had a history of recurrent thrombosis and their age at the time of the first episode. The diagnosis of the thrombotic event was established by objective methods in probands. In the case of relatives, only those with objectively proven thrombotic episodes were considered in the analyses. The localization of thrombosis and the presence of acquired predisposing factors were also noted. The episode was considered spontaneous in the absence of triggering risk factors.

Laboratory methods

Blood was collected 3–6 months after the last thrombotic event in the propositi. The samples were obtained without the influence of oral anticoagulants to prevent interference with protein C and protein S measurements. Should discontinuation of anticoagulants be inadvisable, the samples are collected after a 20-day heparin therapy period. Heparin was not administered the day before sampling. Blood samples were immediately anticoagulated with sodium citrate (0.129 mol/l) and platelet-poor plasma was harvested by centrifugation at 1600 × *g* for 20 min and stored at −40°C until analysis.

Prothrombin time, activated partial thromboplastin time and thrombin time measurements were performed in all the samples to exclude acquired abnormalities of hemostasis or the use of anticoagulants. Antithrombin, heparin cofactor II, plasminogen and amidolytic protein C were determined by using chromogenic substrates from Chromogenix (Stockholm, Sweden). Normal ranges were from 80 to 110% for antithrombin, from 50 to 125% for heparin cofactor II, from 70 to 140% for plasminogen, and from 70 to 130% for amidolytic protein C. Anticoagulant activity of protein C was measured using reagents from Behring (Marburg, Germany; normal = 70–150%). Total protein S was assayed by an ELISA method (Asserachrom Protein S, Stago, Asnières, France). Free protein S was measured by precipitating the C4b-bound fraction with polyethylene glycol and measuring the free protein S in the supernatant using the same ELISA method [26]. The normal range was from 75 to 140% for total protein S and from 75 to 140% for free protein S. In young women (under 45 years), the lower levels for total and free protein S were 65 and 60% respectively [27]. Other antigenic measurements were performed only if the functional assays were below the normal range. Antigenic antithrombin and heparin cofactor II were measured by Laurell's method. Antigenic protein C was measured by an ELISA method (Asserachrom Protein C, Stago). Antigenic plasminogen was determined by radial immunodiffusion. Fibrinogen was quantified according to Clauss' method. Detection of activated protein C resistance was unavailable at the time of this study. The normal ranges listed above are those of the coordinating center (Hospital de Sant Pau, Barcelona) and were obtained from the analysis of 100 healthy blood donors. The coordinating center reanalysed all patients with abnormal or borderline values in the first and in a second (confirmatory) sample. Diagnosis of a deficiency was established if the plasma level of a protein was below the lower limit of its normal range in at least two different samples, and the deficiency was considered to be hereditary if at least one relative had the same protein deficiency. Despite the debate about the subclassification of protein S deficiency, we decided to use the proposal

recommended by the Scientific and Standardization Committee of the International Society on Thrombosis and Haemostasis in Munich, July 1992.

Statistical analysis

The results are expressed as means ± standard deviations for quantitative variables and in percentages with the 95% confidence interval (CI), for qualitative variables. The differences between quantitative variables were analysed by means of the Mann Whitney U-test and differences between qualitative variables by using the χ^2-test. A Kaplan–Meier analysis was used to estimate the thrombosis free survival (in years) of the different groups. The population considered as the reference group was composed of the non-deficient relatives. The age at the time of the thrombotic event in the case of symptomatic individuals and the age at the inclusion in the study of asymptomatic ones were considered to be time variable. Differences between factors were analysed by the log-rank test. The Cox regression method was employed to determine the odds ratios of thrombotic risk with respect to non-deficient individuals, adjusted for sex and type of individual (propositus or relative). The adjustment for type of individual obviates the need for calculating the risks after the exclusion of probands. This approach was used because the number of relatives with thrombosis was low in our population.

