High Risk of Thrombosis in Patients Homozygous for Factor V Leiden (Activated Protein C Resistance)

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Resistance to activated protein C (APC) is a common inherited risk factor for venous thrombosis, which is associated with a mutation in coagulation factor V (factor V Leiden). We investigated the risk of venous thrombosis in individuals homozygous for this abnormality. We determined the factor V Leiden genotype in 471 consecutive patients aged less than 70 years with a first objectively confirmed deep-vein thrombosis and in 474 healthy controls. We found 85 heterozygous and seven homozygous individuals among the cases with thrombosis and 14 heterozygous individuals among the control subjects. The expected number of homozygous individuals among the controls was calculated from Hardy-Weinberg equilibrium and estimated at 0.107 (allele frequency, 1.5%). Whereas the relative risk was increased sevenfold for heterozygous individuals, it was increased 80-fold for homozygous individuals. These patients experienced their thrombosis at a much younger age (31 v 44 years). The homozygous individuals were predominantly women, most likely due to the effect of oral contraceptives. Because of the increased risk of thrombosis with age, the absolute risk becomes most pronounced in older patients, both for heterozygous and homozygous individuals. For the homozygous individuals, the absolute risk may become several percentage points per year. This implies that most individuals homozygous for factor V Leiden will experience at least one thrombotic event in their lifetime.
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PROTEIN C IS A VITAMIN K-dependent protein that inhibits blood coagulation. In its activated form (activated protein C, APC), it exerts its inhibitory action by proteolytic cleavage of the procoagulant proteins factor Va and factor VIIIa. Recently, we have described a common mutation in the APC cleavage site of factor V (factor V Leiden) that is associated with APC resistance.[1]

In APC resistance, the patient's plasma does not exhibit the normal anticoagulant response to addition of APC, as reflected in a prolongation of the activated partial thromboplastin time (APTT).[2] In individuals heterozygous for factor V Leiden, the APTT prolongation is moderately decreased, whereas in homozygous individuals there is little response at all.[3]

APC resistance is known to be a common and strong risk factor for thrombosis.[4,5] It is present in 20% of unselected consecutive patients with deep-venous thrombosis, and in 3% of healthy individuals. In individuals from families referred because of unexplained familial thrombophilia, APC resistance may be found in 40% to 60% of subjects (Svensson and Dahlbäck[5] and Bertina RM, unpublished data, 1993).

Individuals with APC resistance have a sevenfold increased risk of venous thrombosis.[4] Because of the high allele frequency of the mutated factor V gene, homozygous carriers will not be extremely rare as in other types of hereditary thrombophilia. It is unknown whether the homozygous state confers a higher risk than the heterozygous state. We have estimated the risk of thrombosis and the clinical features of patients who were homozygous for factor V Leiden. These patients were identified in a large, population-based, case-control study on deep-venous thrombosis (The Leiden Thrombophilia Study, LETS).[4]

MATERIALS AND METHODS

Study design. The details of the design of LETS have been described previously.[4] We included consecutive patients younger than 70 years who were referred to the Anticoagulation Clinics of Leiden, Amsterdam, and Rotterdam (The Netherlands) for outpatient monitoring of anticoagulant treatment after a first, objectively confirmed episode of deep-vein thrombosis in the absence of known malignant disorders. Patients were seen at least 6 months (range, 6 to 19 months) after the acute thrombotic event. Of eligible patients, 90% were willing to participate in the study.

Each patient was asked to find his own healthy control subject according to these criteria: same sex, approximately same age (less than 5 years difference), no biologic relative, no history of venous thromboembolism, no use of cumarin derivatives for at least 3 months, and no known malignant disorders. Partners of patients were also asked to volunteer as control subjects. When a patient was unable to find a control subject, the first individual from the list of partners with the appropriate age and sex was asked to participate in the study: 225 (47%) of the 474 control subjects were partners of other patients.

Data collection and laboratory analysis. All subjects completed a standard questionnaire that contained questions about the presence of acquired risk situations in the past confined to a specific period before the index date, ie, date of the thrombotic event. As acquired risk situations, we considered surgery, hospitalization without surgery, or prolonged immobilization at home (≥2 weeks)—all in the year preceding the index date—postpartum periods (within 30 days of the index date), and pregnancy at the time of the index date.

