

HIGH PLASMA LEVELS OF FACTOR VIII AND THE RISK OF RECURRENT VENOUS THROMBOEMBOLISM

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ABSTRACT

Background A high plasma level of factor VIII is a risk factor for venous thromboembolism. We evaluated the risk of a recurrence of thrombosis after an initial episode of spontaneous venous thromboembolism among patients with high plasma levels of factor VIII.

Methods We studied 360 patients for an average follow-up period of 30 months after a first episode of venous thromboembolism and discontinuation of oral anticoagulants. Patients who had recurrent or secondary venous thromboembolism, a congenital deficiency of an anticoagulant, the lupus anticoagulant, hyperhomocysteinemia, cancer, or a requirement for long-term treatment with antithrombotic drugs or who were pregnant were excluded. The end point was objectively documented, symptomatic recurrent venous thromboembolism.

Results Recurrent venous thromboembolism developed in 38 of the 360 patients (10.6 percent). Patients with recurrence had higher mean (\pm SD) plasma levels of factor VIII than those without recurrence (182 ± 66 vs. 157 ± 54 IU per deciliter, $P=0.009$). The relative risk of recurrent venous thrombosis was 1.08 (95 percent confidence interval, 1.04 to 1.12; $P<0.001$) for each increase of 10 IU per deciliter in the plasma level of factor VIII. Among patients with a factor VIII level above the 90th percentile of the values in the study population, the likelihood of recurrence at two years was 37 percent, as compared with a 5 percent likelihood among patients with lower levels ($P<0.001$). Among patients with plasma factor VIII levels above the 90th percentile, as compared with those with lower levels, the overall relative risk of recurrence was 6.7 (95 percent confidence interval, 3.0 to 14.8) after adjustment for age, sex, the presence or absence of factor V Leiden or the G20210A prothrombin mutation, and the duration of oral anticoagulation.

Conclusions Patients with a high plasma level of factor VIII have an increased risk of recurrent venous thromboembolism. (N Engl J Med 2000;343:457-62.)

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ELEVATED plasma levels of factor VIII are associated with an increased risk of venous thrombosis.¹⁻³ In the Leiden Thrombophilia Study, plasma levels of factor VIII above 150 IU per deciliter were associated with a quintupled risk of venous thrombosis,¹ an observation that was confirmed by another study from the Netherlands.³ Among patients with venous thrombosis, the prevalence of an elevated plasma level of factor VIII is approximately 20 percent.^{2,3} High factor VIII levels persist over time,^{3,4} are not attributable to an acute-phase reaction,²⁻⁵ and may involve a genetic predisposition.³ The mechanism by which a high factor VIII level leads to thrombosis is unclear.

Recurrent venous thrombosis can be prevented by prophylaxis with oral anticoagulants,^{6,7} but these drugs can cause severe or fatal bleeding.⁸⁻¹⁰ Choosing the optimal duration of prophylaxis entails balancing the risk of recurrent thrombosis after the discontinuation of anticoagulant therapy against the risk of hemorrhagic complications. A small, retrospective study suggested that the risk of recurrent venous thrombosis is high among patients with elevated plasma levels of factor VIII.³ We followed 360 patients who had had a first episode of spontaneous venous thromboembolism to study the effect of high levels of factor VIII on the risk of symptomatic recurrent venous thromboembolism.

METHODS**Patients and Study Design**

The Austrian Study on Recurrent Venous Thromboembolism is an ongoing, prospective study involving patients from four thrombosis centers in Vienna, Austria. The study was approved by the ethics committee of the Vienna University Hospital. Between July 1992 and December 1999, 1259 consecutive patients older than 18 years who had been treated with oral anticoagulants for at least three months after an episode of venous thromboembolism and who provided written, informed consent to participate were enrolled. All the patients had received standard heparin

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therapy to keep the activated thromboplastin time at 1.5 to 2 times the control value or had received subcutaneous low-molecular-weight heparin at therapeutic dosages. Of the 1259 patients, 875 were excluded, for the following reasons: venous thromboembolism before the recent episode (189 patients); surgery, trauma, or pregnancy within the previous three months (224); known deficiency of antithrombin, protein C, or protein S (8); presence of the lupus anticoagulant (16); hyperhomocysteinemia (89); cancer (159); or a requirement for long-term treatment with antithrombotic drugs for reasons other than venous thrombosis (190).

