

Risk of Venous Thromboembolism and Clinical Manifestations in Carriers of Antithrombin, Protein C, Protein S Deficiency, or Activated Protein C Resistance

A Multicenter Collaborative Family Study

Paolo Bucciarelli, Frits R. Rosendaal, Armando Tripodi, Pier Mannuccio Mannucci, Valerio De Stefano, Gualtiero Palareti, Guido Finazzi, Francesco Baudo, Roberto Quintavalla, on Behalf of the GIRTE (Italian Research Group on Inherited Thrombophilia)

Abstract—Deficiencies of antithrombin (AT), protein C (PC) or protein S (PS), and activated protein C resistance (APCR) are very well-established coagulation defects predisposing to venous thromboembolism (VTE). We performed a retrospective cohort family study to assess the risk for VTE in individuals with AT, PC, or PS deficiency, or APCR. Five hundred thirteen relatives from 9 Italian centers were selected from 233 families in which the proband had had at least 1 episode of VTE. We calculated the incidence of VTE in the whole cohort and in the subgroups after stratification by age, sex, and defect. The overall incidence of VTE (per 100 patient-years) in the group of relatives was 0.52. It was 1.07 for AT, 0.54 for PC, 0.50 for PS, 0.30 for APCR, and 0.67 in the group with a double defect. The incidence was associated with age, but not with sex. The mean age at onset was between 30 and 40 years for all the coagulation defects. Women had the peak of incidence in the age range of 21 to 40 years, earlier than men. The lifetime risk for VTE was 4.4 for AT versus APCR, 2.6 for AT versus PS, 2.2 for AT versus PC, 1.9 for PC versus APCR, and 1.6 for PS versus APCR. AT deficiency seems to have a higher risk for VTE than the other genetic defects. There is a relation between age and occurrence of thrombosis for both men and women. The latter had the peak of incidence earlier than the former. (*Arterioscler Thromb Vasc Biol.* 1999;19:1026-1033.)

Key Words: venous thromboembolism ■ antithrombin ■ protein C ■ protein S ■ activated protein C resistance

Inherited thrombophilia is defined as a genetically determined tendency to venous thromboembolism (VTE). It is characterized by onset at an early age, a frequent recurrence, and a family history (each one present in different combination with the others).¹ It has been known for several decades that deficiencies of the natural coagulation inhibitors, such as antithrombin (AT), protein C (PC), and protein S (PS), are associated with inherited thrombophilia.² Several studies have demonstrated that mutations in the genes encoding AT, PC, and PS are strong risk factors for thrombosis, but their prevalence in patients with venous thrombosis is low (3% to 10%).²⁻⁶ Recently, a poor anticoagulant response to activated PC has been described in patients with inherited thrombophilia and is as yet the most common inherited risk factor for thrombosis known (20% to 40% prevalence among patients with venous thrombosis).^{7,8} Activated protein C resistance (APCR) is caused by the presence of a mutant factor V molecule (factor V:Q⁵⁰⁶) in which a single point mutation

leads to a substitution of Arg506 by Gln in 1 of the APC cleavage sites.⁹ Its relatively high frequency in populations of white origin (3% to 13%), and the fact that some homozygotes for APCR remain asymptomatic for a long time led to the conclusion that this coagulation defect is mild in comparison with the inherited deficiencies of the coagulation inhibitors. To date, only 1 clinical study on unselected cases of VTE (Leiden Thrombophilia Study)^{8,10} is available comparing directly APCR and the other coagulation inhibitor deficiencies. In this population-based case-control study, the risk for VTE was increased 7-fold in both PC deficiency and APCR carriers, and increased 2-fold in AT deficiency carriers, but no association between PS deficiency and VTE was demonstrated. The limit of this study is the small size of AT, PC, and PS carriers. A cohort study might assure a sufficient number of subjects also for rare defects, such as those of coagulation inhibitors. For these reasons, the Italian Research Group on Inherited Thrombophilia (GIRTE) has been re-

Received September 21, 1998; revision accepted September 29, 1998.

From the Angelo Bianchi Bonomi Hemophilia and Thrombosis Center (P.B., A.T., P.M.M.), IRCCS Maggiore Hospital and University of Milan, Italy; the Department of Clinical Epidemiology and Hemostasis and Thrombosis Research Center (F.R.R.), Leiden University Medical Center, The Netherlands; the Department of Hematology (V.D.S.), Catholic University of Rome, Italy; the Department of Angiology and Coagulation (G.P.), S Orsola Hospital, Bologna, Italy; the Department of Hematology (G.F.), Riuniti Hospital, Bergamo, Italy; the Hematology Department (F.B.), Niguarda Cà Granda Hospital, Milan, Italy; and the Hemostasis Center (R.Q.), V Medical Department, Regional Hospital, Parma, Italy.

Correspondence to Paolo Bucciarelli Hemophilia and Thrombosis Center, IRCCS Maggiore Hospital, Via Pace 9, 20122 Milano, Italy. E-mail: bucciare@imiucca.csi.unimi.it

© 1999 American Heart Association, Inc.

Arterioscler Thromb Vasc Biol. is available at <http://www.atvbaha.org>

cent
inter
inhe
the
have
coh
inhe
whic
mair
in st
clinic
(3) t
deve

Data

After
receiv
Clinix
inform
conge
(AT, l
main
probat
(numb
deficit
throml
used to
of exp
data v
Bonon
a singl

Study

Familic
particip
least 1
inherite
family
defect)
factors
VTE, v
thrombo
ities, th
portal-n
strumen
venogra
plethysm
giograph
these te
VTE w
conditio
diagnosi
tion of
superfici
Arterial
methods
electroc
ischemic
transient
As ris
anesthes
ceptives
ities), tr
thrombo
was clas
After a
retrospec
avoid bia

cently constituted among clinical centers known to have interest and expertise in the diagnosis and management of inherited coagulation disorders, to form a national register for the study of inherited thrombophilia. Until now, 9 centers have taken part in this project. We performed a retrospective cohort family study of 513 relatives from 233 kindreds with inherited deficiency of AT, PC, or PS, or with APCR, in which the proband had had at least 1 episode of VTE. The main objectives were (1) to assess the lifetime risk for VTE in subjects with inherited thrombophilia, (2) to describe the clinical manifestations in these thrombophilic subjects, and (3) to evaluate the importance of triggering factors for the development of thrombosis.