Blood was collected from the antecubital vein into Sarstedt Monovette tubes (Sarstedt, Nümbrecht, Germany) containing 0.106 mmol/L trisodium citrate. High molecular weight DNA was isolated from leukocytes and stored at 4°C. The presence of the mutant factor V Leiden gene (1691, G → A transition) was determined as previously described.[1,3] By this method we established whether each patient was homozygous normal (GG), heterozygous (AG) for the factor V Leiden mutation, or a homozygous carrier (AA) of this abnormality. The technicians were at all times blinded to the status of the sample, ie, whether it was from a patient or a control subject. Cells for DNA analysis were available for 471 patients and 474 controls.

Analysis and statistics. The frequency of heterozygous and homozygous carriers of the factor V Leiden mutation in cases and controls was compared by simple cross-tabulation. Because in the

analysis of the risk associated with the heterozygous state, sex and age did not appear to be confounding variables (as they were not expected to be for autosomal genetic abnormalities), relative risk estimates for the heterozygous state were obtained by calculation of unmatched exposure odds ratios. A 95% confidence interval (CI95%) was constructed according to Woolf.[6]

The risk associated with the homozygous state could not be estimated in this standard fashion, as no homozygous individuals were found among the controls. Therefore, under the assumption of Hardy-Weinberg equilibrium (in the controls), the expected number of homozygous individuals in a control population was calculated, and the odds ratio was subsequently estimated in the standard fashion. The variance of the (log) odds ratio for the homozygous state was estimated by a modification of the method of Woolf.[6] When each cell of the two-by-two table with cell contents a, b, c, and d is considered to be the realization of a Poisson distribution, the variance of the log(OR) is $1/a + 1/b + 1/c + 1/d$ (as $\text{var}[\ln(x)] = 1/x$).[6] When the number of individuals with GG and AA genotypes are counted in the cases and calculated from Hardy-Weinberg equilibrium for the controls, which requires a quadratic transformation, the odds ratio can be estimated as AA cases/GG cases divided by (A controls/G controls)^2, in which AA and GG are the number of genotypes (individuals), and A and G the number of alleles; the expected number of homozygous controls is (A controls)^2/2(G controls). Thus, the ln(OR) is ln(AA cases) − ln(GG cases) − 2ln(A controls) + 2ln(G controls). The variance can now be estimated by assuming that all parameters have a Poisson distribution, which (as $\text{var}[2\ln(x)] = 2^2/x$) leads to $\text{var}[\ln(\text{OR})] = 1/\text{AA} + 1/\text{GG} + 4/\text{G} + 4/\text{A}$.

The absolute risk for thrombosis for the various genotypes and ages was calculated by first partitioning the total number of person years in the origin population (as derived from census information from the municipal authorities) under the assumption of Hardy-Weinberg equilibrium. Dividing the cases in each subgroup (genotype, age) by these person years leads to estimates of the absolute risks. Subsequently, these crude incidence data were modelled after logarithmic transformation in a weighted least square regression model, with three age classes (0 to 29, 25 years; 30 to 49, 40 years; 50 to 69, 60 years), indicator variables for the heterozygous (0, 1) and the homozygous state (0, 1), weighted for the number of cases in each stratum. This method, in which stratum-specific incidence rates are first estimated and then smoothed (smooth-last[7]) by weighted least square regression, has been described by Grizzle et al.[8] Because for a Poisson distribution the variance of the number of cases equals the number of cases, this closely resembles fitting of a Poisson regression model. The model will lead to more stable estimates than the crude incidence figures, especially for the homozygous state, under the assumption that the incidence rate ratio for the homozygous state (and the heterozygous state) is constant over the age strata [for the log(incidence rate), log(λ)]. This model can be written as: $\log(\lambda) = \alpha + \beta_1 * \text{age} + \beta_2 * \text{AG}(0, 1) + \beta_3 * \text{AA}(0, 1)$, which can subsequently be used to calculate estimates for the absolute risk (by entering all covariate values and the estimated coefficients in this equation) and for the relative risk (as the antilogarithm of the coefficients).

RESULTS

Among 471 patients, we found 85 (18%) who were heterozygous and seven (1.5%) who were homozygous for the defect, whereas the other 379 (80%) did not carry the factor V Leiden mutation. Among the 474 controls, 14 (2.9%) were heterozygous, and all other 460 were normal; there were no homozygous individuals among the controls.

