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Laboratory Evaluation and Clinical Characteristics of 2,132 Consecutive Unselected Patients with Venous Thromboembolism – Results of the Spanish Multicentric Study on Thrombophilia (EMET*-Study)

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Summary

Previous studies on the prevalence of biological abnormalities causing venous thrombosis and the clinical characteristics of thrombotic patients are conflicting. We conducted a prospective study on 2,132 consecutive evaluable patients with venous thromboembolism to determine the prevalence of biological causes. Antithrombin, protein C, protein S, plasminogen and heparin cofactor-II deficiencies, dysfibrinogenemia, lupus anticoagulant and antiphospholipid antibodies were investigated. The risk of any of these alterations in patients with familial, recurrent, spontaneous or juvenile venous thrombosis was assessed. The overall prevalence of protein deficiencies was 12.85% (274/2,132) and antiphospholipid antibodies were found in 4.08% (87/2,132). Ten patients (0.47%) had antithrombin deficiency, 68 (3.19%) protein C deficiency, 155 (7.27%) protein S deficiency, 16 (0.75%) plasminogen deficiency, 8 (0.38%) heparin cofactor-II deficiency and 1 had dysfibrinogenemia. Combined deficiencies were found in 16 cases (0.75%). A protein deficiency was found in 69 of 303 (22.8%) patients with a family history of thrombosis and in 205/1,829 (11.2%) without a history (crude odds ratio 2.34, 95% CI 1.72-3.17); in 119/665 (17.9%) patients with thrombosis before the age of 45 and in 153/1,425 (10.7%) after the age of 45 (crude odds ratio 1.81, 95% CI 1.40-2.35); in 103/616 (16.7%) with spontaneous thrombosis and in 171/1,516 (11.3%) with secondary thrombosis (crude odds ratio 1.58, 95% CI 1.21-2.06); in 68/358 (19.0%) with recurrent thrombosis and in 206/1,774 (11.6%) with a single episode (crude odds ratio 1.78, 95% CI 1.32-2.41). Patients with combined clinical factors had a higher risk of carrying some deficiency. Biological causes of venous thrombosis can be identified in 16.93% of unselected patients. Family history of thrombosis, juvenile, spontaneous and recurrent thrombosis are the main clinical factors which enhance the risk of a deficiency. Laboratory evaluation of thrombotic patients is advisable, especially if some of these clinical factors are present.

Introduction

Thromboembolic disease is a relatively common disease whose incidence may be as high as 1 per 1000 individuals/year in the general population (1). In recent years, abnormalities of the natural anticoagulant pathways in patients and families suffering from thrombophilia have been detected. Antithrombin deficiency (2,3) and alterations of the protein C system (4-12) are the most frequently recognized causes of thrombophilia. Two thirds of protein S is bound to the ß-chain of the C4b-binding protein. Only the free fraction can work as a cofactor for the activated protein C. Three different types of protein S deficiency have been described. According to the nomenclature proposed by Bertina at the ISTH subcommittee meeting in 1991, type I is characterized by a decrease in both total and free protein S, type II by a functional protein defect and type III by a deficiency of free protein S. The molecular difference between type I and type III is controversial. It has been suggested that both types are phenotypic variants of the same genetic disease (13). Recently, a new cause of thrombophilia related to the protein C system was identified: a single point mutation in the factor V gene resulting in an abnormal factor V resistant to degradation by activated protein C (14-16). After this finding, it has been shown that the individuals previously classified as having type II protein S deficiency do not have a defect in the protein S gene but an activated protein C resistance. The reason was that clotting-based functional assay for protein S is affected by the mutated factor V. Other abnormalities such as heparin cofactor-II (17,18) or plasminogen deficiencies (19,20) and dysfibrinogenemia (21) have been involved in thrombophilia, but their role is uncertain (22). Another common acquired cause of thromboembolic disease is antiphospholipid antibodies (23).