The day of discontinuation of oral anticoagulant therapy was defined as the day of enrollment in the study. Three weeks after enrollment, patients were screened for a deficiency of antithrombin, protein C, or protein S and for the presence of the lupus anticoagulant; 24 patients in whom one of these abnormalities was detected at this time were excluded. Blood for measurement of factor VIII levels was also obtained three weeks after the last dose of oral anticoagulant. Patients were seen at three-month intervals during the first year after enrollment and every six months thereafter. They were given detailed written information on the symptoms of venous thromboembolism and were instructed to report to one of the thrombosis centers if such symptoms appeared. At each visit, the medical history was obtained and a physical examination was performed.

Diagnosis of Venous Thromboembolism

The diagnosis of deep-vein thrombosis was established by a positive finding on venography or color duplex ultrasonography (in the case of proximal deep-vein thrombosis). To be considered positive, the venograms had to meet at least one of the following direct or indirect criteria: a constant defect in filling seen on two views; an abrupt discontinuation of visible filling at a constant site in the vein; and the absence of filling in the entire deep-vein system (without external compression), with or without venous flow through collateral veins. With color duplex ultrasonography, at least one of the two following diagnostic criteria for deep-vein thrombosis had to be met: visualization of an intraluminal thrombus in a deep vein and incomplete compressibility or absence of compressibility.

The diagnosis of pulmonary embolism was based on a positive finding on ventilation-perfusion scanning of the lungs according to the criteria of the Prospective Investigation of Pulmonary Embolism Diagnosis.¹¹ Patients with both deep-vein thrombosis and pulmonary embolism were classified as having pulmonary embolism. Venography of the affected leg or arm was performed in all patients with deep-vein thrombosis before the discontinuation of oral anticoagulant therapy.

Outcomes

The end point of the study was the recurrence of symptomatic venous thromboembolism, confirmed by venography or ventilation-perfusion lung scanning according to the above-listed diagnostic criteria. The diagnosis was established by an adjudication committee consisting of independent clinicians and radiologists who were unaware of the presence or absence of risk factors for thrombosis in each patient. Deep-vein thrombosis was considered to have recurred if the patient had a thrombus in the leg or arm other than that affected by the previous thromboembolic event; a thrombus in another deep vein in the same leg or arm as the previous event; or a thrombus in the same venous system as the previous event, with proximal extension of the thrombus (if the upper limit of the original thrombus had been visible) or with a constant filling defect surrounded by contrast medium (if the original thrombus had not been visible).

Laboratory Analysis

After the patients fasted overnight, venous blood was collected in a 1:10 dilution of 0.11 M trisodium citrate. A portion of the collected blood was centrifuged for 20 minutes at 2000×g, and the plasma was stored at -80°C. For measurement of homocysteine, another portion of the collected blood was immediately centrifuged at 1600×g for 20 minutes at 4°C; the plasma was then snap-frozen and stored at -80°C. Genomic DNA was isolated from leukocytes by standard methods. Factor VIII was measured by a one-step clotting assay with use of factor VIII-deficient plasma obtained from Immuno Baxter (Baxter Healthcare, Vienna, Austria) and a fully automated coagulation analyzer (CA 6000, Sysmex, Kobe, Japan). Commercially available, pooled normal plasma (Coag Cal N, Dade Diagnostics, Duedingen, Switzerland) calibrated against World Health Organization standard 91/666 for factor VIII was used.

