Thrombin activatable fibrinolysis inhibitor (TAFI) and the risk of recurrent venous thromboembolism

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Abstract

The impact of fibrinolysis for predicting the risk of recurrent venous thromboembolism (VTE) is low. We prospectively followed 600 patients with a first VTE, and evaluated the thrombin activatable fibrinolysis inhibitor (TAFI) as a risk factor of recurrence. A high TAFI (> 75th percentile of thrombosis patients) was associated with a 2-fold higher risk of recurrence compared with lower levels. The probability of recurrence two years after anticoagulation was 14.5% (95% CI 8.6%-20.4%) among patients with high TAFI and 6.8% (95% CI 4.3%-9.3%) among patients with lower levels (p=0.006). Our data also support the concept of a linkage between fibrinolysis and the coagulation system. Patients with high TAFI had significantly higher levels of factors XI, VIII and IX, and a high risk of recurrence was seen among patients with high TAFI and high levels of one of these factors. The relative risk (RR) of recurrence was highest among patients with high TAFI and high factor XI (2.9; 95% CI 1.3–6.9), high factor VIII (RR 6.5; 95% CI 2.9–14.8), or high factor IX (RR 2.0, 95% CI 1.0–3.9), compared with patients with low levels of TAFI and one of these factors.
Introduction

Venous thromboembolism (VTE) is a chronic and multicausal disease. Many factors that convey a high risk of recurrence including antithrombin deficiency, malignancy, the lupus anticoagulant or high factors VIII and IX, have been identified. In a prospective cohort study from Great Britain, rates of recurrent VTE were not related to the presence or absence of laboratory evidence of heritable thrombophilia (hazard ratio 1.50; 95% confidence interval 0.8-2.8). Data from the Austrian Study on Recurrent Venous Thromboembolism (AUREC) show that in 25% of the patients with recurrent VTE no thrombotic risk factors can be found (unpublished data). These findings suggest that thus far unknown risk factors for (recurrent) VTE still exist. On the other hand, many patients with VTE have more than one thrombophilic condition, and venous thrombosis must therefore be regarded as the consequence of interacting genetic and/or acquired risk factors. For some compound defects, such as double-heterozygosity of factor V Leiden and prothrombin G20210A or high factor VIII and high factor IX, an increased risk of recurrent VTE has been already demonstrated.

Thrombin activatable fibrinolysis inhibitor (TAFI) is a carboxypeptidase plasma zymogen that after being activated removes carboxy-terminal lysine residues of fibrin polymers thereby prohibiting the assembly of plasminogen and tissue plasminogen activator. For the activation of TAFI large amounts of thrombin are required. The amount of factor Xa generated by tissue factor-factor VIIa can only generate small quantities of thrombin because this pathway is quickly shut down by tissue factor pathway inhibitor. The small amounts of thrombin activate factor XI, thereby initiating the intrinsic pathway of the coagulation cascade. Ongoing coagulation activation finally generates - via positive feedback activation – those large amounts of thrombin.
which are required for the activation of TAFI.\textsuperscript{6,7} In this concept of coagulation activation, clot formation and the generation of large amounts of thrombin by factor XI play a central role thereby generating a linkage between activation of coagulation and fibrinolysis via factor XI.

In a prospective cohort study of 600 patients with a first spontaneous VTE, we evaluated TAFI as a risk factor for recurrence and investigated the interaction between the fibrinolytic and the coagulation system on the risk of recurrent VTE.

**Materials and Methods**

**Study population**

All patients who were enrolled in the Austrian Study on Recurrent Venous Thromboembolism (AUREC) were eligible for the present analysis. AUREC is an ongoing prospective multicenter cohort study with the aim to investigate risk factors of recurrent VTE.\textsuperscript{8,9} Between July 1992 and July 2003, 2105 consecutive patients with VTE older than 18 years, who had been treated with oral anticoagulants for at least three months were eligible for the present analysis. 1505 patients were excluded because of previous VTE, surgery, trauma or pregnancy within the previous 3 months; deficiency of a natural coagulation inhibitor, the lupus anticoagulant, cancer, or long-term antithrombotic treatment. The study was approved by the ethics committee of the Vienna University Hospital and the patients had to provide written informed consent before participation in the study.

All patients had received secondary thromboprophylaxis with oral anticoagulants for at least 3 months and entered the study at the time of
discontinuation of oral anticoagulant therapy. Patients were seen at three-month intervals during the first year and every 6 months thereafter. They received written information on the symptoms of VTE and were instructed to report if symptoms occurred.

