Elevated Factor XI Activity Levels Are Associated With an Increased Odds Ratio for Cerebrovascular Events

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Abstract

High levels of factor XI have been implicated as a risk factor for deep venous thrombosis and possibly cardiovascular disease; however, the relationship between elevated factor XI activity and stroke has yet to be established. We retrospectively evaluated factor XI activity, factor XI antigen, and high-sensitivity C-reactive protein (hs-CRP) values in samples from 65 patients with stroke, 13 with transient ischemic attack (TIA), and 17 with venous thrombosis, younger than 55 years with normal prothrombin and partial thromboplastin times who underwent evaluation for a hypercoagulable state. Factor XI activity levels were more than normal in 22% of patients with stroke or TIA and 18% of patients with venous thrombosis, producing odds ratios of 5.3 and 4.1 for stroke or TIA and venous thrombosis, respectively. Factor XI activity levels correlate with factor XI antigen levels by Deming regression analysis (slope, 1.3; R = *0.667), and a lack of correlation of both with hs-CRP suggests that factor XI is not an acute phase reactant. Our findings suggest an association between elevated factor XI activity and stroke.*

Discoveries have led to a new view of the traditional coagulation paradigm in which factor XI is now of pivotal importance. Factor XI is a homodimeric coagulation protein consisting of 2 polypeptide chains of 607 amino acids each and is a part of the intrinsic coagulation pathway. However, it can be activated not only by factor XIIa through the contact pathway as traditionally believed but also by thrombin in a reaction promoted by platelets.[1-3] The fact that factor XI can be activated by thrombin links the intrinsic pathway with the extrinsic pathway, which is activated through exposure of factor VII to tissue factor leading to the activation of factor X and eventual generation of thrombin. The feedback loop thus formed with thrombin activating factor XI results in the generation of sufficient thrombin to convert fibrinogen to a stable cross-linked fibrin clot.

In addition to its role in clot generation, factor XI also has a role in the prevention of clot lysis. This effect is, like the process of clot generation, dependent on the generation of sufficient amounts of thrombin through thrombin-mediated feedback activation of factor XI.[4,5] Generation of sufficient quantities of thrombin leads to activation of thrombin-activatable fibrinolysis inhibitor (TAFI). Activated TAFI removes the carboxy-terminal lysine residues of fibrin polymers, thereby reducing plasminogen binding sites and inhibiting fibrinolysis.[6]

With the heightened role of factor XI in thrombus formation in mind, recent studies suggest that high levels of factor XI are associated with venous thrombosis; however, the relationship between factor XI and arterial vascular disease has yet to be firmly established.[7-9] Two studies have demonstrated an association between elevated factor XI and coronary artery disease, but, to our knowledge, no studies have investigated the relationship of factor XI levels in stroke.[10,11] It is interesting that elevated TAFI levels have been associated with

ischemic stroke.[12-14] We describe an increased prevalence of elevated factor XI activity levels in a group of patients primarily presenting with stroke symptoms.

Materials and Methods

Patients and Reference Populations

We included 95 patients younger than 55 years with normal prothrombin (PT) and partial thromboplastin times (PTT) undergoing consultative evaluation for a hypercoagulable state. Clinical history provided as a part of the evaluation was used to divide the patients into those with stroke, transient ischemic attack (TIA), or venous thrombosis. The time frame from onset of symptoms to the time of blood sample submission was not available. A group of 40 healthy subjects, age and sex matched to the patient population, was used to determine the upper limit of normal for factor XI activity, as defined by the 95th percentile value.

Laboratory Studies

Samples were obtained and stored before analysis according to guidelines previously described.[15] Factor XI activity was measured by a mechanical clot-based method on an STA-R instrument (Diagnostica Stago, Asnieres, France) using factor XI–deficient plasma and modified PTT. Five point calibration curves with a normal and a low control sample within the established range were performed before each run. Factor XI activity levels were deemed valid if results from 2 dilutions agreed within 10%. Factor XI antigen levels were determined by enzyme-linked immunosorbent assay (Affinity Biologicals, Ancaster, Canada). Serum high-sensitivity C-reactive protein (hs-CRP) was determined by automated particle-enhanced immunoturbidimetric assay performed on a Roche/Hitachi model P800 analyzer (Roche Diagnostics, Indianapolis, IN).