Several studies focusing on the laboratory evaluation of patients with thromboembolic disease have been published (24-33). The reported prevalence of different causes of thrombophilia is highly variable, probably due to different patient selection criteria or to geographical reasons.

In a period of 3 years, we performed a prospective multicentric study of consecutive patients with venous thromboembolism. The aim of the study was to assess the prevalence of known biological abnormalities causing thrombophilia and to determine whether some clinical parameters are useful to identify patients with these abnormalities.

Materials and Methods

Patients

From March 1989 to June 1992, 2,154 consecutive patients (from 29 Spanish hospitals listed in the appendix) with acute symptomatic venous thromboembolic disease were included. Patients with venous thromboembolism of any localization, regardless of sex and age, were eligible for recruitment. Patients with a previous history of chronic or acute liver disease or nephrotic syndrome were excluded from the study so as to avoid acquired causes of pro-

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tein deficiencies. Objective diagnostic procedures of the thrombotic event were performed in all patients by each center in accordance with their respective protocols. The diagnosis of deep vein thrombosis of the lower limbs was always objectively established by ultrasonography or ascending venography. Pulmonary embolism was diagnosed by ventilation-perfusion lung scanning or pulmonary angiography. Thrombosis in other unusual sites was diagnosed by computed tomography or magnetic resonance imaging, as a surgical finding or by ophthalmoscopic examination depending on the type of thrombosis and localizations. Patients were asked whether they had suffered previous episodes of venous thromboembolism. If they had recurrent thrombosis, the age at the time of the first episode was recorded. The localization of thrombosis and the recognized predisposing factors were also noted. The episode was considered spontaneous in the absence of risk factors. Patients whose first thrombotic event occurred before the age of 45 years were considered to have juvenile thrombosis. The family history was considered to be positive if at least another first or second degree family member had had venous thrombosis (parents, children, brothers/sisters or grandparents).

Laboratory Methods

Blood was collected 3 to 6 months after the last thrombotic event. Oral anticoagulants were withdrawn and the samples were taken after a washout period of at least 20 days to prevent the effect of oral anticoagulants on protein C and protein S levels. Should discontinuation of anticoagulants be inadvisable, the samples were obtained after a 20-day heparin-therapy period. Heparin was not administered the day before sampling.

Blood samples were obtained from an antecubital vein and immediately anticoagulated with one-tenth volume of 0.129 M. sodium citrate. Platelet poor plasma was harvested by centrifugation at 1600 g for 20 min and stored at -40° C until analysis. Another sample without additives was collected to analyze antiphospholipid antibodies. Prothrombin time, activated partial thromboplastin time and thrombin time were performed in all the samples to detect 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 with the same ELISA method (34). 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 (35). Other antigenic measurements were performed only if 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, Asnières, France).

Table 1 Localization of the first venous thrombotic event

Localization	no.	(%)
Deep vein thrombosis	1208	56.7%
Pulmonary embolism*	870	40.9%
Mesenteric thrombosis	6	0.3%
Renal vein thrombosis	3	0.1%
Upper arms thrombosis	18	0.8%
Superficial thrombophlebitis	11	0.5%
Retinal vein thrombosis	7	0.3%
Intracranial sinus thrombosis	9	0.4%

* Deep vein thrombosis was diagnosed in 338 patients with pulmonary embolism.

Antigenic plasminogen was determined by radial immunodiffusion. Fibrinogen was quantified according to Clauss' method (36). Lupus anticoagulant was detected by using Exner's method (37). Antiphospholipid antibodies were screened by means of an ELISA that uses cardiolipin and phosphatidylserine as antigens (38). Patients whose values were above the mean plus 3 standard deviations where considered as having antiphospholipid antibodies. 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. They were obtained from the analysis of one hundred 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 only if the plasma level of a protein was below the lower limit of its normal range in at least two different samples. Patients whose alteration was corrected in the second sample were considered to have no deficiency.