**Diagnosis of VTE**

The diagnosis of deep vein thrombosis was established by a positive finding on venography or color duplex sonography (in case of proximal deep vein thrombosis of the leg). To be considered positive, the venograms had to meet at least one of the following direct or indirect criteria: a constant filling defect seen on two views; an abrupt discontinuation of the contrast filled vessel at a constant level of the vein; and the absence of filling in the entire deep vein system (without a compression), with or without venous flow through collateral veins. With color duplex ultrasonography, at least one of the two following 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 established either by a positive finding on ventilation-perfusion scanning\textsuperscript{10} or by spiral computed tomography revealing one or several low-attenuation areas that partly or completely fill the lumen of an opacified vessel.

**Study outcome**

The endpoint of the study was recurrent symptomatic deep vein thrombosis confirmed by venography or color duplex sonography (in case of proximal deep vein
thrombosis of the contralateral leg) or recurrent symptomatic pulmonary embolism confirmed by ventilation-perfusion scanning and/or spiral computed tomography according to the aforementioned criteria. Deep vein thrombosis was considered to have recurred if the patient had a thrombus in the leg or arm other than 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). The diagnosis was established by an adjudication committee consisting of independent clinicians and radiologists.

**Blood sampling and laboratory analysis**

In all patients, laboratory testing for the presence of thrombotic risk factors was performed three weeks after discontinuation of oral anticoagulants at the central laboratory. Venous blood was collected after fasting into 1/10 volume of 0.11 mmol/L trisodium citrate and centrifuged for 20 minutes at 2000 g. The plasma was stored at –80°C. Genomic DNA was isolated from leukocytes by standard methods.

TAFI antigen was measured in plasma by use of a commercially available enzyme linked immunoassay (Imuclone® TAFI ELISA, American Diagnostica, Greenwich, CT, USA).

Plasma levels of factor VIII and factor IX were measured by one stage clotting assays as previously described. Factor XI was measured by use of a one stage coagulometric assay using factor XI deficient plasma (Dade Behring, Marburg, Germany).
Antithrombin, protein C, protein S, factor VIII and the diagnosis of a lupus anticoagulant were determined as reported.\textsuperscript{9} Screening for factor V Leiden and for the prothrombin G20210A mutation was carried out as described.\textsuperscript{12,13}

**Statistical Analysis**

Times to recurrence (uncensored observations) or follow-up times in patients without recurrence (censored observations) were analyzed using survival time methods.\textsuperscript{14} The probability of recurrence was estimated according to Kaplan-Meier method.\textsuperscript{15} To test for homogeneity between strata, we applied the log-rank and the generalized Wilcoxon rank sum test.

For categorical analyses, factor VIII was dichotomised at a plasma level of 234 IU/dl (\(= 90^{\text{th}}\) percentile of thrombosis patients), factor IX was dichotomised at a plasma level of 138 IU/dl (\(= 75^{\text{th}}\) percentile of thrombosis patients) and factor XI at a plasma level of 133 IU/dl (\(= 90^{\text{th}}\) percentile of thrombosis patients).

Categorical data were checked for homogeneity using contingency table analyses (\(\chi^2\)-test). For all multivariate analyses, the data were adjusted for potential confounding thrombotic variables, such as age, sex, factor V Leiden, prothrombin G20210A and high factor VIII. Simple descriptive statistics were computed to provide a clear presentation of the data. SAS version 8.02 (SAS Institute, Cary, NC) was used for all analyses and \(p < 0.05\) was considered statistically significant.
Results

Patients

600 patients with a first spontaneous VTE were included at the time of discontinuation of oral anticoagulants. Of these patients, 182 left the study because of a diagnosis of cancer (12 patients) or of antithrombotic therapy for reasons other than VTE (137 patients); 23 (4%) patients were lost for follow-up; 10 patients died, but in none recurrent VTE was the cause of death. These patients were followed until the time of exclusion or death, when the data were censored.