Data and Statistical Analysis

Analysis of variance and a test of multiple proportions (the Holm test) were used to ensure that the reference population was age and sex matched with the test population. The contribution of elevated factor XI activity toward the development of stroke-TIA or venous thrombosis was analyzed by calculating odds ratios as an estimate of relative risk. A multiple logistic regression model was created to evaluate the contributed risk of antithrombin (<73%), functional protein C (<80%), free protein S (females, <50%; males, <64%), homocysteine (females, >1.35 mg/L [>10 µmol/L]; males, >1.62 mg/L [>12 µmol/L]), factor V Leiden (carrier), and prothrombin G20210A mutation (carrier) in populations with stroke or TIA and venous thrombosis using STATA 8 software (StataCorp, College Station, TX). Deming regression analysis of correlation between factor XI activity, factor XI antigen levels, and hs-CRP was performed on EP Evaluator Release 5 software (David G. Rhoads Associates, Kennett Square, PA).

Results

Of 95 patients younger than 55 years with normal PT and PTT values undergoing evaluation for a hypercoagulable condition, 65 had stroke, 13 had TIA, and 17 had venous thrombosis. The age and sex distributions for all patient groups are given in **Table 1**. The analysis of variance and Holm tests demonstrated no significant differences in the ages or male/female ratios of the reference population and the test populations. Despite having normal PT and PTT values, 1 patient in the stroke-TIA group and 1 in the venous thrombosis group were receiving warfarin, 3 patients in the stroke-TIA group and 2 in the venous thrombosis group were receiving heparin, and 4 in the venous thrombosis group were receiving heparin and warfarin.

Factor XI activity in the reference group ranged from 57% to 155% (0.57-1.55) with mean and median levels of 101% (1.01) and 100% (1.00), respectively **Table 2**. The 95th percentile value was 141% (1.41). In the 78 patients with stroke or TIA, factor XI activity ranged from 55% to 675% (0.55-6.75) with mean and median values of 138% (1.38) and 118% (1.18), respectively. Of these 78 patients, 17 (22%) had values higher than the 95th percentile of the reference population ($P < .05$). In the 17 patients with venous thrombosis, factor XI activity ranged from 71% to 196% (0.71-1.96) with mean and median values of 111% (1.11) and 113% (1.13), respectively. Of these 17 patients, 3 (18%) had values higher

Table 1
Age and Sex Distribution of the Populations Evaluated*

	Age (y)			
Factor XI Activity	**Mean ± SD**	**Median**	**Range**	**M/F Ratio**
Reference group (n = 40)	39 ± 9	41	23-55	15:25
Stroke or TIA (n = 78)	42 ± 8	44	20-55	35:43
Venous thrombosis (n = 17)	38 ± 11	37	20-54	7:10

* Age and sex distribution of reference subjects and the test subjects with stroke or transient ischemic attack (TIA) and venous thrombosis in which analysis of variance and the Holm test demonstrated no significant difference in age or sex distribution in the 3 groups ($P > .05$).

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than the 95th percentile of the reference population ($P < .05$). From these data, the unadjusted odds ratios for stroke or TIA and venous thrombosis in patients with factor XI activity higher than the 95th percentile of the reference population are 5.3 and 4.1, respectively.

Alternatively, by using the 98th percentile value of factor XI activity from the reference population (151% [1.51]) as the upper limit of normal, 8 (10%) of 78 patients with stroke or TIA and 3 (18%) of 17 patients with venous thrombosis had elevated activity levels. The odds ratios for stroke or TIA and venous thrombosis calculated from these data are 4.5 and 4.1, respectively.