The homozygous individuals experienced thrombosis at a markedly younger age than the other patients: the median

Table 1. General Characteristics of 471 Thrombosis Patients by Factor V Genotype

	GG	AG	AA
n	379	85	7
Age (yrs)			
Median	46	44	31
Range	15-69	17-69	22-55
Sex			
No. of men (%)	162 (43)	39 (46)	1 (14)
No. of women (%)	217 (57)	46 (54)	6 (86)

age at thrombosis was 31 years compared with 44 years in the heterozygous and 46 years in the patients without the mutation (Table 1).

The clinical course of the deep vein thrombosis in the homozygous patients was unremarkable (see Appendix). All suffered from deep venous thrombosis of the proximal deep veins of the leg. Four were hospitalized for heparinization, and three were treated as outpatients with cumarin derivatives only, according to the regional treatment policies for venous thrombosis.

Six (86%) of the seven homozygous patients were women, compared with 46 (54%) of the heterozygous and 217 (57%) of the individuals without the mutation (Table 2). Also, six of these seven patients had blood group A, compared with 249 (54%) of the other 464 cases. Of the five homozygous women aged 45 years and younger, three used oral contraceptives at the time of the thrombotic event, which was similar to the use of oral contraceptives in all cases (105 of 159, 66%).

In five (71%) of the seven homozygous patients, the thrombosis apparently had occurred spontaneously, and in two there had been a predisposing factor for thrombosis in the year preceding the event (one had hip surgery 20 days before the thrombosis, and one had been admitted to the hospital overnight after giving birth 60 days before the thrombotic event; see Table 2). Among the 85 heterozygous individuals, an acquired risk factor had been present in 25 (29%) patients, and among the normal (GG) patients, in 131 (35%) of 379.

Previous risk situations (operations, pregnancies, hospital admissions) without thrombotic consequences were less frequent in the patients homozygous for factor V Leiden than in the other patients. Still, five of the seven homozygous patients had encountered risk situations in the past without a subsequent thrombosis (two had had surgery, four had given birth to five children).

The seven patients were observed for an average of 2 years without long-term oral anticoagulation after the first thrombotic event. One patient had a recurrent thrombosis (1 in 13.4 years, 7.4% per year recurrence risk for these seven patients). Of the 14 parents of these seven patients, three had a history of venous thrombosis, which is approximately five times higher than expected.[9]

Under Hardy-Weinberg equilibrium, the relative frequency of normals:heterozygotes:homozygotes is $p^2{:}2pq{:}q^2$, where p is the allele frequency of the normal gene and q of the abnormal gene. As $p^2{:}2pq$ was 460/474:14/474, it follows that the allele frequency of factor V Leiden (q) is 0.015. The

Table 2. Detailed Characteristics of Seven Homozygous Patients

Patient No.	Sex	Age (yrs)	APC-SR	Blood Group	OCC*	Predisposing Factors† (days between risk situation and VT)	No. of Parents With History of VT	Arterial Disease‡
90	F	55	1.13	A	N§	N	0	Y
124	F	24	1.14	A	N	Y‖ (60)	0	N
266	F	30	1.20	A	Y	N	0	N
173	F	22	1.14	0	Y	N	0	N
583	F	44	1.23	A	N	Y¶ (20)	1	N
589	F	42	1.21	A	Y	N	1	N
944	M	31	1.19	A	(male)	N	1	N

Abbreviations: APC-SR, APC sensitivity ratio; OCC, oral contraceptives; VT, venous thrombosis.

* Use of oral contraceptives in the month preceding the thrombosis.

† Surgery, hospital admission, immobilization in the year preceding the thrombosis, childbirth 1 month before the thrombosis, pregnancy at the time of the thrombosis.

‡ Angina pectoris, previous myocardial infarction, stroke, or peripheral arterial disease.

§ Menopausal, no use of estrogens.

‖ Overnight hospital stay after giving birth.

¶ Hip surgery.

allele frequencies of $p = 0.985$ and $q = 0.015$ conform to a distribution among 474 unselected individuals of 459.9 (GG), 14.0 (AG), and 0.107 (AA).

The expected number of homozygous individuals (q^2) of 0.107 among 474 controls leads to an odds ratio for the homozygous state of $(7/379)/(0.107/460) = 79.4$. So, the risk of thrombosis for homozygous individuals is almost 80 times increased compared with normal individuals (CI95%, 22 to 289).

