Thrombin-activatable fibrinolysis inhibitor and the risk for recurrent venous thromboembolism

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The impact of fibrinolysis for predicting the risk for recurrent venous thromboembolism (VTE) is low. We prospectively followed up 600 patients with a first VTE and evaluated the thrombin-activatable fibrinolysis inhibitor (TAFI) as a risk factor for recurrence. A high TAFI level (75th or higher percentile in thrombosis patients) was associated with a 2-fold higher risk for recurrence compared with lower levels. The probability of recurrence 2 years after anticoagulation was 14.5% (95% confidence interval [CI], 8.6-20.4) among patients with high TAFI levels and 6.8% (95% CI, 4.3-9.3) among patients with lower levels (P = .006). Our data also support the concept of a linkage between fibrinolysis and the coagulation system. Patients with high TAFI levels had significantly higher levels of factors XI, VIII, and IX, and a high risk of recurrence was seen among patients with high TAFI levels and high levels of one of these factors. The relative risk (RR) for recurrence was highest among patients with high TAFI and high factor XI (RR, 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) levels compared with patients with low levels of TAFI and one of these factors. (Blood. 2004;103:3773-3776)

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Introduction

Venous thromboembolism (VTE) is a chronic and multicausal disease. Many factors that convey a high risk for recurrence, including antithrombin deficiency, malignancy, lupus anticoagulant, and 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 [CI], 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(AUREC) show that in 25% of the patients with recurrent VTE no thrombotic risk factors can be found (V. Pak, unpublished data, July 2003). 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 1 thrombophilic condition, and venous thrombosis must therefore be regarded as the consequence of interacting genetic 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 for recurrent VTE has already been demonstrated.

Thrombin-activatable fibrinolysis inhibitor (TAFI) is a carboxypeptidase plasma zymogen that, after being activated, removes carboxy-terminal lysine residues of fibrin polymers, 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 the tissue factor pathway inhibitor. Small amounts of thrombin activate factor XI, initiating the intrinsic pathway of the coagulation cascade. Ongoing coagulation activation finally generates, through positive feedback activation, those large amounts of thrombin required for the activation of TAFI. 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 coagulation activation and fibrinolysis through 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 for recurrent VTE.

Patients, materials, and methods

Study population

All patients who were enrolled in AUREC were eligible for the present analysis. AUREC is an ongoing, prospective, multicenter cohort study whose aim is to investigate risk factors for recurrent VTE. Between July 1992 and July 2003, 2105 consecutive patients with VTE who were older than 18 years and had been treated with oral anticoagulants for at least 3 months were eligible for the present analysis. 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, 1505 patients were excluded. The ethics committee of Vienna University Hospital approved the study, and 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 3-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). For findings to be considered positive, venograms had to show at least one of the following direct or indirect criteria: constant filling defect seen on 2 views; abrupt discontinuation of the contrast-filled vessel at a constant level of the vein; and absence of filling in the entire deep vein system (without a compression), with or without venous flow through collateral veins. With color duplex ultrasonography, either or both of the 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 by a positive finding on ventilation–perfusion imaging or by one or several low-attenuation areas, revealed by spiral computed tomography, that partially or completely filled the lumen of an opacified vessel.

**Study outcome**

The end point 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 imaging, spiral computed tomography, or both, according to the aforementioned criteria. Deep vein thrombosis was considered to have recurred if the patient had a thrombus in the leg or arm not affected by the previous thromboembolic event; a thrombus in another deep vein in the leg or arm affected by the previous event; or a thrombus in the same venous system affected in 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 3 weeks after the discontinuation of oral anticoagulant therapy. After patients fasted, venous blood was collected into 0.01 vol of 0.11 mM trisodium citrate and was centrifuged for 20 minutes at 2000 × g. The plasma was stored at −80°C. Genomic DNA was isolated from leukocytes using standard methods.