Results

Laboratory screening of families

One hundred and fourteen propositi of the 274 deficient individuals initially detected in the EMET study [25] were recruited for family analysis. Four families with antithrombin deficiency, 35 with protein C, 60 with protein S, four with plasminogen, four with heparin cofactor II, six with combined deficiencies and one family with dysfibrinogenemia were included in the analysis. Protein C plus free protein S were found in four kindreds (all affected individuals carried both defects). The other combined deficiencies were plasminogen plus other deficiencies (protein C and type I protein S), and were considered in the respective groups of the other deficiencies for the analyses. In the case of protein-S-deficient families, although there were families showing both phenotypes, one was absolutely predominant and this was considered to catalogue the entire family. Eleven families with type I protein S deficiency were studied and two had members with type III deficiency. In these two families, 40 individuals were screened, 20 had type I, three type III protein S deficiency and the other 17 were normal. For type III, two out of 49 studied families had members showing type I phenotype. In these two families, 11 individuals were analysed, six were type III, two type I and three normal. The number of deficiencies, whether congenital or not, and the number of studied relatives are shown in Table 1. The differences in the number of relatives between deficiencies not proven versus those proven as hereditary were significant overall and specifically for type III protein S deficiency (see Table 1).

Clinical parameters

The number of thrombotic and asymptomatic individuals with the different types of deficiencies is shown in Table 2. The age at first thrombosis and the number of individuals with recurrent thrombosis are also given. Non-thrombotic individuals were younger than thrombotic patients. There were no

Table 1. Number of families and individuals studied and classification of proven and not-proven as hereditary deficiencies[a]

Deficiencies	No. studied families	No. proven as hereditary (%)	No. not proven as hereditary (%)	No. of relatives studied in families proven as hereditary (mean ± SD)	No. of relatives studied in families not proven as hereditary (mean ± SD)
Antithrombin	4	2 (50)	2 (50)	3.5 ± 2.5	4 ± 0
Protein C	35	24 (69)	11 (31)	7.2 ± 6.0	4.3 ± 1.9
Protein S (type I)	11	10 (91)	1 (9)	9.4 ± 9.3	3
Protein S (type III)	49	27 (55)	22 (45)	5.5 ± 2.2	3.7 ± 2.2 $P < 0.01$
Plasminogen	4	4 (100)	0	6.5 ± 1.7	0
Heparin cofactor-II	4	1 (25)	3 (75)	4	5.7 ± 1.5
Dysfibrinogenemia	1	1 (100)	0	3	0
Combined[b]	6	5 (83)	1 (17)	8.7 ± 2.5	3
Total	114	74 (65)	40 (35)	6.8 ± 5.2	4.0 ± 2.0 $P = 0.001$

[a]Deficiencies not proven as congenital means that the deficiency was not found in other relatives, mainly because an insufficient number of relatives. [b]Combined deficiencies were: protein C plus type III protein S (four cases), protein C plus plasminogen and type I protein S plus plasminogen, each one in a single case. One case of protein C plus type III protein S deficiency was not confirmed as hereditary.

Table 2. Thrombotic and asymptomatic individuals classified by type of deficiency

Deficiencies	No. of thrombotic individuals[a]	No. of asymptomatic individuals	Age at first thrombosis (mean ± SD)	No. of patients with multiple thromboses[b]
Antithrombin	5	4	33.0 ± 20.2	2
Protein C	46	72	38.8 ± 16.6	17
Protein S (type I)	24	35	33.9 ± 16.8	8
Protein S (type III)	51	36	52.5 ± 16.7	11
Plasminogen	5	10	63.6 ± 10.4	0
Heparin cofactor-II	4	2	59.5 ± 25.7	1
Dysfibrinogenemia	1	2	60	0
Combined	9	5	45.7 ± 21.9	5
Non-carriers	5	270	48.8 ± 12.8	0
Overall	150	433	44.7 ± 18.8	44

[a]Propositi are included. [b]Multiple thromboses was defined as more than one episode.

differences as regards sex. The clinical characteristics of thrombotic patients are listed in Table 3.