Table 3 shows the odds ratio for the three age groups when this allele frequency is used to calculate the expected number of homozygous controls in each age group. It is evident that the high relative risk of thrombosis in homozygous individuals diminishes with advancing age. This is in contrast to the relative risk for heterozygous individuals, which is more or less constant over age.

Subsequently, we calculated absolute risks (incidence rates) for the different age groups and genotypes by using data on the age-distribution in the origin population, and under the assumption of Hardy-Weinberg equilibrium. As is shown in Table 3, the incidence increases from only 0.6 per 10,000 per year in the youngest age group with GG genotype, to 16.4 per 10,000 per year for heterozygous individuals in the older age groups. It is also clear from the ratios in Table 3 that at all age groups the risk for homozygous individuals

is much higher than for heterozygous individuals (78 to 176 per 10,000 per year). However, as these figures are based on only seven individuals divided over three age groups, the estimates are unstable and show an unexpected lower incidence in the oldest age group. The regression model we used smooths these estimates, as it assumes a constant relative risk over the age groups. As Fig 1 shows, this model fits excellently for the normal and heterozygous individuals (coefficients: constant, -10.06; age, .0293; AG, 1.96; AA, 4.52). The smoothed incidence estimates for homozygous individuals now increase from 82 per 10,000 person-years in those aged under 30 years to 227 per 10,000 patient-years for those aged 50 to 69 years (Fig 1). These estimates imply that most homozygous patients will experience at least one thrombotic event in their lifetime.

DISCUSSION

Resistance to APC is a common abnormality, with an allele frequency for the mutant factor V gene of about 1.5%. Three percent of the population will be heterozygous, and homozygous individuals can be expected with a prevalence of about 2 per 10,000 births.

In this study we show that homozygous individuals have a high risk of thrombosis, which is considerably higher than the risk in heterozygous individuals. This conclusion is sup-

Table 3. Odds Ratios and Absolute Risk of First Thrombosis by Age

Age (yrs)	Genotype Patients (GG/AG/AA)	Genotype Controls (GG/AG)	E_{AA}	OR_{AA} (CI95%)	OR_{AG} (CI95%)	Person-years	Incidence Rates/10^{-4} yrs* GG	AG	AA
0-29	61/17/2	70/3	.0164	140 (9.5-2,049)	6.5 (1.8-23)	1,134,681	0.6	5.1	78.4
30-49	176/35/4	217/6	.0502	98 (15-652)	7.2 (3.0-17)	1,006,733	1.8	11.8	176.2
50+	142/33/1	173/5	.0400	30 (2.2-428)	8.0 (3.1-21)	682,939	2.1	16.4	110.0

Abbreviations: E_{AA}, expected number of homozygous individuals among controls based on overall Hardy-Weinberg equilibrium (q^2 * $n_{controls in age-stratum}$, $q = .015$); OR_{AA}, odds ratio for homozygous individuals (v homozygous normals); OR_{AG}, odds ratio for heterozygous individuals (v homozygous normals).

* Crude incidence rates, based on the number of observed cases over the number of patient-years, partitioned according to Hardy-Weinberg equilibrium ($q = .015$).
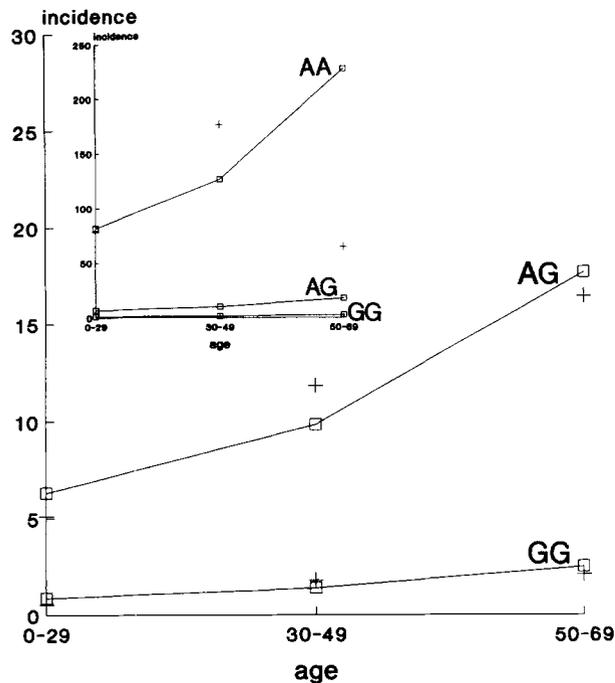
Fig 1. Crude and smoothed incidence rate estimates per 10⁻⁴ years for factor V Leiden genotypes by age. The lowest line shows the estimates for the GG genotype, and the upper line for the AG genotype (in the full figure). The inserted figure also shows the estimates for the AA genotype (homozygous factor V Leiden). +, crude incidence estimates; □, smoothed rates. The smoothed incidence rates per 10,000 person years were as follows: GG: 0.9 (0-29 years), 1.4 (30-49 years), and 2.5 (50-69 years); AG: 6.3 (0-29 years), 9.8 (30-49 years), 17.6 (50-69 years); AA: 81.5 (0-29 years), 126.5 (30-49 years), and 227.3 (50-69 years).

