Elevated Factor IX Activity Is Associated With an Increased Odds Ratio for Both Arterial and Venous Thrombotic Events

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ABSTRACT

Objectives: Elevations of factor IX (FIX) are thought to contribute to thrombotic risk, but this has not been well characterized. We retrospectively sought to determine whether elevated FIX levels are a risk factor for thrombosis in 81 adult subjects younger than 65 years (mean, 47 years) who were referred for evaluation of a hypercoagulable state.

Methods: Patients were classified by arterial transient ischemic attack/stroke (TIA/stroke, n = 62) or venous thromboembolism (VTE, n = 19) events. FIX activity testing was performed on all 81 subjects and a reference group of 40 healthy individuals.

Results: Thirteen (21%) of 62 subjects with TIA/stroke and 5 (26%) of 19 subjects with VTE had elevated FIX activity. Odds ratios for TIA/stroke and VTE in subjects with elevated FIX activity were 3.7 (95% confidence interval [CI], 0.76-17.65) and 6.8 (95% CI, 1.18-39.07), respectively.

Conclusions: Our findings suggest an association between elevated FIX levels and both arterial and venous thrombotic events.

Coagulation factor IX (FIX) is a vitamin K–dependent clotting factor that plays an essential role in the intrinsic pathway of blood coagulation, as shown by the bleeding tendency associated with congenital FIX deficiency (hemophilia B). FIX is activated by the tissue factor—either factor VIIa complex or factor XIa—and plays a key role in thrombin generation by generating factor Xa on the platelet surface, leading to prothrombinase complex assembly and subsequent fibrin formation. Previous studies have suggested that high FIX levels are associated with increased risk of venous thromboembolism (VTE), including deep vein thrombosis (DVT) and pulmonary embolism, and possibly arterial thrombosis. Anticoagulants that lower FIX levels are effective in the prevention of venous and arterial thrombosis.1

Thorough assessment of modifiable thrombotic risk factors in patients with thrombotic events is essential, and
laboratory assessment of inherited thrombotic risk factors may contribute to appropriate patient management and yield useful information for family members. Many risk factors, such as activated protein C (APC) resistance caused by the factor V Leiden mutation (FVL); the prothrombin G20210A mutation (PT20210); hyperhomocysteinemia; and deficiencies of protein C, protein S, and antithrombin have been well characterized and may be included in the laboratory workup of hypercoagulable states. Other potential risk factors are less well defined. For example, elevations of coagulation factors V, VII, VIII, IX, XI, XIII, and von Willebrand factor have been associated with an increased risk of thrombotic events, and elevations of factors VIII, IX, and XI have been identified as inherited traits in families with thrombophilia. However, information on these disorders is limited, and laboratory measurements are not routinely performed as part of thrombophilia evaluations. FIX elevations are among the least characterized of these analytes.

Although there is some evidence that elevated FIX levels contribute to VTE risk, the relationship between FIX and arterial thrombosis is not firmly characterized. FIX activation peptide has been shown to predict acute coronary syndromes, and such activation may play a role in coronary thrombogenesis, not only by continuous thrombin and fibrin generation but also through inhibition of endogenous fibrinolysis via activation of thrombin-activable fibrinolysis inhibitor. Interestingly, elevated thrombin-activable fibrinolysis inhibitor has also been associated with ischemic stroke. To our knowledge, no studies have investigated the relationship between FIX levels and ischemic stroke or transient ischemic attack (TIA). This retrospective study evaluates whether evaluated FIX levels are a risk factor for thrombosis in a group of patients primarily exhibiting stroke symptoms and a subset of patients who also had VTE. Secondary aims included determining the degree of correlation between FIX activity and age and between FIX activity and antigen assays and determining whether FIX is an acute-phase reactant.

Materials and Methods

Subjects and Reference Population

Eighty-one adult subjects younger than 65 years were included in the study. Age 65 years was chosen as a cutoff to minimize the potential influence of atrial fibrillation or malignancy as risk factors for thrombotic events. All subjects were treated for TIA, ischemic stroke, or VTE at Avera McKennan Hospital in Sioux Falls, SD, and underwent testing for thrombotic risk factors at ARUP Laboratories in Salt Lake City, UT. Diagnoses (type of event) were obtained through retrospective chart review by one of the authors (K.K.M.). To exclude the influence of anticoagulant drugs such as warfarin and heparin on FIX activity, only subjects with normal prothrombin time (PT) and activated partial thromboplastin time (aPTT) were included in the study. The study was performed with local institutional review board approval.

A group of 40 healthy subjects was used as a reference group to determine the upper limit of normal for FIX activity and antigen, defined as the 95th percentile of the values found in healthy subjects.