Tests for a deficiency of antithrombin, protein C, or protein S were performed as previously reported.¹² Screening for factor V Leiden and for the G20210A prothrombin mutation was carried out as described.^{13,14} The total homocysteine level was measured by high-performance liquid chromatography (Superspher RP 18 column; mesh size, 4 μm; Waters, Milford, Mass.) under isocratic conditions at room temperature with an acetate buffer (flow rate, 2 ml per minute). Hyperhomocysteinemia was diagnosed when the homocysteine level was above the 95th percentiles (8.8 μmol per liter in women and 11.6 μmol per liter in men) of the levels measured in 73 healthy control subjects who were similar to the study patients with regard to age and sex distribution. The presence of the lupus anticoagulant was assessed according to the criteria of the International Society on Thrombosis and Haemostasis.¹⁵ The level of C-reactive protein was determined by immunologic methods (Quantex CRP Plus, Biokit, Barcelona, Spain). The laboratory technicians were unaware of the patients' characteristics at all times.

Statistical Analysis

Times to recurrence (uncensored observations) or follow-up times in patients without recurrence (censored observations) were analyzed according to survival-time methods.¹⁶ The probability of recurrence was estimated according to the method of Kaplan and Meier.¹⁷ To test for homogeneity among the various groups of patients, we used the log-rank test and the generalized Wilcoxon test. The plasma level of factor VIII was analyzed in Cox proportional-hazards models as a continuous variable and as a dichotomized variable (in a separate analysis) to compare the relative risks of recurrence associated with different levels of factor VIII. The data were adjusted for age, sex, the presence or absence of factor V Leiden, the presence or absence of the G20210A prothrombin mutation, and the duration of oral anticoagulation. Categorical data were checked for homogeneity with the use of contingency-table analyses (by the chi-square test). For numerical operations, SAS software (SAS Institute, Cary, N.C.) was used. Values are given as means ±SD.

Statistical Analysis

RESULTS

Patients

Table 1 shows the base-line characteristics of the 360 patients. The average age of the patients was 48 years, and 52 percent of the patients were women. After the previous episode of venous thromboembolism, the patients had received oral anticoagulants for an average of eight months. One hundred eleven patients (31 percent) were carriers of factor V Leiden, and 32 patients (9 percent) had the G20210A prothrombin mutation. Of the 189 women in the study population, 78 (41 percent) were taking an oral contraceptive when venous thromboembolism developed. After the discontinuation of treatment with oral anticoagulants, the patients were followed for an average of 30 months. A total of 48 patients left the study because they required antithrombotic treatment

TABLE 1. BASE-LINE CHARACTERISTICS OF THE 360 PATIENTS.*

CHARACTERISTIC	VALUE
Age at the time of thromboembolism — yr	48±16
Sex — M/F	171/189
Type of thromboembolism — no. (%)	
Thrombosis in proximal veins of the leg	94 (26)
Thrombosis in distal veins of the leg	120 (33)
Thrombosis in axillary veins	21 (6)
Pulmonary embolism	125 (35)
Duration of oral anticoagulant therapy — mo	8±15
Factor V Leiden — no. (%)	111 (31)
G20210A prothrombin mutation — no. (%)	32 (9)
Observation time — mo	30±22

*Plus-minus values are means ±SD.

for causes other than venous thrombosis (43 patients) or because they were given a diagnosis of cancer (5 patients). Fifty-two patients (14 percent) were lost to follow-up. One patient died of pancreatitis, one of septicemia, and one of gastrointestinal bleeding. Data on these three patients were censored at the time of death.

Recurrence of Venous Thromboembolism

Of the 360 patients in the study population, 38 had recurrent venous thromboembolism (10.6 percent) (deep-vein thrombosis in 24 and pulmonary embolism in 14). Of these 38 patients, 27 were men and 11 were women. They had a shorter observation time than patients without recurrence (12±18 vs. 31±22 months, $P=0.003$). There was no significant difference between the patients with recurrence and those without recurrence with regard to age (52±15 and 48±16 years, respectively), the presence of factor V Leiden (26 percent and 33 percent), the presence of the G20210A prothrombin mutation (13

percent and 9 percent), or the duration of anticoagulation (7±3 and 8±16 months).