Methods

Data Collection

After several meetings about the aims of the study, each center received a floppy disk with a database (made by using the program Clinix 3.1, Softwarehouse of Maggiore Hospital) to fill in with all the information about thrombophilic subjects, ie, subjects with 1 of the congenital deficiencies well established to be associated with VTE (AT, PC, PS, or APCR). The file was divided into the following 5 main parts: patient identification (coded name, age, sex, whether proband or relative, and type[s] of coagulation defect), pedigree (number of family members studied, number of subjects with deficiency, and number of members with thrombosis), history of thrombosis (type of event[s], age at each event, diagnostic methods used to detect thrombosis, and risk factor[s] for each event), history of exposure to risk factors, and laboratory results. All computerized data were returned to the coordinating center (Angelo Bianchi Bonomi Hemophilia and Thrombosis Center of Milan) and entered in a single file.

Study Design

Families were selected based on the probands who came to each participating center between January 1978 and March 1996 with at least 1 episode of VTE. We selected only families for which the inheritance of the coagulation defect was demonstrated (at least 2 family members, including the proband, had to be carriers of the defect). In all cases, history of thrombosis and of exposure to risk factors was recorded before the laboratory diagnosis was made. As VTE, we referred to any episode of deep vein thrombosis (ie, thrombosis affecting the deep veins of the lower and upper extremities, the superior and inferior cava vein, the cerebral veins, and the portal-mesenteric circulation) and/or pulmonary embolism. As instrumental diagnosis of VTE, we accepted all diagnoses made by venography, compression ultrasound, color Doppler, impedance plethysmography, ventilation-perfusion lung scan, pulmonary angiography, CT scan, and MRI scan. Diagnosis made without using these tests was classified as clinical. However, clinically diagnosed VTE was considered "certain" if at least 1 of the following conditions was satisfied: (1) the patient had a recurrence of VTE, diagnosed instrumentally, or (2) there was an objective demonstration of a postphlebotic syndrome after that episode. Diagnosis of superficial vein thrombosis (SVT) was based on clinical symptoms. Arterial thrombosis was diagnosed by clinical signs and objective methods, ie, chest pain, high levels of myocardial enzymes and electrocardiography for myocardial infarction, CT scan or MRI for ischemic strokes, and focal neurological signs during <24 hours for transient ischemic attack.

As risk factors for VTE, we considered surgery (only when total anesthesia was administered), pregnancy, puerperium, oral contraceptives intake, plaster casts (excluding those of the upper extremities), trauma, and immobilization in bed for >10 days. When a thrombosis was not associated with a triggering factor, the episode was classified as "idiopathic."

After a descriptive analysis of our population in toto, we started a retrospective cohort study, considering VTE as the end point. To avoid bias, we focused our attention only on the group of relatives.

For all centers the follow-up extended from the date of birth of subjects to either the date of the first episode of VTE, if any, or April 1996.

Patients

We received information on 1143 subjects from the 9 Italian centers; 178 probands (16 AT, 29 PC, 29 PS, 93 APCR, 1 heparin cofactor II, and 10 with double defect) were excluded because the defect was not demonstrated in other family members; ie, in 124 cases the proband was the only family member studied, whereas in 54 cases at least 1 relative other than the index case was studied, but the inheritance of the defect was not confirmed. Nineteen patients (1 proband and 18 relatives) were excluded because no complete information about history of thrombosis was available. Fifty-six probands and their 144 relatives were not considered in the analysis because the index patient had no episodes of VTE. At the end, 746 eligible subjects with demonstrated inherited thrombophilia were available, ie, 233 probands (31%) and 513 relatives (69%). The average number of study subjects per family was 3.2 (range, 2 to 11 subjects). Total patient-years were 26 151 (6609 for probands and 19 542 for relatives). The number of subjects per type of defect was 129 with AT, 145 PC, 138 PS, 309 APCR, and 25 with a double defect. Of the 513 relatives, 95 had AT, 102 PC, 93 PS, 209 APCR, and 14 had a double defect. Type I deficiency was represented in the 81% of AT, 95% of PC, and 100% of PS, whereas type II deficiency was found in 19% of AT and 5% of PC. Homozygosity was found in 3 patients with PC deficiency (2 probands and 1 relative, all symptomatic), whereas no patient with AT or PS was homozygous. Among the 306 carriers of factor V:Q⁵⁰⁶ mutation, 295 (96%) were heterozygous and 11 (4%) homozygous. Of the latter, 5 were probands (all symptomatic) and 6 were relatives (1 symptomatic and 5 asymptomatic). In 76% of families with AT, 67% with PC, 82% with PS, 75% with APCR, and 82% with double defect, 1 or >1 family member was affected, other than the proband.

In 8% of all subjects with a first episode of VTE, no information was available about the type of diagnosis. In the other cases, the diagnosis was instrumental in 76% and clinical in 24% of VTE. Considering only relatives with at least 1 episode of VTE (n=106), 64 (60%) received instrumental diagnosis and 42 (40%) clinical diagnosis. For 15 of the latter, VTE was in any case considered certain with reference to the inclusion criteria mentioned above. So, for 79 of 106 subjects (75%) VTE was confirmed, and for 27 of 106 (25%) it was uncertain.