Risk of thrombosis

Compared with non-deficient individuals, the risk of thrombosis was found to be increased for antithrombin, protein C or protein S deficiencies, but not for heparin cofactor II or plasminogen deficiencies. The corresponding odds ratios are shown in Table 4. These odds ratios were calculated after adjustment for sex and type of individual (propositus or relative). Significant differences in thrombosis-free survival of deficient individuals and non-carriers were found. At the age of 56 years, 50% of deficient individuals were free of thrombosis (95% CI 51–61). Differences in thrombosis free survival were also noted for some particular deficiencies: the probability of an antithrombin-deficient subject being free of thrombosis at 30 years of age was 50% (95% CI 26–34; Fig. 1). At 46 years of age, 50% (95% CI 39–53) of those with protein C deficiency had at least one manifestation of venous thrombosis (Fig. 2). As for protein S deficiency, 50% (95% CI 28–68) of the deficient individuals had thrombosis at the age of 48 years if the deficiency was type I, or at the age of 61 years if it was type III (95% CI 55–67; Fig. 3). Differences in thrombosis free survival between type I and type III protein S deficiencies tended to be significant ($P = 0.06$), but probably more cases are needed to confirm an early tendency to thrombosis in type-I-deficient individuals. At 40 years of age, 50% (95% CI 3–77) of individuals with combined deficiencies in protein C and protein S had thrombosis (Fig. 4). These differences were maintained when the analyses were performed after

Table 4. Thrombotic risk (expressed as adjusted odds ratio and 95% CI) of different protein deficiencies adjusted for sex and type of individual (propositus or relative)

Deficiencies	Odds ratio (95% CI)
Antithrombin	21.23 (5.71–78.94) $P < 0.001$
Protein C	12.62 (4.75–33.51) $P < 0.001$
Protein S (type I)	19.95 (7.40–53.82) $P < 0.001$
Protein S (type III)	8.11 (2.66–21.99) $P < 0.001$
Plasminogen	NS
Heparin cofactor II	NS
Combined[a]	8.99 (2.79–28.93) $P < 0.001$

NS, not significant. [a]Combined deficiencies of protein C + protein S.

Table 3. Clinical characteristics of thrombotic individuals

	n (%)
Sex	
Men	63 (42%)
Women	87 (58%)
Multiple thromboses[a]	44 (29%)
Age at first thrombosis	44.7 ± 18.8
Spontaneous	53 (35%)
Secondary[b]	97 (65%)
Surgery	34 (23%)
Immobilization	31 (21%)
Pregnancy[c]	27 (20%)
Oral contraceptives[c]	11 (13%)
Varicose veins	18 (12%)
Obesity	11 (7%)
Neoplasms	6 (4%)
Localization	
Deep vein thrombosis	127 (85%)
Pulmonary embolism[d]	38 (25%)
Superficial thrombophebitis	6 (4%)
Mesenteric thrombosis	1 (0.7%)
Intracranial vein thrombosis	1 (0.7%)

[a]Multiple thromboses was defined as more than one episode. [b]Some patients had more than one risk factor. [c]Only women were considered. [d]Deep vein thrombosis was diagnosed in 23 patients with pulmonary embolism.

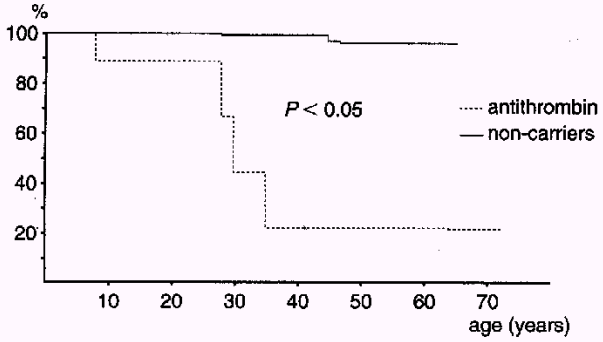

Figure 1. Thrombosis-free survival of antithrombin-deficient individuals compared with non-deficient ones.