Of 6 known risk factors for thrombosis that were analyzed (decreased antithrombin activity, decreased protein C activity, decreased free protein S antigen level, elevated homocysteine level, factor V Leiden carrier, and prothrombin G20210A carrier), a higher proportion of patients with venous thrombosis were positive for all factors than were those with stroke or TIA **Table 3**. In the multivariate analysis, none of the factors significantly contributed to risk for stroke or TIA, and only elevated homocysteine level and carrier status for the prothrombin G20210A mutation were significant risk contributors for venous thrombosis.

Previous studies have measured factor XI by immunoassay as antigenic levels or by enzymatic activity levels, but how one relates to the other has, to our knowledge, yet to be assessed. We found that correlation between factor XI activity and factor XI antigen levels by regression analysis demonstrated a correlation coefficient (R) of 0.667 with a slope of 1.27 **Figure 1**. The Bland-Altman plot of the data, in which the difference between factor XI activity and factor XI antigen level is plotted against factor XI activity, showed a negative mean bias of –12.4%, and the 95% limits of agreement (1.96 SD of the differences) were –62.0% to 37.2%. The scatter on the Bland-Altman plot is distributed randomly, without signs of systematic error.

To establish that factor XI is not an acute phase reactant, we studied the correlation between the acute phase reactant hs-CRP and factor XI activity or factor XI antigen levels in all the patients. Deming regression analysis showed a lack of correlation with correlation coefficients of –0.003 and 0.096 for factor XI activity and factor XI antigen, respectively (data not shown).

Discussion

Stroke is the third leading cause of death in the United States and a major cause of serious, long-term disability among adults.[16] As the US population continues to age, stroke hospitalization

Table 2
Factor XI Activity Levels in Reference Subjects, Patients With Stroke or TIA, and Patients With Venous Thrombosis

	Reference Subjects	Patients With Stroke or TIA	Unadjusted Odds Ratio for Stroke or TIA (95% CI)	Patients With Venous Thrombosis	Unadjusted Odds Ratio for Venous Thrombosis (95% CI)
Factor XI activity (%)					
Mean ± SD	101 ± 23 (1.01 ± 0.23)	138 ± 112 (1.38 ± 1.12)	—	111 ± 36 (1.11 ± 0.36)	—
Median	100 (1.00)	118 (1.18)	—	113 (1.13)	—
Range	57-155 (0.57-1.55)	55-675 (0.55-6.75)	—	71-196 (0.71-1.96)	—
95th percentile	141 (1.41)	—	—	—	—
No. of cases above 95th percentile	2/40 (5%)	17/78 (22%)	5.3 (1.2-24.1)	3/17 (18%)	4.1 (0.6-27.0)

CI, confidence interval; TIA, transient ischemic attack.

Table 3
Multivariate Analysis of Additional Thrombotic Risk Factors in Subjects With Stroke or TIA and Venous Thrombosis*

	Stroke or TIA (n = 78)		Venous Thrombosis (n = 17)	
Thrombotic Risk Factor	**No. (%) of Cases**	***P***	**No. (%) of Cases**	***P***
Antithrombin, <73%	2 (3)	>.05	2 (12)	>.05
Functional protein C, <80%	1 (1)	>.05	2 (12)	>.05
Free protein S, female, <50%; male, <64%	3 (4)	>.05	2 (12)	>.05
Homocysteine, female, >1.35 mg/L (>10 µmol/L); male, >1.62 mg/L (>12 µmol/L)	9 (12)	>.05	11 (65)	**<.05**
Factor V Leiden carrier†	1 (1)	>.05	3 (18)	>.05
Prothrombin G20210A carrier†	0 (0)	UC	4 (24)	**<.05**

TIA, transient ischemic attack; UC, unable to calculate.
* Additional thrombosis risk factors were measured in patient samples by functional assay (antithrombin, protein C), immunoassay (free protein S, homocysteine level), or DNA assay (factor V Leiden, prothrombin G20210A). Bold *P* values indicate statistically significant risk factors.
† All patients were heterozygous carriers.