Blood Collection and Laboratory Methods

All the study subjects (both probands and relatives) received a complete screening for all the coagulation defects that we studied. Also, those patients receiving a diagnosis before 1993 (the year in which APCR was discovered) were contacted again to obtain blood samples and complete the screening. Because of the nature of the study (multicenter retrospective), assays for AT, PC, PS, and APCR changed during the years and in the different centers. However, all the participating centers are expert in the diagnosis of congenital coagulation disorders and participate with proficiency at least annually in quality control laboratory exercises. The diagnostic approach to detect a deficiency of anticoagulant proteins (AT, PC, and PS) followed guidelines previously published in detail.¹¹ In brief, a functional assay was first made and then, if the results of this assay were low, the antigenic concentration of the protein was determined by either enzyme immunoassay or radioimmunoassay, to differentiate type I (quantitative) from type II (qualitative) deficiency. The only exception was for PS deficiency, which was diagnosed based on low levels of free PS antigen. APCR was measured by using an APTT-based clotting method as previously described.¹² In 306 (94%) of the 327 total subjects with APCR the defect was confirmed with a genetic test for demonstration of factor V:Q⁵⁰⁶ mutation,⁹ and in 21 (6%) only the functional test was performed.

Statistical Analysis

The incidence of VTE was estimated by dividing the number of episodes in each group by the total number of patient-years in that group. Only the first thrombotic event of each subject has been

TABLE 1. Characteristics of the Study Population

	Coagulation Defect					Total
	AT	PC	PS	APCR	Double Defect	
No. of families	34	43	45	100	11	233
No. of relatives	95	102	93	209	14	513
Males/females	48/47	52/50	42/51	82/127	8/6	232/281
Age at diagnosis (years)						
Mean	35.7	39.4	38.6	42.0	35.0	39.5
Range	1-73	1-84	3-81	3-95	4-67	1-95
Median	39	40	37	40	33	39
Symptomatic relatives						
No. (%)	38 (40)	35 (34)	30 (32)	42 (20)	4 (29)	149 (29)
Males/females	16/22	12/23	20/10	14/28	3/1	65/84
Type of 1st thrombosis, No. (%)						
VTE	35 (92)	22 (63)	18 (60)	21 (50)	3 (75)	99 (66)
SVT	3 (8)	8 (23)	8 (27)	11 (26)	1 (25)	31 (21)
Arterial thrombosis	0	5 (14)	4 (13)	10 (24)	0	19 (13)

considered. In the calculation of relative risk (RR), we used 95% confidence interval (CI) according to Woolf.¹³ Survival analysis was performed by the Kaplan-Meier method and with the Cox proportional-hazards model, which yields a hazard ratio (and its 95% CI) for VTE, adjusted for age, sex (0 for male and 1 for female), and age of proband at the first episode of VTE (0 for ≤ 20 years, 1 for 21 to 45 years, and 2 for > 45 years). The hazard ratio reflects the relative risk of thrombosis for one defect compared with another one, adjusted for the other variables in the model. Patients with a double defect were excluded from survival analysis.

Results

Clinical Manifestations

Three hundred eighty-two of the 746 subjects of the whole cohort were symptomatic (51%), ie, 233 probands (99 males and 134 females) and 149 relatives (65 males and 84 females). Table 1 shows the main characteristics of the study population of relatives. The prevalence of thrombosis was 29% (149/513). For AT-deficient relatives, VTE occurred most frequently as the first thrombotic event (35 of 38 subjects, 92%), compared with SVT (3 of 38 subjects, 8%), and arterial thrombosis (0 of 38 subjects). For the other groups this difference was less evident; in particular, in APCR relatives 21 of the 42 first episodes (50%) were VTE, 11 of 42 (26%) were SVT, and 10 of 42 (24%) were arterial thromboses. The prevalence of pulmonary embolism at the first episode was 17% for AT, 7% for PC, 10% for PS, and 7% for APCR. No episode of pulmonary embolism was found in the 4 cases with a double defect.

Incidence of VTE

The overall incidence of VTE (per 100 patient-years) was 3.4 for probands and 0.52 for relatives. The mean age at the onset of VTE was 29.4 years (range, 0 to 71 years) for probands and 35.8 years (range, 8 to 81 years) for relatives. Table 2 shows that the age of probands and the age of relatives at the first episode of VTE are positively related, whereas the incidence of VTE in the relatives group is negatively related to the age of probands; the relation is more evident in the last age group of probands (> 45 years). Table 3 gives the incidences of VTE for the strata of defects in the population of relatives. It

shows the highest incidence in the AT group (1.07%/y) and the lowest incidence in the APCR group (0.30%/y). The mean age at the onset was similar for all groups, ranging from 33 to 39 years, except for the group with a double defect, in which it was the lowest (25 years; range, 15 to 41 years). The stratification by sex did not show differences between men and women (incidence, 0.52%/y for both). The mean age at the first episode was 36.5 years (median 36; range, 10 to 69 years) for men and 35.1 years (median 30; range, 8 to 81 years) for women. Table 4 shows incidences of VTE for strata of ages and RR of VTE for each stratum compared with the first age range (≤ 20 years). There was an increased risk for VTE in the second age group (21 to 40 years), and afterward the risk became more or less constant. Table 5 represents the same data after stratification by sex. Women had peak of incidence at a younger age than men (age range, 21 to 40 years versus 41 to 60 years). Differences were found in the age ranges of 21 to 40 for women versus men (0.96%/y versus 0.63%/y; RR=1.5 [95% CI, 0.9 to 2.7]) and 41 to 60 for men versus women (1.1%/y versus 0.63%/y; RR=1.7 [95% CI, 0.85 to 3.4]).