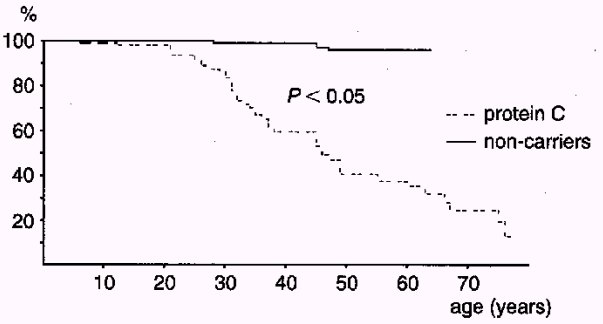

Figure 2. Thrombosis-free survival of protein-C-deficient individuals compared with non-deficient ones.

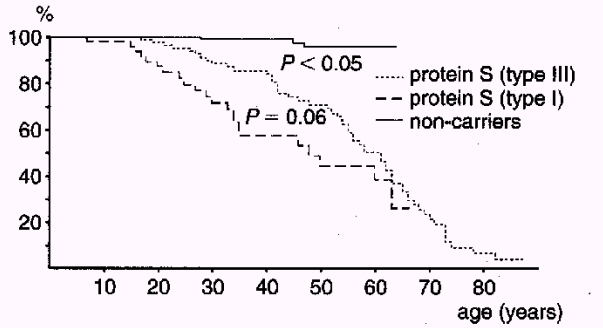

Figure 3. Thrombosis-free survival of protein-S-deficient individuals compared with non-deficient ones. Type I and type III are shown separately. Both are different from non-carriers ($P < 0.05$). No differences between type I and type III were noted ($P = 0.06$).

the exclusion of the probands (data not shown). By contrast, the thrombosis-free survival of heparin cofactor II or plasminogen-deficient individuals was not different from that of unaffected individuals. The cumulated thrombosis-free survival adjusted for sex and type of individual (propositus or relative) is shown in Figure 5. As for specific deficiencies, the

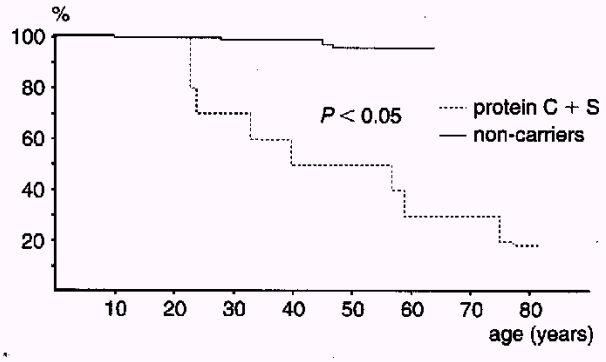

Figure 4. Thrombosis-free survival of individuals with combined deficiencies (protein S plus protein C) compared with non-deficient ones.

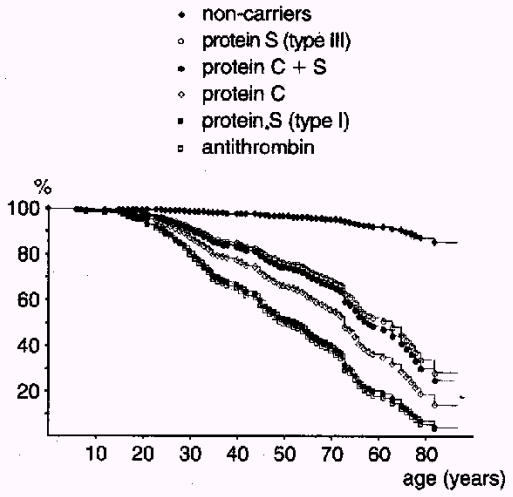

Figure 5. Cumulated thrombosis-free survival adjusted for sex and type of individual (propositus or relative).

thrombosis-free survival was lower for antithrombin than for protein C ($P < 0.04$) or type III protein S deficiencies ($P < 0.01$). There was a tendency of type I protein-S-deficient individuals to have a shorter thrombosis-free survival than type-III-deficient ones ($P = 0.06$). Other differences between thrombosis-free survival were not found, probably because of the small sample.