TAFI and the risk of recurrent VTE

During a mean follow up of 45 months, 83 of 600 (14%) patients had recurrent VTE (57 deep vein thrombosis, 26 pulmonary embolism). Patients with recurrence had higher TAFI levels than patients without recurrence (100 ± 27 IU/dL and 96 ± 24 IU/dL, respectively, p=0.2). When TAFI was analyzed as a continuous variable in a Cox proportional-hazards model, the relative risk (RR) of recurrence was 1.1 [95% confidence interval (CI) 1.01 – 1.19] for each increase of 10 IU/dL in the TAFI level. After adjustment for potentially confounding variables (age, sex, factor V Leiden and prothrombin G20210A), TAFI remained an independent risk factor of recurrence [RR 1.06 (95% CI 1.0 – 1.16)].

We stratified patients into two groups according to their TAFI level (> and < 75th percentile of thrombosis patients). In our cohort, TAFI levels were significantly lower among women (95 ± 24 IU/dL) than among men (99 ± 24 IU/dL; p=0.02). Thus,
the 75th percentile was 114 IU/dL for men and 107 IU/dL for women. The characteristics of patients with high and low TAFI are shown in Table 1. The RR of recurrence was 1.7 (95% CI 1.1 – 2.7) among patients with TAFI ≥ 75th percentile compared with patients with lower levels. At two years, the cumulative probability of recurrence was 14.5% (95% CI 8.6% - 20.4%) among patients with high TAFI compared with 6.8% (95% CI 4.3% - 9.3%) among those with lower levels (p=0.006 Wilcoxon rank sum test).

Interaction of TAFI and intrinsic coagulation factors

Factor XI is regarded to play a pivotal role for generating the amounts of thrombin needed to activate TAFI. We therefore investigated the effect of factor XI on the risk of recurrent VTE among patients with high (≥ 75th percentile) and low TAFI (< 75th percentile). The levels of factor XI were significantly higher among patients with high TAFI compared with those with lower levels (Table 1). Compared with the reference group (patients with TAFI < 75th percentile and factor XI < 90th percentile), the RR of recurrence was 1.6 (95% CI 0.9 – 2.6) and 1.4 (95% CI 0.6 – 3.3) among patients with TAFI ≥ 75th percentile and factor XI < 90th percentile, and TAFI < 75th percentile and factor XI ≥ 90th percentile, respectively. The RR of recurrence was highest (3.0; 95% CI 1.3 – 6.9) among patients with both high TAFI and high factor XI (Table 2). Adjustment for potential confounding variables, including age, sex, factor V Leiden, prothrombin G20210A, and high factor VIII did not substantially influence the result.

We next assessed the relationship between TAFI and other factors of the intrinsic coagulation system, i.e. factor VIII and factor IX, with regard to the risk of recurrence. As shown in Table 1, patients with high TAFI (≥ 75th percentile) had
significantly higher levels of factor VIII and factor IX compared with patients with lower levels (Table 1). In Table 3 and 4 the effect of high factor VIII (> 90th percentile of thrombosis patients) or high factor IX (> 75th percentile of thrombosis patients), respectively, and high TAFI on the RR of recurrence is shown. Compared to the reference group (patients with low TAFI and low factor VIII), the risk of recurrence was 3-fold higher among patients with low TAFI and high factor VIII (RR 2.9; 95% CI 1.4 – 6.2) and was more than 6-fold higher among patients with both high TAFI and high factor VIII (RR 6.5; 95% CI 2.9 – 14.8) after adjustment for age, sex, factor V Leiden and prothrombin G20210 A.

Similarly, a higher risk of recurrent VTE was found in patients with both high TAFI and high factor IX (RR 2.0, 95% CI 1.0 – 3.9) compared with patients with low TAFI and low factor IX (Table 4).

**Discussion**

The impact of defects in the fibrinolytic system for predicting the risk of recurrent VTE has been regarded as low. In a report of the DURAC investigators, increased levels of tissue plasminogen activator antigen or plasminogen activator inhibitor-1 only weakly correlated with the development of a future VTE.\(^6\) In a subsequent study by Crowther et al., no systematic differences in the levels of tissue plasminogen activator antigen and functional plasminogen activator inhibitor-1 were found between patients with or without recurrent VTE.\(^7\) Our study is the first to show a relationship between an impaired fibrinolysis and the risk of recurrent VTE. In 600 patients with a first spontaneous VTE, a TAFI level above the 75th percentile of thrombosis patients was associated with an almost 2-fold higher RR of recurrence.
compared with patients with lower levels. The probability of recurrent VTE two years after discontinuation of secondary thromboprophylaxis was 14.5% in patients with high TAFI and 6.8% in patients with lower levels.

