HIGH LEVELS OF COAGULATION FACTOR XI AS A RISK FACTOR FOR VENOUS THROMBOSIS

Joost C.M. Meijers, Ph.D., Winnie L.H. Tekelenburg, M.Sc., Bonno N. Bouma, Ph.D., Rogier M. Bertina, Ph.D., and Frits R. Rosendaal, M.D.

ABSTRACT

Background   Factor XI, a component of the intrinsic pathway of coagulation, contributes to the generation of thrombin, which is involved in both the formation of fibrin and protection against fibrinolysis. A deficiency of factor XI is associated with bleeding, but a role of high factor XI levels in thrombosis has not been investigated.

Methods   We determined factor XI antigen levels in the patients enrolled in the Leiden Thrombophilia Study, a large population-based, case–control study (with a total of 474 patients and 474 controls) designed to estimate the contributions of genetic and acquired factors to the risk of deep venous thrombosis. Odds ratios were calculated as a measure of relative risk.

Results   The age- and sex-adjusted odds ratio for deep venous thrombosis in subjects who had factor XI levels above the 90th percentile, as compared with those who had factor XI levels at or below that value, was 2.2 (95 percent confidence interval, 1.5 to 3.2). There was a dose–response relation between the factor XI level and the risk of venous thrombosis. Adjustment of the odds ratios for other risk factors such as oral-contraceptive use, homocysteine, fibrinogen, factor VIII, female sex, and older age did not alter the result. Also, when we excluded subjects who had known genetic risk factors for thrombosis (e.g., protein C or S deficiency, antithrombin deficiency, the factor V Leiden mutation, or the prothrombin G20210A mutation), the odds ratio remained the same, indicating that the risk of venous thrombosis associated with elevated levels of factor XI was not the result of one of the known risk factors for thrombosis.

Conclusions   High levels of factor XI are a risk factor for deep venous thrombosis, with a doubling of the risk at levels that are present in 10 percent of the population. (N Engl J Med 2000;342:696-701.)

©2000, Massachusetts Medical Society

FACTOR XI is a component of the intrinsic pathway of coagulation. It can be activated in vitro by contact with a thrombogenic surface, such as glass. Whether surface contact contributes to the activation of factor XI in vivo, however, is uncertain. Factor XI can also be activated by thrombin (Fig. 1), both in the presence and in the absence of negatively charged surfaces.[2][7] Through a feedback mechanism, the thrombin level increases, which is necessary for the formation of fibrin and for protection against fibrinolysis.[7] Thrombin mediates this latter mechanism by activating thrombin-activatable fibrinolysis inhibitor,[8,9] also called procarboxypeptidase B[10] or procarboxypeptidase U.[11] Once activated, it inhibits fibrinolysis by removing C-terminal lysine residues from fibrin, which are essential for the binding and activation of plasminogen.[12]

Factor XI deficiency results in a mild-to-moderate bleeding disorder, especially in tissues with high levels of local fibrinolytic activity (such as the urinary tract, nose, oral cavity, and tonsils).[13,14] On the basis of the in vitro data summarized above, the role of factor XI in hemostasis can be seen as a combination of a procoagulant action (the formation of fibrin) and an antifibrinolytic action (the protection of fibrin).[1] In a rabbit model, the lysis of clots was enhanced by the inhibition of factor XI.[15]

The role of factor XI in thrombosis in humans is unclear. We evaluated the effect of high levels of factor XI on the risk of venous thrombosis as part of the Leiden Thrombophilia Study, a large population-based, case–control study designed to estimate the contributions of genetic and acquired factors to the risk of venous thrombosis.