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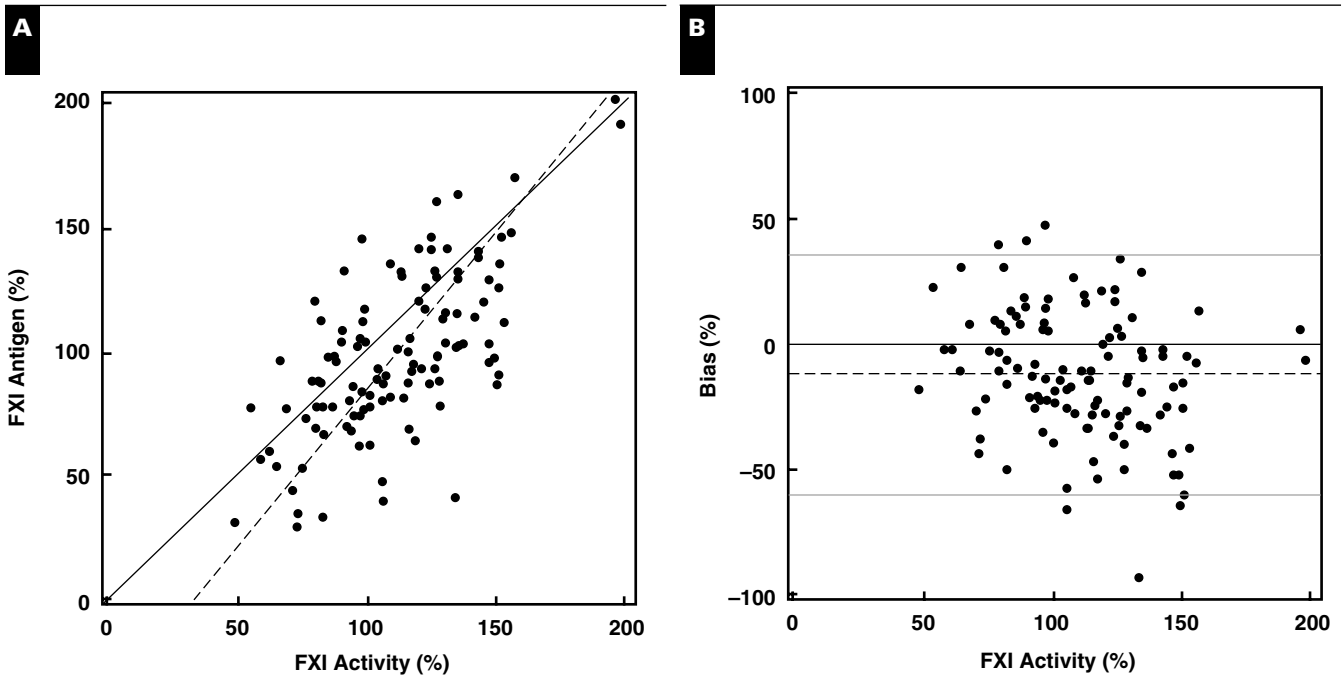

Figure 1 Correlation of FXI antigen and FXI activity levels displayed as a scatter plot (**A**) and Bland-Altman plot (**B**). By Deming regression analysis of the scatter plot, the slope is 1.27; the intercept, –42.1%; and the correlation coefficient (*R*) = 0.667. The Bland-Altman plot shows a negative mean bias (bias = FXI activity minus FXI antigen level) of –12.4% and the 95% limits of agreement (± 1.96 SD of the bias) at –62.0% and 37.2%. These results indicate a fair degree of correlation between the variables with antigen levels more frequently higher than activity levels and a wide range of random error. **A**, Dashed line, Deming regression; dotted line, 1:1 line. **B**, Dashed line, mean bias; solid line, ± 1.96 SD. FXI, factor XI.

rates and the proportion of persons discharged to skilled nursing facilities might increase, and, already, stroke is among the fastest growing expenses for Medicare.[17] A large part of reducing the burden of stroke will be via primary prevention achieved through the identification of at-risk populations.