Survival analysis confirmed these data. The risk for VTE in the AT group, adjusted for age, sex, and age of proband at the time of the first VTE, was 4-fold greater than that in the APCR group (hazard ratio, 4.4 [95% CI, 2.5 to 7.7]; $P<0.0001$), 3-fold than in the PS group (hazard ratio, 2.6 [1.4 to 4.6]; $P=0.002$), and 2-fold than in the PC group (hazard ratio, 2.2 [1.2 to 4.0]; $P=0.01$). The risk was 2 times higher

TABLE 2. Relation Between the Age of Probands and the Age of Relatives at the First Episode of VTE

Age of Probands at First VTE (Years)	Mean Age (range) of Relatives at First VTE (Years)	Median Age of Relatives at First VTE (Years)	Incidence* of VTE in the Group of Relatives
≤ 20 (n=58)	34.3 (8-77)	30	0.62
21-45 (n=151)	35.5 (15-81)	33	0.51
> 45 (n=24)	42.9 (15-67)	40	0.38

*Per 100 patient-years.

TABLE 3. Incidence* of VTE in the Whole Study Cohort and in Different Types of Inherited Coagulation Defects

	Coagulation Defect					Total
	AT	PC	PS	APCR	Double Defect	
Patient-years	3372	4067	3818	8620	451	20 328
No. of cases	36	22	19	26	3	106
Incidence†	1.07	0.54	0.50	0.30	0.67	0.52
Age at first episode (years)						
Mean	35.1	36.2	33.0	39.5	25.0	35.8
Range	10–77	20–54	8–67	15–81	15–41	8–81
Median	35.0	36.0	30.0	37.0	19.0	34.0

*For each subject only the first thrombotic event has been considered.

†Per 100 patient-years.

in the PC group than in the APCR group (hazard ratio, 1.9 [1.1 to 3.4]; $P=0.03$). No difference was found between the PS group and the APCR group (hazard ratio, 1.6 [0.86 to 3.0]; $P=0.14$). The Figure shows thrombosis-free survival curves for carriers of inhibitor deficiencies or APCR.

Considering only relatives with a certain first episode of VTE ($n=79$), the total incidence became 0.41 per 100 patient-years (0.84 for AT, 0.46 for PC, 0.40 for PS, and 0.24 for APCR). Results of survival analysis did not change substantially (data not shown).

Arterial Thromboses

A total of 26 first episodes of arterial thrombosis were recorded in the population of relatives (4 in the AT group, 6 in the PC group, 5 in the PS group, 10 in the APCR group, and 1 in the group with a double defect). The types of clinical manifestation were acute myocardial infarction ($n=10$; 2 AT, 3 PC, 2 PS, and 3 APCR), stroke ($n=8$; 2 AT, 2 PS, and 4 APCR), transient ischemic attack ($n=3$; 1 PC and 2 APCR), peripheral artery occlusion ($n=3$; 1 PC, 1 APCR, and 1 double defect), and bowels infarction ($n=2$; 1 PC and 1 PS). The overall incidence of arterial thrombosis was 0.12%/y. It was 0.09%/y for AT, 0.13%/y for PC, 0.12%/y for PS, 0.11%/y for APCR, and 0.20%/y for the group with a double defect. We found a positive relation between age and incidence of arterial thrombosis (0% for age ≤ 20 years, 0.08%/y for the age range of 21 to 40 years, 0.27%/y for the age range of 41 to 60 years, and 0.78%/y for age >60 years), but the number of cases was too small to draw any firm conclusion (0, 6, 11, and 9, respectively, in the 4 age groups). We obtained similar numbers for both men and women (data not shown).

TABLE 4. Incidence* of VTE After Stratification by Age

Age Ranges (Years)	Patient-Years	No. of Cases	Incidence†	RR	95% CI
≤ 20	9770	18	0.18	1‡	
21–40	6528	53	0.81	4.5	2.6–7.7
41–60	3161	28	0.89	5.0	3.2–7.9
>60	869	7	0.81	4.5	2.0–10.3

*For each subject only the first thrombotic event has been considered.

†Per 100 patient-years.

‡Reference group.

Risk of Thrombosis in High-Risk Situations

Information about risk factors at the time of the first episode of VTE was obtained from 95 of 106 symptomatic relatives (90%). Idiopathic thrombosis occurred in 30 of the 95 cases (32%) (12 of 32 AT [38%], 6 of 20 PC [30%], 3 of 15 PS [20%], 8 of 25 APCR [32%], and 1 of 3 double defect [33%]), and a triggering factor was associated with 65 of the 95 cases of thromboembolic events (68%) (20 of 32 AT [62%], 14 of 20 PC [70%], 12 of 15 PS [80%], 17 of 25 APCR [68%], and 2 of 3 double defect [67%]). Surgery preceded VTE in 28 of 95 cases (29%), puerperium in 12 of 56 (21%), pregnancy in 10 of 56 (18%), whereas immobilization was found in 10 of 95 cases (11%), oral contraceptives in 6 of 56 (11%), and plaster in 6 of 95 (6%). Table 6 shows the distribution of risk factors among the different groups of inherited defects. In most cases of secondary VTE, the event was associated with 1 risk factor only. A concomitant association with 2 risk factors was found in 1 patient with AT (oral contraceptives and plaster), 2 patients with PC (puerperium and gynecologic surgery), 1 patient with PS (orthopedic surgery and immobilization), 1 patient with APCR (general surgery and immobilization), and 1 patient with a double defect (general surgery and immobilization). In 1 subject with PC deficiency, a concomitant association with 3 risk factors was present (orthopedic surgery, immobilization, and oral contraceptives).