TAFI antigen was measured in plasma with the use of a commercially available enzyme-linked immunosassay (Imuclone TAFI ELISA; American Diagnostica, Greenwich, CT). Plasma levels of factor VIII and factor IX were measured by one-stage clotting assays, as previously described.4,11 Factor XI was measured with the use of a 1-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.9 Screening for factor V Leiden and for the prothrombin G20210A mutation was carried out as described.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.14 The probability of recurrence was estimated according to the Kaplan-Meier method.15 To test for homogeneity between strata, we applied the log-rank and the generalized Wilcoxon rank sum test.

**Results**

**Patients**

Six hundred 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 not of recurrent VTE. All patients were followed up until the time of exclusion or death, when the data were censored.

**TAFI and the risk for recurrent VTE**

During a mean follow-up of 45 months, 83 (14%) of 600 patients had recurrent VTE (57 deep vein thrombosis, 26 pulmonary embolism). Patients who experienced recurrence had higher TAFI levels than patients who did not (100 ± 27 IU/dL and 96 ± 24 IU/dL, respectively; P = .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% 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 for recurrence (RR, 1.06; 95% CI, 1.0-1.16).

We stratified patients into 2 groups according to TAFI level (for thrombosis patients, 75th or higher percentile and lower than 75th percentile). In our cohort, TAFI levels were significantly lower among women (95 ± 24 IU/dL) than among men (99 ± 24 IU/dL; P = .02). Thus, the 75th percentile was 114 IU/dL for men and 107 IU/dL for women. Characteristics of patients with high and low TAFI levels are shown in Table 1.

**Table 1. Baseline characteristics of 600 patients with high and low TAFI levels**

| Characteristic                  | Low TAFI (n = 446) | High TAFI (n = 154) | P      |
|--------------------------------|-------------------|---------------------|--------|
| **Women, no. (%)**            | 251 (56)          | 85 (55)             | NS     |
| Age younger than 45 y, no. (%)| 199 (45)          | 57 (37)             | .06    |
| **Type of thromboembolism, no. (%)** |                  |                     |        |
| Proximal leg veins             | 142 (32)          | 64 (42)             | NS     |
| Distal leg veins               | 112 (25)          | 24 (16)             | NS     |
| Auxiliary veins                | 21 (5)            | 6 (4)               | NS     |
| Pulmonary embolism             | 171 (38)          | 60 (39)             | NS     |
| Factor V Leiden, no. (%)       | 130 (29)          | 57 (37)             | .05    |
| Prothrombin G20210A, no. (%)   | 34 (8)            | 15 (10)             | NS     |
| **Factor VIII, IU/dL**         | 159 ± 48          | 179 ± 57            | .0001  |
| Factor IX, IU/dL               | 123 ± 25          | 130 ± 28            | .01    |
| Factor XI, IU/dL               | 102 ± 23          | 107 ± 23            | .03    |

High TAFI levels are those in the 75th or higher percentile; low TAFI levels are those lower than the 75th percentile. NS indicates not significant.

*Values are mean ± SD.
TAFI levels are shown in Table 1. The RR of recurrence was 1.7 (95% CI, 1.1-2.7) among patients with TAFI levels in the 75th or higher percentile compared with patients with levels at lower percentiles. At 2 years, the cumulative probability of recurrence was 14.5% (95% CI, 8.6-20.4) among patients with high TAFI levels compared with 6.8% (95% CI, 4.3-9.3) among those with lower levels (P = .006, Wilcoxon rank sum test; P = .02, log rank test).