Statistical Analysis

Only those abnormalities considered to be hereditary were used for the study of the relationship between protein deficiencies and clinical factors (family history, spontaneous, juvenile and multiple thrombosis). Therefore, patients with antiphospholipid antibodies were not included in the analyses. Frequencies are shown as percentages and 95% confidence intervals (95% CI). All relevant differences are shown as the 95% CI of these differences. A p value less than 0.05 was considered to be significant. The age at the time of the first thrombosis was compared among groups by one-way ANOVA. A hierarchical log-linear model was built to explore the relationship between family history, juvenile, multiple or spontaneous thrombosis, sex and the presence of some deficiency. Starting with a saturated model, a backward stepwise method was used to exclude the uninteresting relationships. The likelihood ratio Chi-square was used to verify goodness-of-fit. A logistic regression method was employed to estimate both crude and adjusted odds ratios of the risk of deficiency. Clinical factors and sex were included as adjustment variables. The improvement of log likelihood was applied to verify goodness-of-fit. Relevant odds ratios are expressed in 95% CI.

Results

The study population consisted of 2,154 consecutive patients with venous thromboembolism. Twenty-two patients were excluded from the analysis because of previously unknown liver cirrhosis. Fifty-two percent (1,101) were men and 48% women (1,031). Their mean age (±SD) was 55.4 ± 1.7 (range 3-89). The age (mean ± SD) at the first thrombosis was 53.3 ± 17.3 (range 2-89). The localizations are shown in Table 1. In 616 (28.9%) patients, the first episode was spontaneous.

Laboratory Screening

The results of the laboratory screening of thrombophilia are summarized in Table 2. A protein deficiency was identified in 274 (12.85%) patients. The most frequent was protein S deficiency, followed by protein C deficiency. Antithrombin, plasminogen, heparin cofactor-II deficiencies and dysfibrinogenemia had a lower prevalence. Combined deficiencies were detected in 16 cases (0.75%). When a patient with a deficiency was identified, all available family members were screened for the same protein deficiency. One hundred and fourteen kindreds could be recruited for family analysis. The number proven as congenital is reflected in Table 3.

Antiphospholipid antibodies were identified in 111 patients (5.21%), and were the only abnormality in 87 (4.08%) of them. Anticardiolipin or antiphosphatidylserine antibodies were detected alone or combined (33 anticardiolipin, 31 antiphosphatidylserine and 47 both) and the isotypes were variable (IgG, IgM or both). Twenty-four patients with antiphospholipid antibodies also had a protein deficiency: 2 antithrom-

Table 2 Number of patients (percentage) with biological defects, levels of the proteins, and age at the first thrombotic event in the studied population

	n (%; 95% CI)	Age at 1st thrombosis mean±SD (95% CI)		levels (%) mean ± SD	
Normal	1771 (83.07; 81.46-84.68)	53.6±16.8	(52.8-54.4)		
Antithrombin	10 (0.47; 0.23-0.86)	33.4±13.7	(23.6-43.2)*	functional	62.0±8.5
				antigen	55.3±11.5
Protein C	68 (3.19; 2.48-4.02)	45.2±19.4	(40.4-49.9)*	coagulative	46.9±14.5
				chromogenic	59.3±13.8
				antigen	57.6±19.9
Protein S (overall)	155 (7.27; 6.21-8.45)	51.9±19.6	(48.8-55.0)	total	85.4±19.5
				free	49.4±15.7
(type I)	34 (1.59; 1.10-2.22)	41.5±19.6	(34.7-48.3)*	total	59.2±9.3
				free	33.8±21.9
(type III)	121 (5.68; 4.73-6.74)	54.8±18.7	(51.5-58.2)	total	92.5±14.9
				free	53.6±9.9
Plasminogen	16 (0.75; 0.43-1.21)	56.8±18.3	(47.0-66.5)	functional	63.4±9.54
				antigen	55.4±15.8
Heparin cofactor-II	8 (0.38; 0.17-0.74)	61.1±17.5	(46.5-75.7)	functional	45.8±4.2
				antigen	42.2±12.4
Dysfibrinogenemia	1 (0.04)	60		functional	0.8 g/l
				antigen	3.1 g/l
Combined deficiencies#	16 (0.75; 0.43-1.21)	54.7±22.0	(43.0-66.4)		
Antiphospholipid antibodies (alone)	87 (4.08; 3.28-5.01)	52.8±17.6	(49.0-56.7)		
All deficiencies¶	274 (12.85; 11.40-14.30)	51.4±19.8	(49.2-53.7)§		
Total	2,132 (100)	53.3±17.3	(52.5-54.0)		