Discussion

One of the main objectives of this study was to establish the lifetime risk for VTE in subjects with 1 of the 4 main coagulation defects related to inherited thrombophilia (AT, PC, PS, and APCR) and to compare the clinical features of the different defects. Particular attention was taken to include only families with documented inherited defects and to avoid

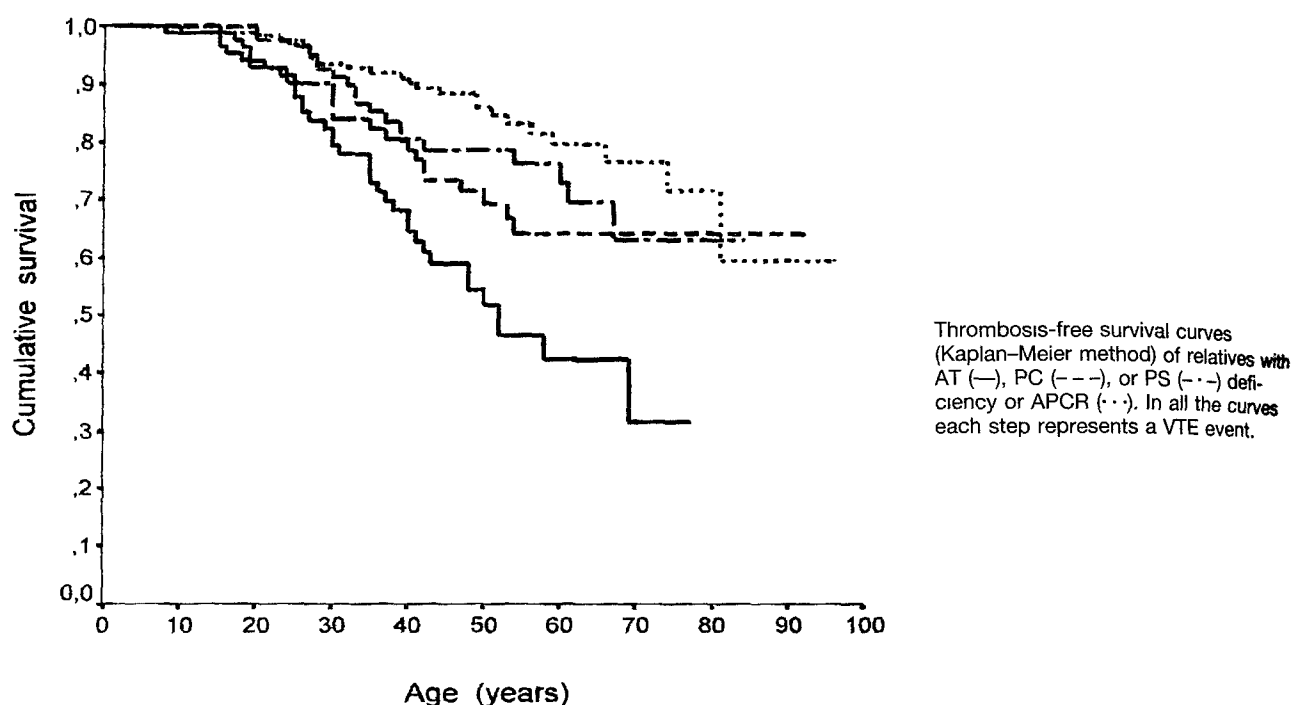
TABLE 5. Incidence* of VTE After Stratification by Age and Sex

Age Ranges (Years)	Men		Women	
	Incidence†	RR (95% CI)	Incidence†	RR (95% CI)
≤ 20	0.25	1‡	0.13	1‡
21–40	0.63	2.5 (1.2–5.3)	0.96	7.4 (3.3–16)
41–60	1.07	4.3 (2.0–9.4)	0.74	5.7 (2.3–14)
>60	0.87	3.5 (1.0–12.6)	0.76	5.8 (1.7–20)

*For each subject only the first thrombotic event has been considered.

†Per 100 patient-years.

‡Reference group.



selection bias, by considering only relatives. Of the 1143 subjects of whom we received data, 746, from 233 kindreds, fulfilled the inclusion criteria. The 513 relatives were included in the study.

The lifetime risk for VTE in the AT group was 4-fold greater than in the APCR group, 3-fold than in the PS group, and 2-fold than in the PC group. It was 2-fold

greater in PC than in APCR, whereas no difference was found between the PS and APCR groups. The probability that a subject with APCR will be free of thrombosis at the age of 45 is 0.88, compared with 0.59 for AT, 0.74 for PC, and 0.79 for PS. This finding is in agreement with the study of Svensson and Dahlbäck,⁷ who found a probability of ≈ 0.89 to be free of VTE at age 45 in his APCR relatives

TABLE 6. Risk Factors Associated With the First Episode of VTE

Risk Factors [No. of Cases/No. of Episodes (%)]	Coagulation Defect					Total
	AT	PC	PS	APCR	Double Defect	
No information	4/36 (11)	2/22 (9)	4/19 (21)	1/26 (4)	0/3 —	11/106 (10)
None	12/32 (38)	6/20 (30)	3/15 (20)	8/25 (32)	1/3 (33)	30/95 (32)
Surgery	5/32 (16)	10/20 (50)	7/15 (47)	5/25 (20)	1/3 (33)	28/95 (29)
Pregnancy*	6/20 (30)	2/14 (14)	1/5 (20)	1/16 (6)	0/1 —	10/56 (18)
Puerperium*	4/20 (20)	4/14 (29)	0/5 —	4/16 (25)	0/1 —	12/56 (21)
Oral contraceptives*	2/20 (10)	1/14 (7)	0/5 —	2/16 (13)	1/1 (100)	6/56 (11)
Plaster	3/32 (9)	1/20 (5)	1/15 (7)	1/25 (4)	0/3 —	6/95 (6)
Immobilization	1/32 (3)	0/20 —	4/15 (27)	4/25 (16)	1/3 (33)	10/95 (11)
Others	0/32 —	0/20 —	0/15 —	1/25 (4)	0/3 —	1/95 (1)

Data are ratios of numbers of patients to numbers of episodes, expressed as percentages in parentheses.