Our data also support the concept of a link between the coagulation and the fibrinolytic systems. For the activation of TAFI, large amounts of thrombin are needed. Thrombin generation via the tissue factor-factor VIIa pathway is quickly blocked by the effects of tissue factor pathway inhibitor. Subsequently, the generation of larger amounts of thrombin required for clot formation and TAFI activation is enhanced by (positive feedback) activation of factor XI. This activation of TAFI can be regarded as a consequence of factor XI activity. Patients with both high TAFI and high factor XI had a 3-fold increased risk of recurrence compared with patients with lower levels and this high risk of recurrence was independent of other thrombotic variables including high factor VIII. We therefore hypothesize that the enhanced risk of recurrence among patients with high TAFI and high factor XI reflects the association between the fibrinolytic system (TAFI) and the coagulation system (factor XI).

The present study provides evidence of a relationship between TAFI, factor VIII and factor IX and an increased risk of recurrence. A synergistic effect between TAFI and high factor VIII on the risk of a first venous thrombosis has been reported by van Tilburg et al., but the association between TAFI and other intrinsic coagulation factors has not been studied either in patients with a first or recurrent venous thrombosis. We have previously shown that high factor VIII is a major risk factor of recurrent VTE and that the risk of recurrence among patients with high factor VIII is further enhanced by high factor IX. In the present analysis, patients with high factor VIII had a substantially greater risk of recurrence than patients with lower levels regardless of the TAFI levels. However, the risk of recurrence was
highest (more than 6-fold) among patients with both high factor VIII and high TAFI. Likewise, a high risk of recurrence was found among patients with both high factor IX and high TAFI. Factor IX is activated by factor XI and it cannot be excluded that this effect of TAFI and factor IX on the risk of recurrence is - at least partially - contributable to factor XI activation. A high factor IX might also be genetically determined or the result of other activation pathways, in which case a factor XI-independent association between TAFI and factor IX must be assumed.

The findings of our study improve the stratification of patients with VTE with regard to their risk of recurrence. The relationship between the fibrinolytic and the coagulation system with regard to the risk of recurrence is a new aspect in the multicausal nature of VTE, and further studies are needed to elucidate the pathomechanisms which modulate the thrombotic phenotype.
References


7. Bajzar L. Thrombin activatable fibrinolysis inhibitor and an antifibrinolytic


Table 1. Base-Line Characteristics of the 600 Patients with high (≥75th percentile) and low (<75th percentile) TAFI

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Low TAFI (n=446)</th>
<th>High TAFI (N=154)</th>
<th>P - Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Females – no. (%)</td>
<td>251 (56%)</td>
<td>85 (55%)</td>
<td>ns</td>
</tr>
<tr>
<td>Age &lt; 45 yrs – no. (%)</td>
<td>199 (45%)</td>
<td>57 (37%)</td>
<td>0.06</td>
</tr>
<tr>
<td>Type of thromboembolism – no. (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>proximal leg veins</td>
<td>142 (32%)</td>
<td>64 (42%)</td>
<td>ns</td>
</tr>
<tr>
<td>distal leg veins</td>
<td>112 (25%)</td>
<td>24 (16%)</td>
<td>ns</td>
</tr>
<tr>
<td>axillary veins</td>
<td>21 (5%)</td>
<td>6 (4%)</td>
<td>ns</td>
</tr>
<tr>
<td>pulmonary embolism</td>
<td>171 (38%)</td>
<td>60 (39%)</td>
<td>ns</td>
</tr>
<tr>
<td>Factor V Leiden – no. (%)</td>
<td>130 (29%)</td>
<td>57 (37%)</td>
<td>0.05</td>
</tr>
<tr>
<td>Prothrombin G20210A – no. (%)</td>
<td>34 (8%)</td>
<td>15 (10%)</td>
<td>ns</td>
</tr>
<tr>
<td>Factor VIII (IU/dL) - mean ± SD</td>
<td>159 ± 48</td>
<td>179 ± 57</td>
<td>0.0001</td>
</tr>
<tr>
<td>Factor IX (IU/dL) - mean ± SD</td>
<td>123 ± 25</td>
<td>130 ± 28</td>
<td>0.01</td>
</tr>
<tr>
<td>Factor XI (IU/dL) - mean ± SD</td>
<td>102 ± 23</td>
<td>107 ± 23</td>
<td>0.03</td>
</tr>
</tbody>
</table>
**Table 2.** Relative Risk (RR) of recurrent VTE for categories of TAFI [< (= low) and ≥ (= high) 75th percentile] and factor XI [< (= low) and ≥ (= high) 90th percentile]