* p<0.001: – antithrombin deficiency versus the following groups: nondeficient, type III protein S, plasminogen and heparin cofactor-II deficiencies
– protein C deficiency versus the following groups: nondeficient and type III protein S deficiency
– type I protein S deficiency versus the following groups: nondeficient and type III protein S deficiency

§ p<0.05: deficient versus nondeficient patients

associations were: protein C plus protein S (11 cases), antithrombin plus protein C (2 cases), protein S plus heparin cofactor-II, protein C plus plasminogen and protein S plus plasminogen, each in a single case

¶ patients with antiphospholipid antibodies are not included

bin, 5 protein C, 14 free protein S. Lupus anticoagulant was positive in only 12 patients. The percentage of patients with antiphospholipid antibodies in the group of patients with low levels of free protein S was significantly higher than the percentage of patients with only antiphospholipid antibodies (12.3% versus 4.7%, difference 7.6%, 95% CI 1.9-13.3, p<0.005).

Clinical Parameters

The main clinical factors considered in this survey were: sex, family history, juvenile, multiple and spontaneous thrombosis. The log-linear analysis showed that all these variables, except sex, were clearly associated with the presence of a protein deficiency.

Family history of thrombosis was recorded in 303 patients (14.2%, 95% CI 12.7-15.7). In this group of patients, the frequency of deficient individuals was higher than in the group without family history (22.8% versus 11.2%; difference 11.6%, 95% CI 6.6-16.6 p<0.01) (Table 4). Data on specific deficiencies are shown in Table 5. The risk of protein C, protein S or combined deficiencies was higher in patients with a family history of thrombosis.

The mean ages at the first thrombosis in patients with different defects are shown in Table 2. Overall, patients with non-acquired deficiencies were slightly but significantly younger than patients without abnormalities. Deficiencies were detected in 17.9% of younger patients

Table 3 Number of deficiencies proven to be congenital

	no. of propositi	no. of studied families	no. of proven as congenital (%)
Antithrombin	10	3	1 (33)
Protein C	68	35	24 (69)
Protein S (type I)	34	11	10 (91)
Protein S (type III)	121	49	27 (55)
Plasminogen	16	4	4 (100)
Heparin cofactor-II	8	4	1 (25)
Dysfibrinogenemia	1	1	1 (100)
Combined deficiencies	16	7	6 (86)
Total	274	114	74 (65)

and in 10.7% of older ones (difference 7.2%, 95% CI 3.9-10.5, p<0.01) (Table 4). Moreover, patients with antithrombin, type I protein S or protein C deficiencies had their first thrombotic event at a younger age than non-deficient patients (Tables 2 and 5).

The frequency of deficiencies was slightly higher in the group with spontaneous thrombosis (16.7% versus 11.3%; difference 5.4% 95% CI 2.1-8.7 p<0.05) (Table 4). As for specific deficiencies, patients with spontaneous thrombosis showed an increased risk of protein C, type I protein S or heparin cofactor-II deficiencies, and a tendency to have

Table 4 Risk of a protein deficiency in patients who have one of the main clinical factors