*Only female cases.

group.
pective
inciden
found i
previou
a lower
PS, wh
vis.^{16,17}
PC def
vein th
with A
tomatic
exposu
carrier
also re
single-
cases.

One
not co
differ
differ
throm
Carrie
easier
PS) an
will a
is cru
in ble
from
tion,²
defic
ical
diffe
of 2
this
only
episc
cons
ies.⁸
case
subj
A
(mu
enco
diag
diff
(2)
eve
exp
2 fa
we
tio
test
qua
pat
ger
risl
"co
sub
de

group. A similar result was obtained recently in a retrospective family study,¹⁴ in which the estimated annual incidence of VTE in APCR carriers was 0.45%, near that found in our study (0.30%). These figures may explain previous observations¹⁵ that homozygosity for APCR gives a lower risk of thrombosis than homozygosity for PC and PS, which frequently causes severe neonatal thrombosis.^{16,17} Also in our study, all 3 homozygous patients with PC deficiency were symptomatic, and 1 of them had deep vein thrombosis at birth; of the 11 homozygous patients with APCR, 6 were symptomatic and 5 were still asymptomatic (2 of them were >60 years old), despite their exposure to many risk factors. A higher risk for VTE in AT carriers than in subjects with PC and PS deficiency was also reported in previous studies,^{18,19} as well as in a recent single-center prospective study but with a small number of cases.²⁰

One possible limitation in our study is that cases were not consecutive, and selection criteria could have been different over the years for each single center and in different centers. This implies that the apparent severity of thrombophilia depends on how families were selected. Carriers of common inherited defects (eg, APCR) are easier to find than those with rare defects (eg, AT, PC, and PS) and selection on severity may be less strong; then they will appear less severely affected. Evidence that selection is crucial comes from the reported absence of thrombosis in blood donors with PC deficiency and their relatives,²¹ from the different age at onset because of patient selection,²² from some case reports about homozygous PC-deficient carriers with absence or moderately severe clinical symptoms,^{23,24} and from those studies that report a different risk of thrombosis in patients with an association of 2 inherited coagulation abnormalities.²⁵⁻²⁹ In our study this problem has been reduced by focusing our attention only on families in which the proband had had at least 1 episode of VTE. The results might be different if we consider unselected cases, as reported in previous studies.^{8,10} But the main disadvantage of selecting consecutive cases is the time demanded if an appropriate number of subjects with rare defects must be selected.

Another possible limitation is the nature of this study (multicenter retrospective). This design could have influenced our conclusions in 2 ways, ie, (1) the laboratory diagnosis has been made by using different assays during different periods of time and in different centers, and (2) there could be a possible recall bias for thrombotic events, if they are not objectively documented, and for exposure to risk factors. The inclusion criterion of at least 2 family members with the coagulation defect suggests that we avoided possible acquired defects, also in those situations in which we did not have confirmation by genetic tests. Furthermore, all centers are proficient in laboratory quality control exercises in which they regularly participate. Recall bias should be equally represented in all the genetic defects for cases of thrombosis and exposure to risk factors. In fact, selection of only relatives with a "certain" episode of VTE (79 of 106) did not affect substantially the results.

We did not contemplate a parallel study of VTE incidence in individuals without genetic defects. To get a

general idea of the different risk for VTE between subjects with a genetic defect and the general population, we considered the study by Nordström et al,³⁰ which showed in a community-wide study that the annual incidence of first VTE is ≈ 1.0 per 1000. Taking this example as a baseline risk, the risk for VTE for AT deficiency carriers would be ≈ 11 -fold greater than this, whereas it would be 5-fold for PC and PS deficiency carriers and 3-fold for APCR carriers.

The incidence rate of VTE could be underestimated because some asymptomatic subjects might have had subclinical thrombosis that no one was able to diagnose. This problem has also been postulated by Anderson et al,³¹ but there is no particular reason to think that it is represented more in one inherited defect than in the others.

The mean age of relatives at the time of the first episode of VTE was similar in all the groups of coagulation defects, excluding that with a double defect. This is in agreement with recent reports regarding the age at onset in relatives with APCR and PC.²² In most of the other studies the age at onset of VTE was lower than the age in our study, and the prevalence of symptomatic subjects was higher, perhaps because both probands and relatives were considered in the analysis, leading to a selection bias.^{18,19,32,33} Moreover, the age of relatives at the time of the first episode of VTE and the incidence of thrombosis can change according to the age of the probands at the first VTE event (Table 2). This could be because a selection of subjects from families with a different risk for VTE (higher in families in which the proband had had the thrombosis at a younger age), probably because there is a co-segregation of other unknown inherited defects.

This study gives a clear demonstration that the risk for VTE increases with age after age 20, but it becomes constant after age 40. This is in agreement with the finding that the mean age at onset of VTE is in the fourth decade. Ridker et al³⁴ also demonstrated an increased risk for VTE with age in factor V:Q⁵⁰⁶ carriers. Women had the peak of risk earlier than men (age range, 21 to 40 years versus 41 to 60 years); pregnancy/puerperium and oral contraceptives play an important role in the earlier onset of VTE for women.