METHODS

Patients and Controls

The methods by which blood samples were obtained and data were collected have been described elsewhere.[16][19] We enrolled consecutive patients under the age of 70 years who had had a first episode of deep venous thrombosis (objectively confirmed by impedance plethysmography, Doppler ultrasonography, compression ultrasonography, or contrast venography) between 1988 and 1993 and who were not known to have cancer. The patients were selected from the files of three anticoagulation clinics in the Netherlands (in Leiden, Amsterdam, and Rotterdam). These clinics monitor the anticoagulant treatment of virtually all patients in three well-defined regions. Each patient was asked to find a neighbor or friend of the same sex and age (within five years) without deep venous thrombosis who was willing to participate as a control subject. Partners of patients were also asked to volunteer as control subjects. If a patient was unable to find a control subject, the first person on the list of partners who was the same age and sex as the patient was asked to participate in the study. A total of 225 of the 474 control subjects (47 percent) were partners of other patients. The study protocol was approved by the Leiden University ethics committee, and all participants gave informed consent.
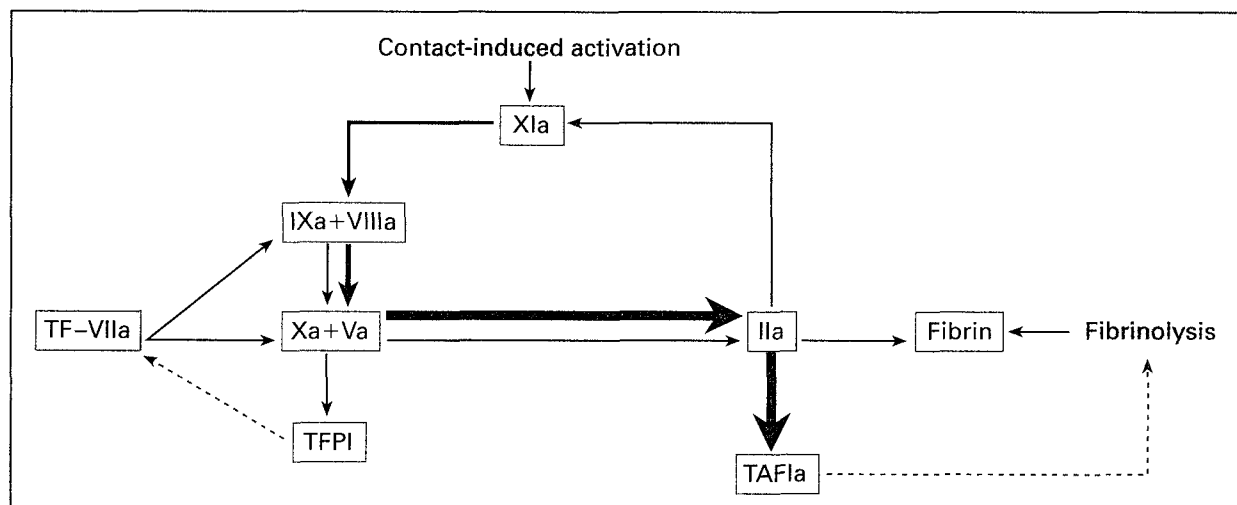
Figure 1. Model of Blood Coagulation.

Coagulation is initiated by tissue factor (TF), which binds to factor VIIa. This complex activates factor IX or factor X, which leads to the activation of thrombin (factor IIa) and the formation of fibrin. Tissue-factor–dependent coagulation is rapidly inhibited by tissue-factor–pathway inhibitor (TFPI). Coagulation is maintained through the activation of factor XI by thrombin. Through the intrinsic tenase complex (factors IXa and VIIIa) and the prothrombinase complex (factors Xa and Va), the additional thrombin required to down-regulate fibrinolysis is generated by the activation of thrombin-activatable fibrinolysis inhibitor (TAFI). Activated TAFI (TAFIa) down-regulates fibrinolysis by removing C-terminal lysine residues involved in the binding and activation of plasminogen. Although the contact system is a potent activator of the coagulation system in vitro, it is not a physiologic activator of coagulation. The solid arrows indicate activation, and the broken arrows inhibition. The solid arrow from factor Xa to TFPI indicates that factor Xa has to form a complex with TFPI and that this complex then inhibits TF–VIIa. The increasing width of the arrows indicates the cascade effect. Modified from Bouma et al.[1]

Laboratory Studies

Blood was collected in tubes containing 0.106 M trisodium citrate. Plasma was prepared by centrifugation at $2000 \times g$ at room temperature and was stored at $-70°C$. Blood samples were obtained from patients at least six months after the thrombotic event and at least three months after the discontinuation of treatment with an oral anticoagulant. Plasma samples were available for 473 of the 474 patients and for all 474 controls.