Recurrent Venous Thromboembolism and Levels of Factor VIII

Patients with recurrent venous thromboembolism had higher plasma levels of factor VIII than those without a recurrence (182±66 vs. 157±54 IU per deciliter, $P=0.009$). When factor VIII was analyzed as a continuous variable in a Cox proportional-hazards model, the relative risk of recurrence was 1.08 (95 percent confidence interval, 1.04 to 1.12; $P<0.001$) for each increase of 10 IU per deciliter in the level of factor VIII. After adjustment for age, sex, the presence or absence of factor V Leiden, the presence or absence of the G20210A prothrombin mutation, and the duration of oral anticoagulation, higher levels of factor VIII remained an independent risk factor for recurrence (relative risk per increase of 10 IU per deciliter, 1.07; 95 percent confidence interval, 1.02 to 1.12; $P=0.001$). This analysis assumed a graded relation between levels of factor VIII and the risk of recurrence.

To evaluate whether this relation was linear or whether there was a threshold level of factor VIII for an increase in the risk of recurrence, we calculated the relative risk associated with each of several different ranges of factor VIII levels (Table 2). The risk of recurrence was almost seven times as great among patients with factor VIII levels exceeding the 90th percentile of the values in the study population as among patients with levels in the reference range (below the 25th percentile). The relation between factor VIII levels exceeding the 90th percentile and the risk of recurrence was even stronger after adjustment for age, sex, the presence or absence of factor V Leiden, the presence or absence of the G20210A prothrombin mutation, and the duration of anticoagulation (Table 2).

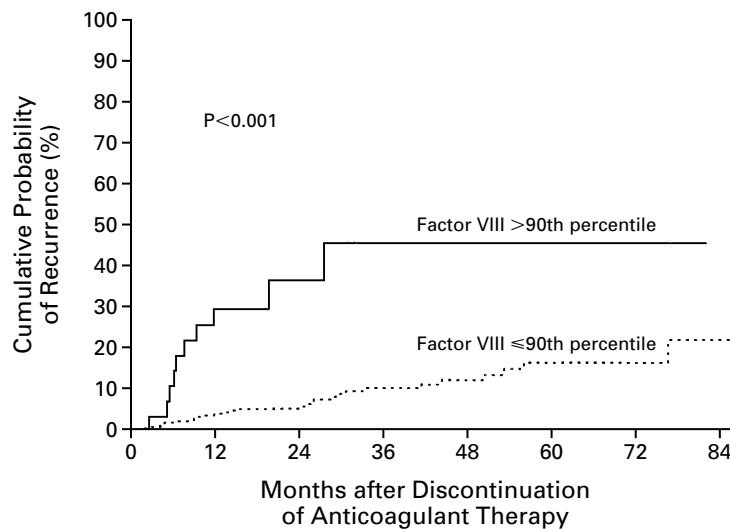
According to Kaplan–Meier analysis, there was a

TABLE 2. RELATIVE RISK OF RECURRENT VENOUS THROMBOEMBOLISM ACCORDING TO THE PLASMA LEVEL OF FACTOR VIII.*

FACTOR VIII (IU/dl)	PERCENTILE	NO. OF PATIENTS	NO. OF RECURRENCES	UNIVARIATE RELATIVE RISK (95% CI)	MULTIVARIATE RELATIVE RISK (95% CI)†
<120	<25th	94	6	1.0	1.0
120–150	25th–50th	90	11	1.6 (0.5–4.5)	1.6 (0.6–4.4)
151–192	51st–75th	88	8	1.7 (0.6–4.8)	1.5 (0.5–4.6)
193–234	76th–90th	52	3	0.9 (0.2–3.7)	0.9 (0.2–4.3)
>234	>90th	36	10	6.6 (2.4–18.4)	11.4 (3.1–42.5)

*CI denotes confidence interval.

†Multivariate relative risks were calculated with adjustment for age, sex, the presence or absence of factor V Leiden, the presence or absence of the G20210A prothrombin mutation, and the duration of anticoagulation.