The type of first thrombotic manifestation was different among the groups of inherited defects. In the AT group, VTE was mostly represented, SVT was rare, and arterial thrombosis absent, whereas in PC and PS groups the prevalence of SVT and arterial thrombosis was higher, as described in previous studies.^{18,19,32} In patients with APCR, the prevalence of VTE and that of SVT were near those in PC- and PS-deficient patients (50% and 26%, respectively), in agreement with a recent study³⁵ that showed similar clinical manifestations in APCR, PC, and PS deficiency, despite a later occurrence of the first event in APCR.

We conclude that the risk for VTE in carriers of inherited AT, PC, and PS deficiencies or APCR is related to age. The age at onset is earlier in women than in men. The risk seems to be higher in AT deficiency than in the other inherited coagulation abnormalities. Selection of families in which probands have different ages at onset of VTE can affect the age at onset and the incidence of

thrombosis of relatives coming from those different families. Recruiting relatives from kindreds with inherited thrombophilic defects can lead to an overestimation of the thrombotic risk because of such mutations, as further unknown genetic defects may co-segregate in these families. In our study we considered family members of patients with at least 1 episode of VTE; 90% of the first events in the index cases occurred before age 45. Therefore, it should be emphasized that these findings must be applied only to familial thrombophilia, but for consecutive cases the results might be different. Because in our study the follow-up ended in April 1996, we could not consider the other common genetic determinant of VTE, the novel G20210A prothrombin gene mutation.³⁶ In the near future we will evaluate in a family study the importance of this mutation, alone and in combination with the other defects, as a cause of VTE.

Acknowledgment

This study was supported by a grant from Istituto Superiore di Sanità (Progetto Sanguine). We thank all the participating GIRTE Centers for their cooperation in this study. We are also very grateful to Dr Eugenia Biguzzi from the Angelo Bianchi Bonomi Hemophilia and Thrombosis Center, Maggiore Hospital, Milan, and to Drs Luca Pizzocaro and Rosalba Donato from the Softwarehouse of Maggiore Hospital, Milan, for their help with the computerization of the data.

Appendix

List of Participants

Coordinating Center: Paolo Bucciarelli, Armando Tripodi, Pier Mannuccio Mannucci (A Bianchi Bonomi Hemophilia and Thrombosis Center, IRCCS Maggiore Hospital and University of Milan); Valerio De Stefano, Katia Paciaroni, Giuseppe Leone (Hematology Department, Catholic University, Rome); Gualtiero Palareti, Cristina Legnani (Angiology and Coagulation Diseases Division, S Orsola Hospital, Bologna).

Guido Finazzi, Stefana Marziali (Hematology Department, Riuniti Hospitals, Bergamo); Francesco Baudo, Rosaria Redaelli (Hematology Department, Niguarda Cà Granda Hospital, Milan); Roberto Quintavalla (Hemostasis Center, V Medical Department, Regional Hospital, Parma); Mauro Berrettini, Alessandra Bura (Internal and Vascular Medicine, University of Perugia); Pier Luigi Antignani, Anna Rita Todini (Angiology Department, S Camillo Hospital, Rome); Nicola Ciavarella, Loredana Mendolicchio (Coagulation Department, Policlinico Hospital, Bari).