Clinical feature		no. of patients	no. of deficiences (%)	Crude odds ratio (95% CI)	Adjusted odds ratio§ (95% CI)
Family history of thrombosis	yes	303	69 (22.8)	2.34 (1.72-3.17) p<0.001	2.03 (1.48-2.78) p<0.001
	no	1829	205 (11.2)		
Age at first thrombosis	<45 yrs.	665	119 (17.9)	1.81 (1.40-2.35) p<0.001	1.74 (1.33-2.28) p<0.001
	>45 yrs.	1425	153 (10.7)		
Type of first thrombosis	spontaneous	616	103 (16.7)	1.58 (1.21-2.06) p<0.001	1.78 (1.35-2.35) p=0.003
	secondary	1516	171 (11.3)		
Multiple thrombosis	yes	358	68 (19.0)	1.78 (1.32-2.41) p<0.001	1.60 (1.17-2.18) p<0.001
	no	1774	206 (11.6)		
Sex	women	1031	144 (14.0)	1.21 (0.94-1.56) NS	1.31 (0.96-1.61) NS
	men	1101	130 (11.8)		

NS: not significant

§ Adjusted for all other variables (including sex).

Table 5 Risk of a specific deficiency in accordance with the presence of a clinical factor expressed as adjusted odds ratio (95% CI)

deficiencies	number	Family history (yes versus no)	Age at first thrombosis (<45 yrs. versus >45 yrs.)	Spontaneous versus secondary thrombosis	Multiple versus single thrombosis	Sex (women versus men)
Antithrombin	10	0.53 (NS)	24.77 (3.10-198.04) (p<0.003)	1.89 (NS)	0.53 (NS)	3.17 (NS)
Protein C	68	1.98 (1.11-3.52) (p=0.02)	3.05 (1.83-5.07) (p<0.001)	1.73 (1.02-2.97) (p<0.05)	2.37 (1.39-4.04) (p<0.002)	1.12 (NS)
Protein S, (type I)	34	2.89 (1.39-6.04) (p<0.005)	3.58 (1.75-7.32) (p<0.001)	2.23 (1.09-4.59) (p<0.03)	1.57 (NS)	1.69 (NS)
Protein S (type III)	121	1.61 (1.01-2.59) (p<0.05)	1.15 (NS)	1.44 (NS) (p=0.07)	1.52 (NS) (p=0.07)	1.61 (1.10-2.36) (p=0.01)
Plasminogen	16	2.36 (NS)	0.79 (NS)	2.73 (0.99-7.51) (p=0.051, NS)	1.14 (NS)	1.27 (NS)
Heparin cofactor-II	8	2.97 (NS)	0.35 (NS)	6.04 (1.19-30.58) (p=0.03)	0.60 (NS)	0.20 (NS)
Combined	16	6.73 (2.46-18.41) (p<0.001)	0.87 (NS)	1.71 (NS)	1.52 (NS)	1.47 (NS)

NS: not significant.

plasminogen defects. Risk factors associated with secondary thrombosis were: previous surgery (664 cases), immobility (654), varicose veins (261), obesity (203), neoplasms (109), pregnancy (89), contraceptive pill intake (61) and congestive cardiac disease (68). There was no relationship between these factors and the presence of deficiencies except for an increased risk of antiphospholipid antibodies in women with pregnancy-related thrombosis (odds ratio 3.77; 95% CI 1.23-11.53 p = 0.01), and of protein S deficiency (both types) in women with thrombosis while taking contraceptives (odds ratio 2.41; 95% CI 1.07-5.39 p = 0.03).

Seventeen percent of the patients underwent more than one thrombotic event and the frequency of deficiencies was higher in this group (19.0% versus 11.6%; difference 7.4% 95% CI 3.1-11.7 p<0.01) (Table 4). As for the type of deficiency, the risk of protein C deficiency was increased in patients with recurrent events (Table 5).