In the present study, levels of factor XI activity higher than the 95th percentile of healthy individuals were associated with an increased odds ratio of 5.3 for stroke in a population of patients younger than 55 years. Use of an age- and sex-matched reference population deemed this effect independent of age and sex, and multivariate analysis demonstrated that this risk also was independent of antithrombin deficiency, protein C or protein S deficiency, elevated serum homocysteine level, factor V Leiden mutation, and prothrombin G20210A mutation. In addition, high factor XI levels did not correlate with hs-CRP levels, indicating that the high levels were not likely secondary to factor XI being an acute phase reactant.

Previous studies identifying elevated factor XI as a risk factor for venous thrombosis or cardiovascular disease used antigenic or functional factor XI assays.[7-11] Our results show a fair degree of correlation between factor XI activity and antigen levels (R = 0.667; slope, 1.27) by Deming regression analysis, suggesting that increased activity is related to a quantitative increase in the factor XI protein rather than increased enzymatic activity. The negative mean bias of –12.4% indicates that antigen levels were more frequently higher than activity levels, and the pattern of random scatter on the Bland-Altman plot indicates a lack of systematic error. These findings are likely due to increased sensitivity of the functional assay to random preanalytic sources of error such as sample deterioration. Because factor XI activity seems dependent on antigen levels, it could be argued that measuring factor XI antigen may be a more sensitive and robust method.

A small proportion (18%) of patients included in this study had a diagnosis of venous thrombosis, and our analysis supports previous reports that elevated factor XI is associated with venous thrombosis.[7] Multivariate analysis showed that elevated homocysteine levels and the prothrombin G20210A mutation were significant risk contributors for venous thrombosis. Likely owing to the small sample, the other known venous thrombotic risk factors did not reach statistical significance in the multivariate model.

The present study provides evidence that elevated factor XI activity is associated with stroke. Ischemic stroke often is due to large artery atherothromboembolism, a process that begins with acute arterial thrombosis developing on the surface of a ruptured atheromatous plaque or as a consequence of endothelial cell erosion. The same process of acute arterial

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thrombosis may lead to myocardial ischemia, and the relationship between stroke and coronary artery disease has been well established.[18] The fact that elevated factor XI also has been associated with coronary heart disease[10,11] supports our findings and suggests that elevated factor XI levels may have a role in promoting acute arterial thrombosis.

A limitation of this study lies in the fact that stroke is a heterogeneous disease composed of ischemic stroke secondary to arterial occlusion (≈80%), primary intracerebral hemorrhage (≈15%), and subarachnoid hemorrhage (≈5%).[19] Within the ischemic stroke category, in the white population, approximately 50% of cases are due to atherothromboembolism, 25% to intracranial small artery disease, 20% to embolic events from the heart, and 5% to other rare causes.[19] Without the availability of detailed clinical diagnoses in this study, we can only speculate that elevated factor XI is associated with ischemic atherothromboembolic stroke. However, for the following reasons, the likelihood of the other sources of stroke is much lower. First, atrial fibrillation accounts for many of the embolic cardiac events, but, by selecting a population younger than 55 years, we biased against this risk. Second, venous thromboembolism may account for a number of the strokes in our series; however, their proportion is likely small because the likelihood of paradoxical embolism through a patent foramen ovale is thought to be low.[20] In addition, the results of our multivariate analysis do not support this concept through clear demonstration of a lack of significant contribution of venous thrombosis risk factors in the stroke-TIA population. Lack of detailed clinical information about our study patients also precluded evaluation of conventional stroke risk factors such as cigarette smoking, diabetes, and obesity. Adjustment for these factors in the multivariate analysis likely would have lowered the odds ratio for stroke.

This retrospective analysis reveals a previously undescribed association between elevated factor XI activity and stroke. Further prospective studies of this relationship are warranted and may be important for identifying at-risk populations who could benefit from primary prevention.

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