NO. OF PATIENTS AT RISK		0	12	24	36	48	60	72	84
Factor VIII >90th percentile	35	19	7	4	4	2	1	0	
Factor VIII ≤90th percentile	325	258	181	112	78	50	22	3	

Figure 1. Kaplan–Meier Estimates of the Risk of Recurrent Venous Thromboembolism According to the Plasma Level of Factor VIII.

The probability of recurrent thrombosis was greater among patients with factor VIII levels above the 90th percentile than among patients with factor VIII levels at or below the 90th percentile ($P < 0.001$ by the Wilcoxon test and by the log-rank test).

clear divergence between the rate of recurrence among patients with factor VIII levels above the 90th percentile and the rate among patients with lower levels, throughout the period of observation ($P < 0.001$ by the Wilcoxon test and by the log-rank test) (Fig. 1). At 24 months, the probability of recurrence was 37 percent (95 percent confidence interval, 16 to 57 percent) among patients with factor VIII levels above the 90th percentile, as compared with 5 percent (95 percent confidence interval, 2 to 8 percent) among patients with levels of factor VIII at or below the 90th percentile. According to the univariate analysis, factor VIII levels above the 90th percentile conferred a relative risk of recurrence of 5.5 (95 percent confidence interval, 2.7 to 11.4; $P < 0.001$). After adjustment for age, sex, the presence or absence of factor V Leiden, the presence or absence of the G20210A prothrombin mutation, and the duration of anticoagulation, the relation between factor VIII levels above the 90th percentile and the risk of recurrence was even stronger (relative risk, 6.7; 95 percent confidence interval, 3.0 to 14.8; $P < 0.001$).

To evaluate the possibility that the level of factor VIII had increased because of an acute-phase reaction (such as inflammation), we correlated the levels of factor VIII with the corresponding levels

of C-reactive protein. No significant relation was detected.

DISCUSSION

Our prospective study shows that a high plasma level of factor VIII is a strong risk factor for recurrent venous thromboembolism. In 360 consecutive patients with a single episode of spontaneous venous thromboembolism, the risk of recurrence was almost seven times as great among patients with factor VIII levels above the 90th percentile of the values in the study population as among those with lower levels.

The relation between factor VIII and the risk of recurrence was nonlinear; factor VIII levels above the 90th percentile conferred a particularly high risk. This observation in patients with recurrent venous thrombosis is in contrast to the findings of the Leiden Thrombophilia Study, in which a linear relation between factor VIII levels and the risk of thrombosis was found in patients who had had a single episode of venous thrombosis.¹

The risk of recurrent venous thromboembolism depends on the number of risk factors present and their severity. The risk is high among patients who have had more than one thromboembolic episode¹⁸ or who have cancer, the lupus anticoagulant,^{19,20} hy-

perhomocysteinemia,²¹ or a congenital deficiency of a natural inhibitor of coagulation.²² Patients with these risk factors need prolonged prophylaxis, and such patients were therefore excluded from our study. We also excluded patients with thrombosis related to surgery, trauma, or pregnancy, whose risk of recurrence is low.

The overall rate of recurrence of venous thromboembolism in our study (approximately 5 percent per year) was similar to that reported in a Swedish study⁶ but lower than that in a recent study from Canada (approximately 20 percent during the first year).⁷ This discrepancy may well be explained by differences in the populations of patients selected. In contrast to the Canadian study, ours included patients with a low risk of recurrence, such as patients who had distal-vein thrombosis, and excluded patients at high risk, such as those who had the lupus anticoagulant or who had had a previous episode of recurrent venous thromboembolism, even when the previous episode was the result of trauma or surgery. The longer duration of oral anticoagulation in our patients may also have reduced the risk of recurrence. Of the women in our study, 41 percent had had the first thrombotic event while taking an oral contraceptive and had subsequently stopped using it. This change may also have contributed to the lower risk of recurrence among our patients than among those in other studies.