**Interaction of TAFI and intrinsic coagulation factors**

Factor XI is thought to play a pivotal role in generating the amounts of thrombin needed to activate TAFI. We therefore investigated the effect of factor XI on the risk for recurrent VTE among patients with high (75th or higher percentile) and low TAFI (lower than 75th percentile). The levels of factor XI were significantly higher among patients with high TAFI compared than among those with lower levels (Table 1). Compared with the reference group (patients with TAFI levels lower than the 75th percentile and factor XI levels lower than the 90th percentile), the RR of recurrence was 1.6 (95% CI, 0.9-2.6) among patients with TAFI levels in the 75th or higher percentile and factor XI levels lower than the 90th percentile and 1.4 (95% CI, 0.6-3.3) among patients with TAFI levels lower than the 75th percentile and factor XI levels in the 90th or higher percentile. The RR of recurrence was highest (3.0; 95% CI, 1.3-6.9) among patients with high TAFI levels and high factor XI levels (90th or higher percentile). The levels of factor XI were significantly higher among patients with TAFI levels in the 75th or higher percentile compared than among those with lower levels (Table 1). In Tables 3 and 4, the effect of high factor VIII (higher than 90th percentile for thrombosis patients) or high factor IX (75th or higher percentile for thrombosis patients) and high TAFI levels on the RR for recurrence are shown. Compared with the reference group (patients with low TAFI and low factor VIII levels), the risk for recurrence was 3-fold higher among patients with low TAFI and high factor VIII levels (RR, 2.9; 95% CI, 1.4-6.2) and was more than 6-fold higher among patients with high TAFI and high factor VIII levels (RR, 6.5; 95% CI, 2.9-14.8) after adjustment for age, sex, factor V Leiden, and prothrombin G20210A.

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

**Discussion**

The impact of defects in the fibrinolytic system for predicting the risk for recurrent VTE has been regarded as low. In a report of the Duration of Anticoagulation (DURAC) Trial Study Group, increased levels of tissue plasminogen activator antigen or plasminogen activator inhibitor-1 only weakly correlated with the development of a future VTE. 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. Our study is the first to show a relationship between impaired fibrinolysis and the risk for recurrent VTE. In 600 patients with a spontaneous VTE, a TAFI level above the 75th percentile of IX) with regard to the risk for recurrence. As shown in Table 1, patients with high TAFI levels (75th or higher percentile) had significantly higher levels of factor VIII and factor IX than did patients with lower levels (Table 1). In Tables 3 and 4, the effect of high factor VIII (higher than 90th percentile for thrombosis patients) or high factor IX (75th or higher percentile for thrombosis patients) and high TAFI levels on the RR for recurrence are shown.
thrombosis patients was associated with an almost 2-fold higher RR for recurrence compared with patients with lower levels. The probability of recurrent VTE 2 years after the discontinuation of secondary thromboprophylaxis was 14.5% in patients with high TAFI levels 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 through 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.\(^5\)\(^7\) Thus, the activation of TAFI can be regarded as a consequence of factor XI activity. Patients with high TAFI and high factor XI levels had a 3-fold increased risk for recurrence compared with patients with lower levels, and this high risk for recurrence was independent of other thrombotic variables, including high factor VIII levels. We hypothesize that the enhanced risk for recurrence among patients with high TAFI and high factor XI levels 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 levels and an increased risk for recurrence. A synergistic effect between TAFI and high factor VIII levels on the risk for a first venous thrombosis has been reported by van Tilburg et al.,\(^10\) but the association between TAFI and other intrinsic coagulation factors has not been studied in patients with a first or with recurrent venous thrombosis. We have previously shown that a high factor VIII level is a major risk factor for recurrent VTE\(^3\) and that the risk for recurrence among patients with high factor VIII levels is further enhanced by high factor IX.\(^3\) In the present analysis, patients with high factor VIII levels had substantially greater risk for recurrence than patients with lower levels regardless of TAFI levels. However, the risk for recurrence was highest (more than 6-fold) among patients with both high factor VIII and high TAFI levels. Similarly, a high risk for recurrence was found among patients with high factor IX and high TAFI levels. Factor IX is activated by factor XI, and it cannot be excluded that this effect of TAFI and factor IX on the risk for recurrence contributes, at least partially, to factor XI activation. A high factor IX level might also be genetically determined or might be 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 for recurrence. The relationship between the fibrinolytic and the coagulation systems with regard to the risk for recurrence is a new aspect in the multicausal nature of VTE, and further studies are needed to elucidate the pathomechanisms that modulate the thrombotic phenotype.

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