Although no differences were observed as regards sex (Table 4), the risk of type III protein S deficiency was higher in women (Table 5). The frequencies of spontaneous and multiple thrombosis were higher in men (36.6% versus 20.6%, difference 16.0%, 95% CI 12.2-19.8, p<0.01 for spontaneous thrombosis; and 18.3% versus 15.1%, difference 3.2%, 95% CI 0.05-6.4, p<0.05 for multiple thrombosis). Pregnancy or contraceptive pill use did not account for the slightly higher frequency of secondary thrombosis found in women.

No relationship between the localization of the first event (Table 1) and deficiencies could be demonstrated. Unusual sites of thrombosis were very infrequent and tended to occur in younger patients without triggering factors, but no statistical differences were found.

Table 6 indicates the risk of a deficiency when two or more of the more representative clinical factors are associated. Patients who had more than one clinical feature were compared with patients lacking these same clinical characteristics. The log-linear analysis showed that one of the most important combinations was: family history, juvenile and multiple thrombosis. The risk of a deficiency increases with the accumulation of clinical factors. Interestingly, a weak albeit significant association of family history with juvenile thrombosis, family history with recurrent thrombosis and male sex with spontaneous thrombosis could also be demonstrated in the group of nondeficient patients.

Table 6 Risk of a protein deficiency in patients with combined clinical factors

Clinical factors		no. of patients	no.of deficiencies (%)	Crude OR (95 % CI)	Adjusted OR§ (95 % CI)
TFH + ST	yes	92	30 (32.6)	4.30 (2.68-6.89)	3.61 (2.37-6.49)
	no	1305	132 (10.1)		
TFH + JT	yes	137	41 (29.9)	3.89 (2.58-5.86)	3.53 (2.90-4.31)
	no	1263	125 (9.9)		
TFH + MT	yes	77	26 (33.8)	4.33 (2.63-7.14)	3.24 (2.12-4.94)
	no	1548	163 (10.5)		
JT + ST	yes	159	41 (25.8)	3.40 (2.25-5.16)	3.11 (2.07-4.67)
	no	982	91 (9.3)		
JT + MT	yes	138	36 (26.1)	3.18 (2.08-4.86)	2.79 (1.87-4.15)
	no	1212	121 (10.0)		
TFH + JT + ST	yes	39	18 (46.2)	9.10 (4.64-17.82)	6.30 (3.84-10.32)
	no	871	75 (8.6)		
TFH + ST + MT	yes	26	10 (38.5)	5.92 (2.62-13.37)	5.76 (3.50-9.49)
	no	1120	107 (9.6)		
TFH + JT + MT	yes	45	22 (48.9)	9.70 (5.22-18.05)	5.65 (3.53-9.02)
	no	1081	97 (9.0)		
JT + ST + MT	yes	42	14 (33.3)	5.38 (2.71-10.67)	4.96 (3.02-8.16)
	no	846	72 (8.5)		
TFH + JT + ST + MT	yes	16	9 (56.2)	15.21 (5.47-42.30)	10.06 (5.78-17.48)
	no	757	59 (7.7)		

TFH: thrombotic family history, JT: juvenile thrombosis, ST: spontaneous thrombosis, MT: multiple thrombosis
§ Adjusted for all other variables (including sex).

Discussion

The diagnosis of a deficiency of a natural coagulation inhibitor is of clinical relevance not only to the patient himself, but also to deficient asymptomatic relatives and offspring. The diagnosis is important since it determines the type and duration of treatment as well as a more intense prophylaxis in future thrombotic risk situations. Even more important is the diagnosis for asymptomatic relatives. Knowledge of a thrombotic risk may help to prevent further thrombotic events.

The main aim of this study was to establish the prevalence of biological abnormalities causing thrombophilia in a cohort of consecutive Spanish patients with venous thrombosis recruited country-wide. We found that 12.85% of the patients had some protein deficiency. Data on the prevalence of these deficiencies are conflicting and rates from 4.5 to 32.2 percent have been reported (24-33) (Table 7). There are marked discrepancies that could be attributed to different patient selection criteria or differences in establishing the diagnosis of a deficiency state. In general, two types of studies may be considered: studies with young patients or those who belong to families that are prone to thrombosis, and studies with unselected patients. Although geographic and genetic reasons could also account for these variations, the high prevalences reported in earlier studies are probably due to patient preselection.