ported by the young age at which the homozygous individuals experience their first thrombotic event.

It is clear that the risk of thrombosis in homozygous factor V Leiden is nowhere near the risk of thrombosis in homozygous protein C or protein S deficiency; these abnormalities lead to neonatal purpura fulminans.[10,11] All of the individuals with homozygous factor V Leiden lived until adulthood before the first thrombotic event, and one even until late middle age. Most of the homozygous individuals had experienced risk situations in the past without thrombosis; for most of these situations (pregnancy, puerperium), no anticoagulant prophylaxis will have been prescribed. This shows that APC resistance should be seen as a quantitative defect (decreased inactivation rate of factor Va) rather than a qualitative defect (no protein C activity), as in homozygous protein C deficiency.

A remarkable finding in this study was the predominance of women among the homozygous patients. Among these homozygous women, the use of oral contraceptives was as prevalent as among the other female thrombosis patients. From this, it follows that the relative risk associated with oral contraceptive use is the same for homozygous women as for women with the other genotypes (under the reasonable assumption that among the healthy control subjects oral contraceptive use is not associated with the factor V genotype:

the exposure odds among the cases divided by the exposure odds of the controls—the odds ratio—will be the same). This implies that the already greatly increased risk of a homozygous woman is again multiplied four- to sixfold when she uses the pill,[12] and so the absolute increase in risk due to oral contraceptives is much larger in women homozygous for the factor V Leiden mutation than for other women.[13] This shows that oral contraceptives have the same synergistic effect with the homozygous factor V Leiden mutation as we have recently reported for the heterozygous state.[12] The incidence rates of Table 3 and Fig 1 are overall data, and so the rates will be somewhat lower for those who do not use oral contraceptives, at 50 to 100 per 10,000 person years in the homozygous individuals. These rates clearly demonstrate the different effect of oral contraceptives for the various genotypes: for the youngest women with the GG genotype, use of oral contraceptives with a relative risk of 4 will only lead to 1 or 2 additional cases per 10,000 woman years. In the homozygous women, use of oral contraceptives will lead to 200 or more additional cases per 10,000 woman years.

The relative risk for heterozygous individuals appears constant for the different age groups. This observation has to be considered in the light of a background incidence that increases with age. This implies, as we show in Fig 1, that the absolute risk of thrombosis, or the absolute risk added by APC resistance, becomes substantial for older heterozygous individuals.

It may be noted that our overall estimate for the incidence rate, at about 2 per 10,000 per year, is lower than the usual estimates of about 0.5 to 1 per 1,000 person years.[9,14] This discrepancy is most easily explained by the age limits in our study (less than 70 years), the restriction to confirmed thromboses, the exclusion of patients with malignancies, and the restriction to first thrombotic events.

The homozygous patients had a risk of thrombosis that was 80 times increased, which leads to an overall incidence of about 1% per year. The observed decrease of the rate in the oldest age groups has two explanations, other than chance. First, there might be few older homozygous individuals in the population who had not already experienced a first thrombotic event, although this would only explain part of the decrease. Second, the effect of oral contraceptives, which will not be present in the oldest age group, but will be greatest in the age group 30 to 49 years, where genotype, age, and pill-use all combine and result in the observed peak. Obviously, the number of homozygous patients in our study does not allow for formal subgroup analysis. Therefore, as an overall figure, the incidence figures that were recalculated from the weighted regression model seem the best estimate of the risk, which becomes over 2% per year in the patients aged 50 years and older.