Factor XI antigen was measured by enzyme-linked immunosorbent assay with a monoclonal antibody (XI-5)[20] used for capture and a rabbit polyclonal antibody[21] used for detection. The results are expressed as a percentage, with 100 percent equivalent to the factor XI antigen level in pooled plasma samples from 40 healthy volunteers. Each sample was assayed on a single occasion at two dilutions in duplicate. The intraassay and interassay coefficients of variation were 4.6 and 7.0 percent in 14 and 70 assays, respectively. All antigen measurements were performed during a three-month period between 1998 and 1999, without knowledge of whether the sample was from a patient or a control subject. Protein C, protein S, and antithrombin were also measured, since deficiencies of these proteins are associated with an increased risk of venous thrombosis.[16]

Statistical Analysis

We performed two sets of analyses: one to identify the determinants of factor XI levels, and the other to establish the contribution of high plasma factor XI levels to the risk of thrombosis. Determinants of factor XI levels were examined in the controls, since they represented the general population. We used a comparison of means and linear regression, and we report means, medians, and regression coefficients, with 95 percent confidence intervals for these coefficients.

The contribution of an elevated factor XI level to the risk of

venous thrombosis was analyzed by calculating odds ratios as estimates of the relative risk. Since cutoff points for factor XI plasma values are essentially arbitrary, we used percentiles of the distribution among controls as cutoff points. We used the 90th and 95th percentiles and also performed an analysis by quartiles. In all these analyses, the group with the lowest factor XI levels (i.e., those at the 90th percentile or below, the 95th percentile or below, and the 25th percentile or below, respectively) served as the reference category for odds ratios. Ninety-five percent confidence intervals were calculated according to the method of Woolf[22] or were derived from the logistic model. We used multivariate modeling by unconditional logistic regression to adjust for sex and age and other putative confounding variables. Age was analyzed as a continuous variable and as a dichotomous variable (<30 years, 30 to 50 years, or >50 years). Other variables included in the multivariate model were oral contraceptives (no use or use just before the thrombotic episode), factor V Leiden (noncarrier or carrier [AA and AG]),[23] the prothrombin G20210A mutation (noncarrier or carrier),[24] C-reactive protein (continuous variable), homocysteine (>18.5 μmol per liter or ≤18.5 μmol per liter), factor VIII:C (>150 IU per deciliter or ≤150 IU per deciliter), and fibrinogen (>4 g per liter [11.8 μmol per liter] or ≤4 g per liter). Adjustment for thrombophilic abnormalities was performed in a separate analysis. We determined whether the odds ratio increased linearly with increasing levels of factor XI by comparing two logistic models: one that assumed a linear trend and one that did not (the dummy-variable model). The two models were compared with the likelihood-ratio test, which has a chi-square distribution.

To assess the effect on public health of a high factor XI level as a risk factor for venous thrombosis, we calculated the population attributable risk. This risk indicates the proportion of all events attributable to the risk factor under study, on the basis of certain assumptions, including the assumption that the association is causal. One can also view the population attributable risk as the num-

ber of events that will be prevented if the risk factor is removed. It is defined as the difference between the overall incidence of the event and the incidence in persons who do not have the risk factor and is expressed as a proportion of the overall incidence. The population attributable risk is derived from the relative risk and the prevalence of the risk factor.[25]

RESULTS

The mean age of the patients and controls was 45 years, and the ratio of the males to females was approximately 3:4 (Table 1). The mean (±SD) factor XI level in the control subjects was 97.0±19.5 percent, as compared with 104.2±22.6 percent in the patients (P<0.001), and was slightly higher in female controls than in male controls (Table 2). Figure 2 shows the distribution of factor XI levels in patients and controls. The levels were normally distributed and increased with age; there was a 0.19 percent increase (95 percent confidence interval, 0.07 to 0.30 percent) with each additional year of age. As Table 2 shows, mean factor XI levels in the control subjects were 7 percent lower in those under 30 years of age than in those over 50 years.