Laboratory Studies

The panel ordered for clinical testing in all subjects included tests for APC resistance with reflex of abnormal results to FVL mutation testing with polymerase chain reaction (PCR), PCR for the PT20210 mutation, homocysteine measurement, and factor VIII activity. Tests for less common thrombotic risk factors, such as inherited protein C, protein S, and antithrombin deficiencies, were not performed. PT and aPTT were measured at the time of clinical testing and were used to apply inclusion criteria. Additional testing was performed for FIX activity and antigen levels on surplus samples that had been stored frozen at –70ºC until the time of testing. FIX activity testing was performed on platelet-poor plasma from all 81 patients using a partial thromboplastin time–based (STA PTT-A, Diagnostica Stago, Asnieres, France) mechanical clotting method on a STA-R instrument (Diagnostic Stago) and FIX-deficient plasma. FIX antigen testing was performed on a subset of 50 patients using enzyme-linked immunosorbent assay (Diagnostic Stago). Results for FIX activity and antigen were expressed as percentage of normal. High-sensitivity C-reactive protein testing was performed on a subset of 51 patients with available serum samples using an immunoturbidimetric method on a Roche/Hitachi P800 analyzer (Roche Diagnostics, Indianapolis, IN).

Data and Statistical Analysis

Correlation between variables was assessed by calculating the Spearman ρ. Estimates of optimal prediction thresholds were determined using receiver operator characteristic (ROC) curves. The contribution of elevated FIX activity toward the development of TIA/stroke or VTE was analyzed by calculating odds ratios (ORs) as derived from logistic regression. P values less than .05 were considered statistically significant. All statistical analyses were performed using SAS software, version 9.1 (SAS Institute, Cary, NC).

Results

Eighty-one subjects were included in the study. Of these, 62 had arterial events, either TIA (n = 26) or stroke (n = 36),
and 19 had VTE, including 10 with DVT and 9 with pulmonary embolism. Subject characteristics, such as age and sex, are presented in Table II. The mean age of all study subjects was 47 years (standard deviation [SD], 11; range, 19-64 years; 59% male), and the mean age for the reference group was 31 years (SD, 9; range, 20-55 years; 48% male). Although the reference group was younger on average than the study subjects, there was no correlation between age and FIX activity, allowing us to compare FIX values between the two groups.

Figure 1. No significant sex differences were seen between the study and reference groups.

FIX activity in the reference group ranged from 87% to 176%, with mean and median values of 133% (SD = 20) and 132%, respectively. The 95th percentile value of the reference group (169%) was used to define elevated FIX activity. Two of 40 normal subjects had FIX values that would be defined as elevated. In the 62 subjects with arterial events, FIX activity ranged from 90% to 249%, with mean and median values of 152% (SD = 29) and 153%, respectively. Of these 62 subjects, 13 (21%) had values higher than the 95th percentile of the reference group. In 19 patients with VTE, FIX activity ranged from 111% to 222%, with mean and median values of 164% (SD = 28) and 160%, respectively. Of these 19 subjects, 5 (26%) had values higher than the 95th percentile of the reference group. From these data, the unadjusted ORs for TIA/stroke and VTE in subjects with elevated FIX activity were 3.7 (95% confidence interval [CI], 0.76-17.65) and 6.8 (95% CI, 1.18-39.07), respectively. FIX was also evaluated as a continuous variable. For each 1% increase in FIX activity, the increased percent risk for TIA/cerebrovascular accidents (CVA) was 3.0% (95% CI, 1.1%-5.0%) and the increased percent risk for VTE was 5.8% (95% CI, 2.5%-9.3%). Table II summarizes FIX activity results.

An ROC curve was constructed to determine which FIX activity value best predicts TIA/stroke. The optimal cutoff was determined to be 139%, with values more than 139% having 73% sensitivity, 70% specificity, and area under the curve (AUC) of 0.7. The ROC curve for FIX activity to predict VTE demonstrated an optimal cutoff value of 154%, with 79% sensitivity, 85% specificity, and AUC of 0.8.

Previous studies have measured FIX antigen using an immunoassay or measurement of FIX activity (as in our study). However, the correlation between methods is not well evaluated. We tested FIX antigen using an enzyme-linked immunosorbent assay in a subset of 50 subjects and compared FIX antigen and activity values. Antigen values were consistently lower than activity values with means of 96% and 158%, respectively, and area under the curve (AUC) of 0.7. The ROC curve for FIX activity to predict VTE demonstrated an optimal cutoff value of

### Table I

<table>
<thead>
<tr>
<th>Age and Gender Characteristics of Study Subjects</th>
<th>Age, y</th>
<th>Mean ± SD</th>
<th>Median</th>
<th>Range</th>
<th>Males (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TIA/stroke (n = 62)</td>
<td>48 ± 10</td>
<td>49</td>
<td>9-63</td>
<td>56</td>
<td></td>
</tr>
<tr>
<td>VTE (n = 19)</td>
<td>46 ± 13</td>
<td>51</td>
<td>24-64</td>
<td>68</td>
<td></td>
</tr>
<tr>
<td>All subjects (n = 81)</td>
<td>47 ± 11</td>
<td>49</td>
<td>19-64</td>
<td>59</td>
<td></td>
</tr>
</tbody>
</table>

TIA, transient ischemic attack; VTE, venous thromboembolism.