According to our results and those of Tabernero (29), antithrombin deficiency is an exceptional situation in our environment since its prevalence is lower than that reported by other groups (table 7), and only doubles the prevalence in the general population (0.2%) (39). The prevalence of protein C deficiency in our study was found to be similar to the one reported in other series of unselected patients (7, 28, 32, 33) (Table 7) and 8 times higher than in the general population (1 in 250) (40). One of the most striking findings was the high prevalence of protein S deficiency, especially type III. Other studies have found lower rates of protein S deficiency, but the majority of them only report type I protein S deficiency or do not specify the type (Table 7). In patients with type III protein S deficiency, the female:male ratio was 1.33. Koster (7), in their patients with protein S deficiency, found a female:male ratio of 8.5. This difference is probably due to the fact that normal

Table 7 Main studies on the prevalence of protein deficiencies in patients with thrombosis. The number in parentheses represents the percent of the total

	Briët[24]	Gladson[25]	Engesser[27]	Heijboer[28]	Tabernero[29]	Bick[30]	Melissari[31]	Malm[32]	Pabinger[33]	EMET study
Number of patients	113	141	203	277	204	100	382	439	680	2,132
Deficiencies	(32.2)	(15.3)	(18.5)	23 (8.3)	9 (4.5)	18	90 (23.6)	24 (5.5)	48 (7.1)	274 (12.85)
Antithrombin	(4.4)	(3)	(3)	3 (1.1)	1 (0.5)	8	20 (5.2)	3 (0.7)	19 (2.8)	10 (0.47)
Protein C	(11.5)	6 (4.3)	(6.8)	9 (3.2)	3 (1.5)	2	35 (9.2)	10 (2.3)	17 (2.5)	68 (3.19)
Protein S (overall)	(13.2)	7 (5.0)	(7.8)	6 (2.2)	3 (1.5)	8	29 (7.6)	9 (2.1)	9 (1.3)	155 (7.27)
(type I)	ND	7 (5.0)	ND	ND	3 (1.5)	ND	ND	3 (0.7)	9 (1.3)	34 (1.59)
(type III)	ND	ND	ND	ND	0	ND	ND	6 (1.4)	ND	121 (5.68)
Plasminogen	(0.7)	(2)	ND	4 (1.4)	2 (1.0)	0	3 (0.8)	2 (0.5)	ND	16 (0.75)
Heparin cofactor-II	(0.7)	ND	ND	ND	ND	0	ND	ND	ND	8 (0.38)
Dysfibrinogenemia	(1.7)	(1)	(0.9)	ND	ND	0	3 (0.8)	ND	ND	1 (0.04)
Combined deficiencies	ND	ND	ND	1 (0.4)	ND	ND	ND	ND	3 (0.4)	16 (0.75)

young women have lower levels of protein S than older women and men (35). In our study, a cut-off point adjusted for age and sex was used. One recent study supports the idea that type I and type III protein S deficiencies could be different phenotypic variants of the same genetic disease (13). If this were true, the combination of both type I and type III protein S deficiencies would be the second cause of thrombophilia in Spain (7.27%) after activated protein C resistance (see below). The role of plasminogen and heparin cofactor-II deficiencies in thrombosis is unclear. Furthermore, their prevalence is low in our series. The determination of plasminogen and heparin cofactor-II should therefore not be considered in the initial screening of thrombophilia, but measured in a second step. The presence of only one case of dysfibrinogenemia in our series suggest that its detection is anecdotal in our environment. Fibrinogen has been related to arterial thrombosis (41), and recently it has also been identified as a possible risk factor for venous thrombosism (42). Since alterations other than coagulative properties could be implicated, we cannot exclude the presence of other abnormalities with our methodology. Sixteen patients had mixed deficiencies. The most frequent ones were protein C and protein S deficiencies. In our series, half the patients had a family history of thrombosis. Some series reported no patients with combined deficiencies. Given the relatively high frequency of protein C and protein S deficiencies in the general and thrombotic populations, it seems reasonable to assume that mixed deficiencies would be detected more frequently.