The 90th percentile of the factor XI levels in the control group was 120.8 percent. Of the 473 patients, 92 (19 percent) had values that exceeded this cutoff point, as compared with 47 of the 474 subjects in the control group (10 percent, by definition). The unadjusted odds ratio for deep venous thrombosis in patients with a factor XI level above the 90th percentile (Table 3), as compared with those who had a lower value, was 2.2 (95 percent confidence interval, 1.5 to 3.2). The sex- and age-adjusted odds ratio was also 2.2 (95 percent confidence interval, 1.5 to 3.2). When the cutoff point was set at the 95th percentile (130.2 percent), the odds ratio was 2.3 (95 percent confidence interval, 1.4 to 3.9).

The relative risk of venous thrombosis associated with a factor XI level above the 90th percentile was slightly higher in men (odds ratio, 3.2; 95 percent confidence interval, 1.7 to 6.3) than in women (odds ratio, 1.8; 95 percent confidence interval, 1.1 to 2.8); the difference was not statistically significant (P= 0.15). The relative risk was similar in different age groups: 2.5 (95 percent confidence interval, 0.8 to 7.4) for patients younger than 30 years of age, 2.0 (95 percent confidence interval, 1.1 to 3.4) for those between 30 and 50 years, and 2.4 (95 percent confidence interval, 1.3 to 4.4) for those older than 50 years.

To determine whether there was a dose–response relation between the factor XI level and the risk of thrombosis, we stratified the subjects according to the factor XI level, and odds ratios were calculated for venous thrombosis in the patients at the higher levels as compared with those at the lowest level. As shown in Figure 3, the relative risk of thrombosis increased with the factor XI level, indicating a continuous dose–response relation. Indeed, a linear model described the relative risk associated with increasing levels of factor

XI as well as a less stringent model with dummy variables (P>0.5).

We performed separate analyses adjusted for each of several putative confounding variables. The odds ratio associated with a factor XI level that exceeded the 90th percentile was 2.2 (95 percent confidence interval, 1.5 to 3.3) when adjusted for oral contraceptive use, 2.3 (95 percent confidence interval, 1.5 to 3.3) when adjusted for a homocysteine level higher than 18.5 μmol per liter, 2.1 (95 percent confidence interval, 1.4 to 3.1) when adjusted for a fibrinogen level higher than 4 g per liter, 1.9 (95 percent confidence interval, 1.3 to 2.8) when adjusted for a factor VIII level higher than 150 IU per deciliter, 2.1 (95 percent confidence interval, 1.4 to 3.1) when adjusted for the C-reactive protein level, 2.2 (95 percent con-

TABLE 1. CHARACTERISTICS OF PATIENTS WITH VENOUS THROMBOSIS AND CONTROL SUBJECTS.

CHARACTERISTIC	PATIENTS (N=473)	CONTROLS (N=474)
Age (yr)		
Mean	45.0	44.7
Median	45.3	45.3
Range	14–69	14–72
Sex (M/F)	202/271	202/272
Protein C, <0.67 U/ml (no.)*	15	4
Protein S, <0.67 U/ml (no.)*	5	6
Antithrombin, <0.80 U/ml (no.)*	5	1
Factor V Leiden (no.)†	92	14
Prothrombin G20210A (no.)‡	29	11

*Data are for abnormal results of tests with two separate samples from each patient.

†Eight patients were homozygous carriers, and all the carriers among the controls were heterozygous.

‡All carriers were heterozygous.

TABLE 2. FACTOR XI LEVELS IN CONTROL SUBJECTS ACCORDING TO SEX AND AGE.