Discussion

Natural anticoagulant deficiencies are of clinical relevance not only to the patient, but also to other asymptomatic relatives and offspring who may carry the deficiency. The diagnosis determines the type and duration of treatment as well as a more intense prophylaxis in future thrombotic risk situations.

Knowledge of a thrombotic risk may help to prevent further thrombotic events, especially in affected asymptomatic relatives. Several studies have been carried out to estimate the actual risk of thrombosis in these deficiencies. All available data are based mainly on uncontrolled retrospective studies which, whilst demonstrating a higher risk of thrombosis in deficient versus non-deficient individuals [3,4,6,11] have not provided reliable estimates of the actual risk of thrombosis. An important multicenter case–control study [the European Prospective Cohort On Thrombophilia (EPCOT)] is currently being conducted [28]. One of its most important objectives is to determine the risk of thrombosis in carriers of prothrombotic deficiencies. In most studies, participants belong to families with a high risk of thrombosis and may be aware of a thrombotic tendency in their families. This could result in overstating the history of thrombotic events. However, in our study, the relatives did not know whether or not the proband was affected at the time of the thrombotic episodes since all the individuals, probands and relatives were newly recruited for the EMET study.

The available estimates of the risk of thrombosis in antithrombin deficiency are scant. An analysis of pooled data in the literature [3] gave approximately a 50% thrombosis-free survival at the age of 27 years. Despite the low number of antithrombin-deficient individuals in Spain [25], a similar analysis of our patients was carried out and the age of a 50% thrombosis-free survival was found to be very similar. The risk of thrombosis in protein-C-deficient individuals was found to be 50% at the age of 45 years in a study where the deficient state of each individual was confirmed by molecular analysis of the protein C gene [6]. Our data were obtained on the basis of phenotypic findings (confirmed protein C deficiency in plasma), but the estimates of thrombosis-free survival was similar (50% at the age of 46 years). Available data on protein S deficiencies were supplied by Zöller *et al.* [11] who, whilst demonstrating an increased risk of thrombosis in affected individuals, found no differences among type I or type III. In our study, no differences were observed either. As regards combined deficiencies, we were able to analyse those who had protein S plus protein C deficiencies, since the combinations were plasminogen plus other deficiencies and were included in the respective groups of the other deficiencies. Earlier data about combined deficiencies were supplied by the studies of Pabinger *et al.* [4] and Zöller *et al.* [11]. Despite the small number of cases, an increased risk of thrombosis in these patients was found at an earlier age. This was also the case with our patients, whose thrombosis-free survival was shortened. The calculations of the risk of thrombosis showed that plasminogen or heparin cofactor-II-deficient individuals did not run an increased risk of thrombosis. This is in agreement with the view that the role of these deficiencies in thrombophilia is uncertain [19]. All these data suggest that individuals with antithrombin, protein C and protein S deficiencies without previous thrombotic events have an increased risk of thrombosis.

Although it was not our intention to establish a reliable risk of thrombosis, we do think that these results are helpful and, moreover, they are consistent with findings in other geographical zones. It should be pointed out, however, that one problem encountered by physicians who perform family studies in Spain is the poor compliance of patients. Our study shows that the number of relatives screened is important for establishing a diagnosis of familial deficiency. It is occasionally necessary for the physician to stress the importance of studying a sufficient number of relatives belonging to thrombophilic pedigrees in order to identify affected individuals.

There are currently no well-designed studies that establish how and when thromboembolic prophylaxis must be undertaken in affected individuals. Nor is there any information about whether the hemorrhagic risk of long-term anticoagulant treatment outweighs the benefit of antithrombotic prophylaxis. At present, the most reasonable course of action is to carry out thromboprophylaxis in risk situations only [4,29].

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Appendix

The Estudio Multicéntrico Español de Trombofilia (EMET) group consists of:

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