<table>
<thead>
<tr>
<th></th>
<th>No. of Patients (%)</th>
<th>RR univariate (95% CI)</th>
<th>RR multivariate* (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAFI low</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FXI low</td>
<td>405 (67)</td>
<td>1†</td>
<td>1†</td>
</tr>
<tr>
<td>TAFI low</td>
<td>40 (7)</td>
<td>1.4 (0.6-3.3)</td>
<td>1.4 (0.6-3.2)</td>
</tr>
<tr>
<td>FXI high</td>
<td>134 (22)</td>
<td>1.6 (0.9-2.6)</td>
<td>1.4 (0.8-2.3)</td>
</tr>
<tr>
<td>TAFI high</td>
<td>21 (4)</td>
<td>3.0 (1.3-6.9)</td>
<td>2.4 (1.03-5.8)</td>
</tr>
<tr>
<td>FXI high</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* adjusted for age, sex, factor V Leiden, prothrombin G20210A and high factor VIII

† reference group
Table 3. Relative Risk (RR) of recurrent VTE for categories of TAFI [< (= low) and > (= high) 75th percentile] and factor VIII [< (= low) and > (= high) 90th percentile]

<table>
<thead>
<tr>
<th>Category</th>
<th>No. of Patients (%)</th>
<th>RR univariate (95% CI)</th>
<th>RR multivariate 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAFI low, FVIII low</td>
<td>412 (69)</td>
<td>1†</td>
<td>1†</td>
</tr>
<tr>
<td>TAFI low, FVIII high</td>
<td>33 (6)</td>
<td>4.0 (1.9-8.1)</td>
<td>2.9 (1.4-6.2)</td>
</tr>
<tr>
<td>TAFI high, FVIII low</td>
<td>129 (21)</td>
<td>1.6 (0.9-2.7)</td>
<td>1.3 (0.8-2.3)</td>
</tr>
<tr>
<td>TAFI high, FVIII high</td>
<td>26 (4)</td>
<td>4.4 (2.1-9.3)</td>
<td>6.5 (2.9-14.8)</td>
</tr>
</tbody>
</table>

* adjusted for age, sex, factor V Leiden, and prothrombin G20210A
† reference group
Table 4. Relative Risk (RR) of recurrent VTE for categories of TAFI [< (= low) and > (= high) 75\(^{th}\) percentile] and factor IX [< (= low) and > (= high) 75\(^{th}\) percentile]

<table>
<thead>
<tr>
<th></th>
<th>No. of Patients (%)</th>
<th>RR univariate (95% CI)</th>
<th>RR multivariate* (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAFI low</td>
<td>338 (56)</td>
<td>1†</td>
<td>1†</td>
</tr>
<tr>
<td>FIX low</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TAFI low</td>
<td>108 (18)</td>
<td>1.7 (1.0-3.0)</td>
<td>1.4 (0.8-2.5)</td>
</tr>
<tr>
<td>FIX high</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TAFI high</td>
<td>103 (17)</td>
<td>1.6 (0.9-3.0)</td>
<td>1.5 (0.8-2.8)</td>
</tr>
<tr>
<td>FIX low</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TAFI high</td>
<td>51 (9)</td>
<td>2.7 (1.4-5.2)</td>
<td>2.0 (1.0-3.9)</td>
</tr>
<tr>
<td>FIX high</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* adjusted for age, sex, factor V Leiden, and prothrombin G20210A
† reference group
Legend to the Figure

**Figure 1: Kaplan-Meier method estimate of the risk of recurrent VTE according to the level of TAFI.** The probability of recurrent VTE after discontinuation of secondary thromboprophylaxis was significantly higher among patients with TAFI ≥ 75th percentile than among patients with lower levels (p=0.006 by the Wilcoxon rank sum test and p=0.02 by the log rank test).
No. of Patients at Risk

<table>
<thead>
<tr>
<th>TAFI ≥ 75th percentile</th>
<th>154</th>
<th>123</th>
<th>100</th>
<th>65</th>
<th>41</th>
<th>27</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAFI &lt; 75th percentile</td>
<td>446</td>
<td>387</td>
<td>331</td>
<td>261</td>
<td>195</td>
<td>139</td>
</tr>
</tbody>
</table>
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