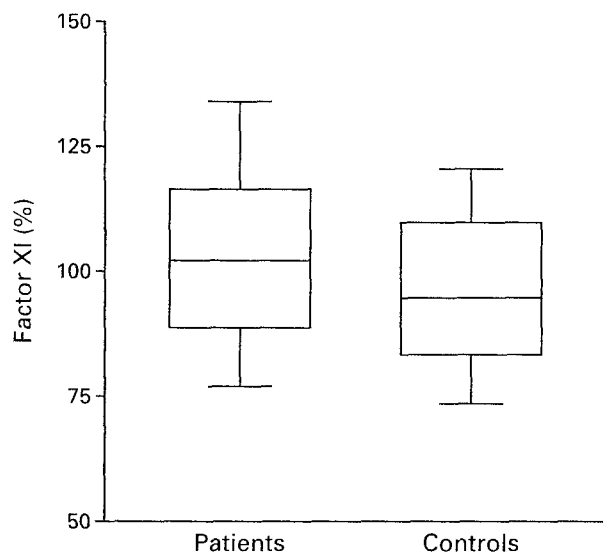| VARIABLE	NO. OF SUBJECTS	FACTOR XI
		%
Overall	474	97.0±19.5
Sex		
Male	202	95.2±19.4
Female	272	98.3±19.5
Age		
<30 yr	77	91.8±18.9
30–50 yr	224	97.3±19.1
>50 yr	173	99.0±20.0

Figure 2. Factor XI Levels in Patients and Control Subjects

In each box plot, the lower and upper bars represent the 10th and 90th percentiles, respectively; the lower and upper ends of the box represent the 25th and 75th percentiles, respectively, and the line inside the box represents the median factor XI level.

TABLE 3. RISK OF THROMBOSIS ACCORDING TO FACTOR XI LEVEL.

FACTOR XI*	PATIENTS (N=473)	CONTROLS (N=474)	ODDS RATIO (95% CI)†
≤90th Percentile	381	427	1 0
>90th Percentile	92	47	2 2 (1 5–3 2)
≤95th Percentile	423	451	1 0
>95th Percentile	50	23	2 3 (1 4–3 9)

*The 90th and 95th percentiles of factor XI levels in control subjects were 120 8 and 130 2 percent, respectively, with 100 percent equivalent to the factor XI antigen level in pooled plasma samples from 40 healthy volunteers

†The odds ratio is for the higher percentile as compared with the lower percentile CI denotes confidence interval

fidence interval, 1.5 to 3.2) when adjusted for factor V Leiden, and 2.3 (95 percent confidence interval, 1.6 to 3.3) when adjusted for prothrombin G20210A. All these odds ratios were also adjusted for age and sex.

When patients with known genetic risk factors for thrombosis (i.e., protein C deficiency, protein S deficiency, antithrombin deficiency, the factor V Leiden mutation, or the prothrombin G20210A mutation) were excluded from the analysis, the odds ratio for venous thrombosis in patients with factor XI levels exceeding the 90th percentile remained 2.2 (95 percent confidence interval, 1.5 to 3.3).
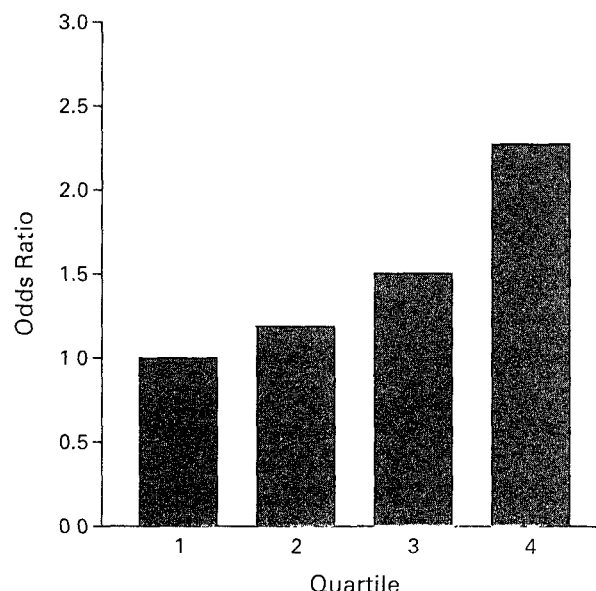

Figure 3. Odds Ratio for Thrombosis According to the Factor XI Level.