### Table II

<table>
<thead>
<tr>
<th>Factor IX Activity Values in Reference and Study Subjects</th>
<th>Reference Subjects</th>
<th>TIA/Stroke</th>
<th>Unadjusted OR for TIA/Stroke (95% CI = 0.76-17.65)</th>
<th>VTE</th>
<th>Unadjusted OR for VTE (95% CI = 1.18-39.07)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>40</td>
<td>62</td>
<td>-</td>
<td>19</td>
<td>-</td>
</tr>
<tr>
<td>Factor IX activity (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>133 ± 20</td>
<td>152 ± 29</td>
<td>-</td>
<td>164 ± 28</td>
<td>-</td>
</tr>
<tr>
<td>Median</td>
<td>132</td>
<td>153</td>
<td>-</td>
<td>160</td>
<td>-</td>
</tr>
<tr>
<td>Range</td>
<td>87-176</td>
<td>90-249</td>
<td>-</td>
<td>111-222</td>
<td>-</td>
</tr>
<tr>
<td>No. of cases above 95th percentile</td>
<td>2 (5)</td>
<td>13 (21)</td>
<td>3.7a</td>
<td>5 (26)</td>
<td>6.8a</td>
</tr>
</tbody>
</table>

CI, confidence interval; OR, odds ratio; TIA, transient ischemic attack; VTE, venous thromboembolism.

* For each 1% increase in factor IX activity, the increased percent risk for TIA/cerebrovascular events was 3.0% (95% CI, 1.1%-5.0%) and the increased percent risk for VTE was 5.8% (95% CI, 2.5%-9.3%).
Regression analysis demonstrated relatively good correlation between antigen and activity with a correlation coefficient ($R$) of 0.760. Figure 4 demonstrates this relationship.

Because our study population presented with thrombotic events, it is important to determine whether FIX is an acute-phase reactant that could be responsible for higher FIX values in study subjects compared with healthy controls. We studied the relationship between the acute-phase reactant high-sensitivity C-reactive protein and FIX activity in a subset of 51 patients and found a lack of strong correlation, with an $R$ value of 0.398. Figure 5 illustrates this finding.

Evaluation of the contributed risk of common thrombotic risk factors, including APC resistance/FVL mutation, the PT20210 mutation, and elevated homocysteine, showed that few subjects carried the FVL and PT20210 mutations. Only five (8.1%) of 62 subjects with TIA/stroke demonstrated APC resistance and were heterozygous for FVL (no homozygotes were identified), and two (3.2%) of 62 subjects were heterozygous for the PT20210 mutation. There were no compound heterozygotes positive for both mutations. One (5.3%) of 19 subjects with VTE demonstrated APC resistance and was homozygous for FVL, and no subjects carried the prothrombin G20210A mutation. Elevated homocysteine was a frequent finding: 20 (24.7%) of 81 total subjects, 14 (22.6%) of 62 patients with TIA/stroke, and six (31.6%) of 19 patients with VTE had elevated homocysteine, which was identified as a significant risk factor for both venous and arterial thrombosis ($P < .05$). Because factor VIII is a well-established acute-phase reactant and because the time frame between thrombotic event and blood draw was not precisely known in every subject, factor VIII values performed as part of the clinical testing panel were not considered in the analysis.

Figure 2 shows the receiver operating characteristic curve constructed to determine which factor IX activity value best predicts transient ischemic attack/stroke, showing maximum efficiency cutoff at 139% (73% sensitivity, 70% specificity, and area under the curve of 0.7030).

Figure 3 shows the receiver operating characteristic curve constructed to determine which factor IX activity value best predicts venous thromboembolism, showing an optimal cutoff value of 154% (79% sensitivity, 85% specificity, and area under the curve of 0.8112).

Figure 4 illustrates the correlation between factor IX (FIX) activity and antigen on a subset of 50 samples, demonstrating good correlation with an $R$ value of 0.760.