The prevalence of antiphospholipid antibodies in our venous thrombotic patients was high (5.21%), compared with the prevalence of persistent anticardiolipin antibodies in Spanish blood donors, which has been found to be 1.4% (43). No relationship with underlying chronic or immunologic diseases was detected. Occasionally, they were found to be associated with other deficiencies, probably because of their high prevalence, but they were significantly more associated with free protein S deficiency. Although this association has already been reported (44), there is no definitive explanation for this finding. Antiphospholipids have been related to recurrent abortion or foetal death (45), but data on this parameter at the time of abortion was unavailable for our patients, and a possible relationship could not be excluded. Antiphospholipids were also more prevalent in women with pregnancy-related thrombosis. This finding support their determination in this clinical setting.

The risk of thrombosis in patients with deficiencies of antithrombin (2, 3), protein C (4, 6, 7) or protein S (4, 11) has been well established. Since this study was not designed with an asymptomatic control group, an assessment of the thrombotic risk could not be carried out. However, it was possible to assess the risk of a deficiency in a population of thrombotic patients when the following clinical factors were considered: family history, juvenile, spontaneous and multiple thrombosis. Our results suggest that the *risk of deficiency* is higher if one factor is taken into account (Table 4). These four factors tend to be independent because the crude and adjusted odds ratios are similar. Moreover, the risk of deficiency increases when they are combined (Table 6). A recent work (46) analyzed the importance of family history in the evaluation of thrombophilia in a population-based study and showed that the finding of a positive family history must be considered with caution. In our series of thrombotic patients, however, there is an increased risk if a relative (first or second degree) has had thrombosis. Thus, family history of thrombosis could be an important clinical marker for thrombophilia. Spontaneous or recurrent thrombosis is also a factor which could enhance the risk of deficiency. Data on the significance of these factors are contradictory (29, 33), but in our patients the risk of deficiency was higher. Finally, another main clinical factor to be borne in mind is the age at the first thrombosis. The prevalence of deficiencies in studies with only selected young patients was higher than in unselected patients. Clinicians generally believe that young patients suffering from thrombosis (28, 29, 33), especially in the absence of triggering factors (29), probably carry a deficiency. This is in line with our findings. In our patients, the risk of deficiency was higher in patients under 45 years of age. When patients with more than one clinical factor were analyzed, the risk of deficiency increased dramatically as the factors accumulated. The clinical data was analyzed without the detection of activated protein C resistance. If this factor (or other unknown genetic or acquired factors) were included in the analysis, probably the overall result could not be essentially different because the clinical profile of thrombophilic patients has remained essentially the same after the discovety of this factor. One interesting finding was that in the group of nondeficient individuals, family history had a weak but significant association with age at the first thrombosis and recurrent thrombosis. This association suggests that there are probably other unknown genetic-related causes of thrombophilia. New functional assays and genetic linkage analysis in thrombotic families may help to find new pathogenic pathways of thrombosis (47, 48).

Although the activated protein C resistance was unknown when this study was performed, data available today shows that its prevalence in Spanish thrombotic patients is 14.53% (unpublished data). If we consider all the acquired and hereditary abnormalities which are known to be responsible for a thrombophilic state, the overall prevalence in our country could be as high as 31.46%. Moreover, family history, age at the first event, spontaneous and recurrent thrombosis are the main clinical factors that alone or combined increase the probability of a deficiency in unselected thrombotic patients. Thus, a laboratory assessment is advisable, especially when one or more of the above mentioned clinical factors are present.

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APPENDIX

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