The patients and control subjects were stratified in quartiles according to the factor XI level (first quartile, ≤83.3 percent, second quartile, ≤94.8 percent; third quartile, ≤110 0 percent; and fourth quartile, >110.0 percent), with 100 percent equivalent to the factor XI antigen level in pooled plasma samples from 40 healthy volunteers. Odds ratios for thrombosis were calculated in the patients in the second, third, and fourth quartiles, as compared with those in the first quartile. The 95 percent confidence intervals were 0.8 to 1.8 for the second quartile, 1.0 to 2 2 for the third, and 1.7 to 3.3 for the fourth.

In the most fully adjusted model (adjusted for age, sex, a high level of factor VIII, a high fibrinogen level, hyperhomocysteinemia, use of oral contraceptives, factor V Leiden, the prothrombin 20210A allele, and the C-reactive protein level), the odds ratio associated with a factor XI level that exceeded 120.8 percent (90th percentile) was 1.9 (95 percent confidence interval, 1.3 to 2.9). This result indicated that the risk of thrombosis associated with a high level of factor XI was not the result of one of these underlying abnormalities.

DISCUSSION

We found that a high level of factor XI (defined as a value above the 90th percentile of the distribution of values in control subjects) was a risk factor for venous thrombosis, with a relative risk of 2.2 (95 percent confidence interval, 1.5 to 3.2) as compared with lower levels. Our findings were not due to the effects of oral-contraceptive use, sex, or age. Also, known genetic risk factors, such as protein C deficiency, protein S deficiency, antithrombin deficiency, the factor V Leiden mutation, or the prothrombin G20210A

mutation, did not explain the increased risk of thrombosis associated with a high level of factor XI. High factor XI levels were associated with a slightly higher relative risk of thrombosis for men than for women. Since there is no obvious biologic mechanism for such a sex-specific difference in the effect of factor XI on the risk of thrombosis, we think this finding may well have been due to chance.

The relative risk associated with a high factor XI level did not vary according to age. Venous thrombosis is strongly associated with age, with annual incidence rates increasing from less than 1 case per 10,000 young adults to nearly 1 per 100 elderly persons.[26] When a similar relative risk is applied to these incidence rates, the result, in absolute terms, is a larger effect of a high level of factor XI in the elderly. This effect is compounded by the increase in factor XI levels with increasing age.

The relative risk of 2.2 associated with a factor XI level present in 10 percent of the population leads to the conclusion that a high factor XI level is an important contributor to the overall burden of venous thrombosis. On the basis of these data, the population attributable risk of thrombosis is 11 percent (i.e., 11 percent of all cases of thrombosis in the general population may be attributable to high factor XI levels).[25]

Our understanding of the role of factor XI in hemostasis has changed considerably over the past 20 years. Initially, factor XI was thought to be involved in the initiation of coagulation, as a component of the contact system. The contact system consists of factor XI, factor XII, prekallikrein, and high-molecular-weight kininogen, and the system is activated in vitro when these proteins are exposed to a negatively charged surface such as glass. However, the role of the contact system in coagulation in vivo is doubtful, because of the absence of bleeding complications in patients with deficiencies of contact factors other than factor XI and the absence of a physiologic activating surface.

Factor XI deficiency results in a wide range of bleeding manifestations, from asymptomatic bleeding to injury-related bleeding that requires multiple transfusions.[27,28] Unlike hemophilia A or B, factor XI deficiency is rarely manifested as spontaneous bleeding; the associated bleeding usually occurs after trauma, surgery, or other challenges to hemostasis.[29] This finding indicates that factor XI is activated during coagulation by one or more enzymes other than factor XIIa. The finding that thrombin can activate factor XI[2,3] has led to a revised model of coagulation[3,30] in which factor XI contributes to the secondary generation of thrombin (in contrast to the primary generation of thrombin that is due to the extrinsic pathway of coagulation). This revised model explains the particularly severe bleeding complications in patients with a deficiency of factor VIII or IX, because these fac-

tors are involved in both primary and secondary thrombin generation. However, this model does not explain the predominance of bleeding in tissues with high levels of local fibrinolytic activity (the urinary tract, nose, oral cavity, and tonsils) in patients with factor XI deficiency.[13,14] The location of the bleeding may be explained, however, by the role of secondary thrombin generation in the regulation of fibrinolysis[7] through the activation of thrombin-activatable fibrinolysis inhibitor.[8,9]