The most common congenital risk factors for deep-vein thrombosis — the presence of factor V Leiden and the presence of the G20210A prothrombin mutation — were unknown at the time our study began. In our patient population as well as several other cohorts,^{7,23-26} neither factor V Leiden nor the G20210A prothrombin mutation conferred a risk of recurrence that was higher than that in patients without the genetic defect. Carriers of either of these mutations were therefore not excluded from our analysis. Nevertheless, to avoid any confounding effect resulting from the presence of these mutations in some patients, the relative risk of recurrent thromboembolism was adjusted for these two genetic defects; a high level of factor VIII remained a strong and independent risk factor after this adjustment.

The duration of oral anticoagulation affects the risk of recurrent venous thrombosis. The rate of recurrence was significantly lower among patients treated with oral anticoagulants for six months than among those who received a six-week course of therapy.⁶ In another study, the rate of recurrence among patients who received oral anticoagulants for three months was higher than that among patients who were treated longer.⁷ In our study, the duration of oral anticoagulation varied from three months to several years (in a small group of patients) and averaged eight months. The duration of anticoagulation was similar in patients with and patients without recurrence (seven and eight months). Moreover, after adjustment

for the duration of anticoagulation, a high level of factor VIII remained an independent and strong risk factor for recurrent venous thromboembolism.

Plasma levels of factor VIII increase in patients with inflammation, cancer, or pregnancy. Patients with these conditions were therefore excluded from the study. Factor VIII was measured in blood that was obtained an average of more than six months after the initial episode of venous thromboembolism, thus ruling out the possibility that the factor VIII level was affected by the first episode. Moreover, no correlation was found between factor VIII levels and levels of C-reactive protein, which is a sensitive indicator of an acute-phase reaction. These findings, which are in keeping with those of others,²⁻⁵ indicate that a high level of factor VIII is a cause rather than a consequence of venous thrombosis. This concept is also supported by the observation that among patients with venous thrombosis, high levels of factor VIII persist over time.^{3,4}

The factor VIII levels in our patients were higher than those in the patients examined in the study from Leiden.¹ In that study, approximately 25 percent of patients with a first episode of venous thrombosis had a factor VIII level above 150 IU per deciliter; in our study, the threshold value for the 75th percentile was 192 IU per deciliter. This difference may be due to the positive correlation between age and factor VIII levels, with particularly high levels present in elderly persons.²⁷ Although our patients were only slightly older than those in the Leiden study, the proportion of patients older than 70 years who were excluded from that study was relatively high (10 percent).¹ The factor VIII levels in our patients correspond well to those found by Kraaijenhagen et al.,³ in whose study the 75th percentile of factor VIII levels was 175 IU per deciliter among patients with thrombosis and a mean age of 55 years.

How long should a patient be treated with oral anticoagulants? The decision depends on the patient's risk of recurrent thromboembolism and his or her risk of severe bleeding due to anticoagulation. The annual incidence of serious bleeding complications associated with anticoagulant therapy ranges from 1 to 2 percent, a value that decreases over time during the course of treatment and that increases with age. Approximately 25 percent of severe hemorrhagic complications are fatal.⁸⁻¹⁰ The risk of recurrent thrombosis depends on the presence of acquired or congenital risk factors and declines over time. Roughly 5 percent of the recurrences are fatal.²⁸ Using these numbers, one could calculate that long-term oral anticoagulation would benefit the subgroups of patients in which the annual incidence of recurrence is above 10 percent, such as those who have already had a recurrence of venous thromboembolism or who have the lupus anticoagulant.^{18,20} Our study shows that among patients with high factor VIII levels, the risk

of recurrence is higher than 10 percent per year. Prospective trials are needed to investigate the value of prolonged anticoagulant therapy in patients with high factor VIII levels, but until these data are in hand, extended prophylaxis must be considered after a single episode of spontaneous venous thromboembolism in such patients. We believe that the high prevalence of elevated levels of factor VIII and the risk of recurrent venous thrombosis associated with such high levels warrant the measurement of factor VIII during routine screening for thrombophilia.

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