We conclude that APC resistance caused by homozygous factor V Leiden leads to a high risk of deep venous thrombosis. This thrombosis appears not to occur before adulthood and may not even become apparent in risk situations such as pregnancy and puerperium. Therefore, although we are convinced that these patients should receive short-term prophylaxis with anticoagulants in risk situations, we do not feel that, without the prospective follow-up data we intend

to gather, lifelong prophylaxis in individuals homozygous for factor V Leiden is recommended.

APPENDIX: CLINICAL SUMMARIES

Patient 90. A 55-year-old woman (height, 1.66 m; weight, 70 kg) with a history of angina complained of increasing pain and swelling of her leg over a 3-week period. She did not smoke. She was menopausal and did not receive hormonal replacement therapy. Her brother had a history of deep venous thrombosis. There had been no precipitating event. Contrast venography showed a thrombus extending over 15 cm in the femoral vein. She received heparin and cumarins and was discharged after 15 days. Treatment with oral anticoagulants was continued for 1 year.

Patient 124. This 24-year-old woman (height, 1.75 m; weight, 87 kg) complained of intermittent pain in her left leg since giving birth 60 days before admission. She smoked 25 cigarettes per day and had not yet restarted oral contraceptives. Family history was negative for venous thrombosis in her parents and siblings. At examination she had a painful, swollen, and red left leg and a mild fever (38.5°C, 101.3°F). Doppler sonography showed the absence of flow in the left femoral vein. She was treated with heparin and cumarins and was discharged after 10 days. Treatment with oral anticoagulants was continued for 3 months.

Patient 266. This 30-year-old woman (height, 1.69 m; weight, 79 kg) had noticed a sharp pain in the left calf the day before admission. She recalled a spell of painful breathing 4 weeks previously. She smoked 25 cigarettes per day and used oral contraceptives. None of her parents and siblings had a history of venous thrombosis. Doppler sonography showed deep vein thrombosis of the popliteal vein, and a ventilation-perfusion scan showed perfusion defects indicating multiple pulmonary emboli. She was treated with heparin and cumarins and was discharged after 10 days. Treatment with oral anticoagulants was continued for 7 months.

Patient 173. This 22-year-old woman (height, 1.75 m; weight, 87 kg) had a large hematoma that, on ultrasound examination, had been diagnosed as a ruptured Baker's cyst of the left knee. Treatment consisted of elastic stockings. The left lower leg remained intermittently swollen, and the skin took on a shiny appearance the day before presentation. She was a nonsmoker and used oral contraceptives. The family history was negative in parents and siblings. Ultrasound examination showed deep vein thrombosis of the popliteal vein. She was treated with heparin and cumarins and discharged after 10 days. Oral anticoagulant treatment was continued for 4 months.

Patient 583. This 44-year-old woman (height, 1.77 m; weight, 74 kg) had elective hip surgery with several days of immobilization. She received subcutaneous heparin and was discharged after 6 days, at which time anticoagulation was discontinued. She complained of a painful, swollen right leg, but a first impedance plethysmography (IPG) was negative; a second IPG, 2 weeks after discharge, showed deep vein thrombosis. She smoked 25 cigarettes per day and did not use oral contraceptives. Her mother had also had deep vein thrombosis. She was treated as an outpatient with cumarins, which were continued for 4 months.

Patient 589. This 42-year-old woman (height, 1.57 m; weight, 62 kg) developed extensive thrombophlebitis of her left leg during a sunbathing vacation in the Mediterranean. There had been no obvious precipitating event. Complaints of pain and swelling of the leg worsened after her return to The Netherlands, and an IPG 3 weeks after the initial complaint showed deep vein thrombosis of the left leg. She smoked cigarettes and used oral contraceptives. The family history was positive with regard to venous thrombosis in her father. She was treated as an outpatient with cumarins, which were continued for 3 months.

Patient 944. This 31-year-old man (height, 1.84 m; weight, 83 kg) developed left calf pain after a long drive on his motorcycle. Over a period of several weeks, both his upper and lower leg became swollen, red, and painful. He smoked 15 cigarettes per day. The family history was positive; his father had experienced venous thrombosis in the past. An IPG confirmed deep venous thrombosis. He was treated as an outpatient with cumarins for 3 months. Three weeks after cumarin treatment was discontinued, he again experienced a swollen and painful left leg, which by phlebography was shown to be caused by thrombi in the deep veins. He was admitted to the hospital and treated with heparin and cumarins.

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