The role of factor XI in coagulation is twofold: by generating thrombin, it both contributes to the formation of fibrin and helps protect fibrin from rapid proteolysis.[1] It was therefore our hypothesis that with high levels of factor XI, the secondary generation of thrombin would be enhanced or sustained, leading to a prolonged down-regulation of fibrinolysis and therefore a risk of thrombosis. The risk of thrombosis associated with high levels of factor XI may be explained by the role of factor XI in the down-regulation of fibrinolysis. The K_m for the activation of factor XI by thrombin is 50 nM,[3] which is within the range of the plasma level of factor XI (30 to 60 nM).[3] This suggests that, at least kinetically, an increase in the factor XI level should lead to an increased rate of activation.

The first abnormalities in the clotting system that were found to be associated with an increased risk of venous thrombosis were deficiencies of antithrombin, protein C, and protein S. These deficiencies are generally the result of major genetic disruptions that lead to loss of the protein's natural anticoagulant activity. Prothrombotic abnormalities that are the result of more subtle genetic alterations and a gain of function include factor V Leiden (a mutated factor V, which is less sensitive to inactivation)[31] and increased levels of three clotting factors (factor VIII,[18] prothrombin,[24] and fibrinogen[32]). Until now, the prothrombotic effects of elevated levels of these coagulation proteins were thought to be due to prolonged formation of fibrin as a result of excessive generation of thrombin. At least in the case of factor VIII and prothrombin, however, an alternative explanation may be that the sustained generation of thrombin results in prolonged down-regulation of fibrinolysis through the activation of thrombin-activatable fibrinolysis inhibitor.

In the case of prothrombin, the risk of thrombosis is associated with a mutation in the gene (G20210A) that may act through elevated levels.[24] We are currently investigating whether elevated levels of factor XI may also be genetically determined.

In conclusion, an elevated factor XI level is a risk factor for venous thrombosis. We postulate that a high level of factor XI causes thrombosis through sustained generation of thrombin, which leads to the protection of fibrin from proteolysis.

REFERENCES

1 Bouma BN von dem Borne PAK Meijers JCM Factor XI and protection of the fibrin clot against lysis — a role for the intrinsic pathway of coagulation in fibrinolysis Thromb Haemost 1998 80 24 7

2 Naito K Fujikawa K Activation of human blood coagulation factor XI independent of factor XII factor XI is activated by thrombin and factor XIi in the presence of negatively charged surfaces J Biol Chem 1991 266 7353 8

3 Gailani D Broze GJ Jr Factor XI activation in a revised model of blood coagulation Science 1991 253 909 12

4 von dem Borne PAK Koppleman SJ Bouma BN Meijers JCM Surface independent factor XI activation by thrombin in the presence of high molecular weight kininogen Thromb Haemost 1994 72 397 402

5 Cawthern KM van t Veer C Lock JB DiLorenzo ME Branda RF Mann KG Blood coagulation in hemophilia A and hemophilia C Blood 1998 15 4581 92

6 Oliver JA Monroe DM Roberts HR Hoffman M Thrombin activates factor XI on activated platelets in the absence of factor XII Arterioscler Thromb Vasc Biol 1999 19 170 7

7 von dem Borne PAK Meijers JCM Bouma BN Feedback activation of factor XI by thrombin in plasma results in additional formation of thrombin that protects fibrin clots from fibrinolysis Blood 1995 86 3035 42

8 Von dem Borne PAK Bajzar L Meijers JCM Nesheim ME Bouma BN Thrombin mediated activation of factor XI results in a thrombin activatable fibrinolysis inhibitor dependent inhibition of fibrinolysis J Clin Invest 1997 99 2323 7

9 Nesheim ME Wang W Boffa M Nagashima M Morser J Bajzar L Thrombin thrombomodulin and TAFI in the molecular link between coagulation and fibrinolysis Thromb Haemost 1997 78 386 91