References

- Lane DA, Mannucci PM, Bauer KA, Bertina RM, Bochkov NP, Boulyjenkov V, Chandy M, Dahlbäck B, Ginter EK, Miletich JP, Rosendaal FR, Seligsohn U. Inherited thrombophilia: part 1. *Thromb Haemost.* 1996;76:651-662.
- Allaart CF, Briët E. Familial venous thrombophilia. In: Bloom AL, Forbes CD, Thomas DP, Tuddenham EGD, eds. *Haemostasis and Thrombosis*. 3rd ed. London, UK: Churchill Livingstone; 1994: 1349-1360.
- De Stefano V, Finazzi G, Mannucci PM. Inherited thrombophilia: pathogenesis, clinical syndromes, and management. *Blood.* 1996;87: 3531-3544.
- Pabinger I, Brucker S, Kyrle PA, Schneider B, Korninger H, Niessner H, Lechner K. Hereditary deficiency of antithrombin III, protein C and protein S: prevalence in patients with a history of venous thrombosis and criteria for rational patient screening. *Blood Coagul Fibrinolysis.* 1992; 3:547-553.
- 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.* 1991;36: 249-254.
- Ben-Tal O, Zivelin A, Seligsohn U. The relative frequency of hereditary thrombotic disorders among 107 patients with thrombophilia in Israel. *Thromb Haemost.* 1989;61:50-54.
- Svensson PJ, Dahlbäck B. Resistance to activated protein C as a basis for venous thrombosis. *N Engl J Med.* 1994;330:517-522.
- Koster T, Rosendaal FR, de Ronde H, Briët E, Vandenbroucke JP, Bertina RM. Venous thrombosis due to poor anticoagulant response to activated protein C: Leiden Thrombophilia Study. *Lancet.* 1993;342: 1503-1506.
- Bertina RM, Koeleman BPC, Koster T, Rosendaal FR, Dirven RJ, de Ronde H, van der Velden P, Reitsma P. Mutation in blood coagulation factor V associated with resistance to activated protein C. *Nature.* 1994; 369:64-67.
- Koster T, Rosendaal FR, Briët E, van der Meer FJM, Colly LP, Trienekens PH, Poort SR, Vandenbroucke JP. Protein C deficiency in a controlled series of unselected out-patients: an infrequent but clear risk factor for venous thrombosis: Leiden Thrombophilia Study (LETS). *Blood.* 1995;85:2756-2761.
- Mannucci PM, Tripodi A. Laboratory screening of inherited thrombotic syndromes. *Thromb Haemost.* 1987;57:247-251.
- Dahlbäck B, Carlsson M, Svensson PJ. Familial thrombophilia due to a previously unrecognized mechanism characterized by poor anticoagulant response to activated protein C: prediction of a cofactor to activated protein C. *Proc Natl Acad Sci U S A.* 1993;90:1004-1008.
- Woolf B. On estimating the relation between blood group and disease. *Am Hum Genet.* 1955;19:251-253.
- Middeldorp S, Henkens CMA, Koopman MMW, van Pampus ECM, Hamulyák K, van der Meer J, Prins MH, Büller HR. The incidence of venous thromboembolism in family members of patients with factor V Leiden mutation and venous thrombosis. *Ann Intern Med.* 1998;128: 15-20.
- Rosendaal FR, Koster T, Vandenbroucke JP, Reitsma PH. High risk of thrombosis in patients homozygous for factor V Leiden (activated protein C resistance). *Blood.* 1995;85:1504-1508.
- Marlar RA, Montgomery RR, Madden RM. Homozygous protein C deficiency. In: Bertina RM, ed. *Protein C and related proteins*. Edinburgh, UK: Churchill Livingstone; 1988:182.
- Mahasandana C, Suvatte V, Marlar RA, Manco-Johnson M, Jacobson LJ, Hathaway WE. Neonatal purpura fulminans associated with homozygous protein S deficiency. *Lancet.* 1990;335:61-62.
- De Stefano V, Leone G, Mastrangelo S, Tripodi A, Rodeghiero F, Castaman G, Barbui T, Finazzi G, Bizzi B, Mannucci PM. Clinical manifestations and management of inherited thrombophilia: retrospective analysis and follow-up after diagnosis of 238 patients with congenital deficiency of antithrombin III, protein C, protein S. *Thromb Haemost.* 1994;72:352-358.
- Pabinger I, Schneider B, for the GTH Study Group on Natural Inhibitors. Thrombotic risk in hereditary antithrombin III, protein C, or protein S deficiency: a cooperative, retrospective study. *Arterioscler Thromb Vasc Biol.* 1996;16:742-748.
- Finazzi G, Barbui T. Different incidence of venous thrombosis in patients with inherited deficiencies of antithrombin III, protein C and protein S. *Thromb Haemost.* 1994;71:15-18.
- Miletich J, Sherman L, Broze G. Absence of thrombosis in subjects with heterozygous protein C deficiency. *N Engl J Med.* 1987;317: 991-996.
- Lensen RPM, Rosendaal FR, Koster T, Allaart CF, de Ronde H, Vandenbroucke JP, Reitsma PH, Bertina RM. Apparent different thrombotic tendency in patients with factor V Leiden and protein C deficiency due to selection of patients. *Blood.* 1996;88: 4205-4208.
- Sharon C, Tirindelli MC, Mannucci PM, Tripodi A, Mariani G. Homozygous protein C deficiency with moderately severe clinical symptoms. *Thromb Res.* 1986;41:483-488.
- Tripodi A, Franchi F, Krachmalnicoff A, Mannucci PM. Asymptomatic homozygous protein C deficiency. *Acta Haematol.* 1990;83: 152-155.
- Koeleman BPC, Reitsma PH, Allaart CF, Bertina RM. Activated protein C resistance as an additional risk factor for thrombosis in protein C-deficient families. *Blood.* 1994;84:1031-1035.
- Zöller B, Bernstdotter A, García de Frutos P, Dahlbäck B. Resistance to activated protein C as an additional genetic risk factor in hereditary deficiency of protein S. *Blood.* 1995;85:3518-3523.
- Koeleman BPC, van Rump D, Hamulyák K, Reitsma PH, Bertina RM. Factor V Leiden: an additional risk factor for thrombosis in protein S deficient families? *Thromb Haemost.* 1995;74:580-583.

- 28 van Boven HH, Reitsma PH, Rosendaal FR, Bayston TA, Chowdhury V, Bauer KA, Scharrer I, Conard J, Lane DA Factor V Leiden (FV R506Q) in families with inherited antithrombin deficiency *Thromb Haemost* 1996,75 417-421
- 29 Phillips MD Interrelated risk factors for venous thromboembolism *Circulation* 1997,95 1749-1751
- 30 Nordstrom M, Lindblad B, Bergqvist D, Kjellstrom T A prospective study of the incidence of deep-vein thrombosis within a defined urban population *J Intern Med* 1992,232 155-160
- 31 Anderson FA, Wheeler B, Goldberg RJ, Hosmer DW, Patwardhan NA, Jovanovic B, Forcier A, Dalen JE A population-based perspective of the hospital incidence and case-fatality rates of deep vein thrombosis and pulmonary embolism *Arch Intern Med* 1991,151 933-938
- 32 Tripodi A, Mannucci PM, the Ad Hoc Study Group A survey of inherited thrombotic syndromes in Italy *Res Clin Lab* 1989,19 67-74
- 33 Pabinger I, Kyrle PA, Heisteringer M, Eichinger S, Wittmann E, Lechner K The risk of thromboembolism in asymptomatic patients with protein C and protein S deficiency a prospective cohort study *Thromb Haemost* 1994,71 441-445
- 34 Ridker PM, Glynn RJ, Miletich JP, Goldhaber SZ, Stampfer MJ, Hennekens CH Age-specific incidence rates of venous thromboembolism among heterozygous carriers of factor V Leiden mutation *Ann Intern Med* 1997,126 528-531
- 35 Leroy Matheron C, Levent M, Pignon JM, Mendonça C, Gouault Heilmann M The 1691 A mutation in the factor V gene relationship to activated protein C (APC) resistance and thrombosis in 65 patients *Thromb Haemost* 1996,75 4-10
- 36 Poort SW, Rosendaal FR, Reitsma PH, Bertina RM A common genetic variation in the 3'-untranslated region of the prothrombin gene is associated with elevated plasma prothrombin levels and an increase in venous thrombosis *Blood* 1996,88 3698-3703