Figure 5 shows the correlation between factor IX activity and high-sensitivity C-reactive protein (hs-CRP) as an acute-phase reactant in a subset of 51 samples, showing no strong correlation with an $R$ value of 0.398.
Discussion

High plasma concentrations of factors II, VIII, IX, and XI have been associated primarily with increased risk of venous thrombosis, whereas elevated concentrations of factors V and VII and von Willebrand factor have been associated with increased risk of arterial thrombosis.1 Two primary case control studies5,6 published in 2000 evaluated FIX levels in VTE. In the first study of 426 patients with DVT, more than 20% of subjects with FIX antigen levels higher than 129 IU/dL (129% of normal) had a twofold increased risk of DVT (95% CI, 1.3-3.2) after adjusting for age, sex, oral contraceptive use, and high levels of other vitamin K–dependent clotting factors. They also found increased risk with increasing FIX levels and further increased risk when combined with increased factor VIII levels (eightfold increased risk).6 In the second study of 66 patients with VTE, FIX activity levels above 150% of normal were associated with a 2.34-fold increased risk (95% CI, 1.26-4.35) after adjusting for use of hormone therapy and other factors.5 A nested case-control study evaluated the association of coagulation factor values and VTE in 462 VTE cases and found an OR for elevated FIX of 1.4.13 This is in agreement with our finding of an unadjusted OR for venous thrombosis of 6.8 (95% CI, 1.18-39.07) in subjects with elevated FIX and 5.8% increased odds of VTE with each 1% incremental increase of FIX activity (95% CI, 2.5%-9.3%). High FIX levels and risk of recurrent VTE have also been studied on a very limited basis with conflicting results;7,16 In 2002, a College of American Pathologists consensus panel concluded that, although FIX elevations may be associated with an increased risk of VTE, routine laboratory measurements are not currently recommended because of limited data.2

As previously mentioned, very little is known about elevated FIX levels and the risk of arterial thrombosis. A previous prospective study investigated the activation of clotting factors IX and XI, activation of the contact coagulation factors, and the common pathway of coagulation in 50 patients with acute myocardial infarction (AMI), 50 patients with unstable angina, and 50 patients with stable angina. FIX peptide values were significantly higher in patients with AMI and unstable angina compared with patients with stable angina (P < .01), demonstrating activation of clotting factors IX and XI in patients with acute coronary syndromes.17 In our study, elevated FIX activity was associated with an increased OR of 3.7 for TIA/stroke. Although the 95% CI for this OR is wide and crosses 1, this likely represents a limitation of evaluating FIX as a dichotomous variable. When FIX was evaluated as a continuous variable, we found that for each 1% increase in FIX activity, the increased percent risk for TIA/CVA was 3.0% (95% CI, 1.1%-5.5%). This yielded a much narrower CI, which supported the significance of our findings. Lack of correlation of FIX values with age or sex suggests that the risk is independent of these variables. The previously identified relationship between elevated FIX and AMI supports our findings for TIA/stroke, providing additional evidence for elevated FIX as a risk factor in arterial thrombosis. Further identification of risk factors for arterial thrombosis may contribute to preventive efforts for these disorders, which represent major causes of morbidity and mortality.

In clinical laboratories, FIX levels are most commonly measured as FIX activity by clot-based assays, although both activity and antigenic methods are represented in the literature. Our results showed a good correlation between the activity and antigen assays, suggesting that increased FIX protein is present rather than gain of function. Either method appears to be a viable way to evaluate FIX in future studies. Our studies also show that FIX is not an acute-phase reactant.

In the present study, elevated homocysteine was the most common thrombotic risk factor identified and appeared to be a significant risk factor for both venous and arterial thrombosis. However, our study is limited by small numbers of subjects and the fact that the laboratory evaluation did not include all established thrombotic risk factors, precluding our ability to perform a thorough multivariate analysis. Because risk of thrombosis is thought to be cumulative or multiplicative in subjects with multiple risk factors, identification of additional thrombotic risk factors could provide useful information. In addition, our study used retrospective chart review and reference laboratory testing, which precluded comprehensive clinical information availability for our subjects; this could have elucidated the time frame of the samples relative to disease presentation and other risk factors such as obesity, smoking, and diabetes.

Although there is a growing body of evidence that elevations of coagulation factors contribute to thrombotic risk, many questions remain to be answered before such measurements become a routine component of thromboembolic risk evaluation with significant contribution to treatment decisions. Challenges include age-related differences in values for some coagulation factors, acute-phase reactions affecting certain factors, and imperfect laboratory methods of measurement. For example, proficiency testing surveys for factor assays frequently reveal between-laboratory coefficients of variation of at least 10% to 20% for measurement of samples with normal factor activities. Factor activities have historically been measured to detect bleeding disorders caused by deficiencies, rather than to detect elevations; the correlation between degree of deficiency and bleeding risk is fairly well established. However, this is not the case for factor elevations in which the optimal cutoffs to define elevations corresponding to thrombotic risk are currently unknown.

To the best of our knowledge, our study is one of the first to provide evidence that elevated FIX activity is associated with TIA/stroke. Prospective studies evaluating the
relationship between high FIX values and thrombotic events are needed to confirm the association and determine whether evaluation of FIX levels could contribute to preventive efforts or care of these patients.

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References