10 Eaton DL Malloy BE Tsai SP Henzel W Drayna D Isolation molecular cloning and partial characterization of a novel carboxypeptidase B from human plasma J Biol Chem 1991 266 21833 8

11 Wang W Hendriks DF Scharpe SS Carboxypeptidase U a plasma carboxypeptidase with high affinity for plasminogen J Biol Chem 1994 269 15937 44

12 Wang W Boffa MB Bajzar L Walker JB Nesheim ME A study of the mechanism of inhibition of fibrinolysis by activated thrombin activatable fibrinolysis inhibitor J Biol Chem 1998 273 27176 81

13 Asakai R Chung DW Davie EW Schgsohn U Factor XI deficiency in Ashkenazi Jews in Israel N Engl J Med 1991 325 153 8

14 Berliner S Horowitz I Martinowitz U Brenner B Schgsohn U Dental surgery in patients with severe factor XI deficiency without plasma replacement Blood Coagul Fibrinolysis 1992 3 465 8

15 Minnema MC Friederich PW Levi M et al Enhancement of rabbit jugular vein thrombolysis by neutralization of factor XI in vivo evidence for a role of factor XI as an anti fibrinolytic factor J Clin Invest 1998 101 10 4 [Erratum J Clin Invest 1998 101 917]

16 Koster T Rosendaal FR Briet E et al Protein C deficiency in a controlled series of unselected outpatients an infrequent but clear risk factor for venous thrombosis Blood 1995 85 2756 61

17 Koster T Rosendaal FR de Ronde H Briet E Vandenbroucke JP Bertina RM Venous thrombosis due to poor anticoagulant response to activated protein C Leiden Thrombophilia Study Lancet 1993 342 1503 6

18 Koster T Blann AD Briet E Vandenbroucke JP Rosendaal FR Role of clotting factor VIII in effect of von Willebrand factor on occurrence of deep vein thrombosis Lancet 1995 345 152 5

19 Vandenbroucke JP Koster T Briet E Reitsma PH Bertina RM Rosendaal FR Increased risk of venous thrombosis in oral contraceptive users who are carriers of factor V Leiden mutation Lancet 1994 344 1453 7

20 Wuillemin WA Minnema M Meijers JCM et al Inactivation of factor XIa in human plasma assessed by measuring factor XIa protease inhibitor complexes major role for C1 inhibitor Blood 1995 85 1517 26

21 Bouma BN Vlooswijk RAA Griffin JH Immunologic studies of human coagulation factor XI and its complex with high molecular weight kininogen Blood 1983 62 1123 31

22 Woolf B On estimating the relation between blood group and disease Ann Hum Genet 1955 19 251 3

23 Rosendaal FR Koster T Vandenbroucke JP Reitsma PH High risk of thrombosis in patients homozygous for factor V Leiden Blood 1995 85 1504 8

24 Poort SR 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 703

25 Rothman KJ Modern epidemiology Boston Little Brown 1986 35 40

26 Nordstrom M Lindblad B Bergqvist D Kjellstrom T A prospective study of the incidence of deep vein throm bosis within a defined urban population J Intern Med 1992 232 155 60

27 Rapaport SI Proctor RR Patch MJ Yettra M The mode of inheritance of PTA deficiency evidence for the existence of major PTA deficiency and minor PTA deficiency Blood 1961 18 149 65

28 Schgsohn L High gene frequency of factor XI (PTA) deficiency in Ashkenazi Jews Blood 1978 51 1223 8

29 Idem Factor XI deficiency Thromb Haemost 1993 70 68 71

30 Davie EW Fujikawa K Kisiel W The coagulation cascade initiation maintenance and regulation Biochemistry 1991 30 10363 70

31 Bertina RM Koeleman BPC Koster T et al Mutation in blood coagulation factor V associated with resistance to activated protein C Nature 1994 369 64 7

32 Koster T Rosendaal FR Reitsma PH van der Velden PA Briet E Vandenbroucke JP Factor VII and fibrinogen levels as risk factors for venous thrombosis a case control study of plasma levels and DNA polymorphisms — the Leiden Thrombophilia Study (LETS) Thromb Haemost 1994 71 719 22