Thrombin activatable fibrinolysis inhibitor and the risk for deep vein thrombosis

Nico H. van Tilburg, Frits R. Rosendaal, and Rogier M. Bertina

Thrombin activatable fibrinolysis inhibitor (TAFI, or procarboxypeptidase B) is the precursor of a recently described carboxypeptidase that potently attenuates fibrinolysis. Therefore, we hypothesized that elevated plasma TAFI levels induce a hypofibrinolytic state associated with an increased risk for venous thrombosis. To evaluate this hypothesis, we developed an electroimmunoassay for TAFI antigen and used this assay to measure TAFI levels in the Leiden Thrombophilia Study, a case–control study of venous thrombosis in 474 patients with a first deep vein thrombosis and 474 age- and sex-matched control subjects. In 474 healthy control subjects, an increase of TAFI with age was observed in women but not in men. Oral contraceptive use also increased the TAFI concentration. TAFI levels above the 90th percentile of the controls (> 122 U/dL) increased the risk for thrombosis nearly 2-fold compared with TAFI levels below the 90th percentile (odds ratio, 1.7; 95% confidence interval, 1.1-2.5). Adjustment for various possible confounders did not materially affect this estimate. These results indicate that elevated TAFI levels form a mild risk factor for venous thrombosis. Such levels were found in 9% of healthy controls and in 14% of patients with a first deep vein thrombosis. Elevated TAFI levels did not enhance the thrombotic risk associated with factor V Leiden but may interact with high factor VIII levels. (Blood. 2000;95: 2855-2859)

Introduction

During normal hemostasis there is a balance between coagulation and fibrinolysis. Disturbances of this balance may result in a bleeding disorder or in a thrombotic tendency. Activated protein C (APC), the endproduct of the protein C anticoagulant pathway,[1,2] plays an important role in maintaining this balance through its anticoagulant[3,4] and profibrinolytic[5-8] properties. In fact, part of its profibrinolytic action was found to be related to its anticoagulant properties, and the existence of a plasma component was proposed that, on activation by thrombin, would act as an inhibitor of t-PA–dependent fibrinolysis.[9] Bajzar et al[10] have isolated this protein, the thrombin activatable fibrinolysis inhibitor (TAFI). It appeared to be identical to plasma procarboxypeptidase B[11] and procarboxypeptidase U.[12]

Detailed biochemical studies revealed that TAFI can be converted to an active carboxypeptidase by enzymes such as trypsin, thrombin, and plasmin.[10,11] Activation of TAFI by thrombin is increased more than 1000-fold in the presence of its cofactor thrombomodulin.[13,14] After activation, TAFI suppresses fibrinolysis through the removal of carboxy terminal lysine residues that appear during proteolysis of the fibrin polymers and that serve an important role in assembling the components of the fibrinolytic system on the fibrin surface.[15,16] Together these properties of TAFI make it an important negative regulator of fibrinolytic efficiency in vitro.[17] Therefore, we hypothesized that high-plasma TAFI levels are a risk factor for venous thrombosis. To test this hypothesis we made use of a large population-based case–control study on venous thrombosis, the Leiden Thrombophilia Study (LETS).[18,19]

Materials and methods

Purification of TAFI

Fresh frozen plasma (acid citrate dextrose anticoagulant) was thawed and adsorbed with 2% (wt/vol) aluminum hydroxide suspension for 20 minutes at room temperature. After centrifugation 500 mL adsorbed plasma was passed through a lysine–Sepharose column (40 mL bed volume) equilibrated in 50 mmol/L triethanolamine and 100 mmol/L NaCl, pH 7.4 (TEA–NaCl). The column was washed with the same buffer until the A_{280} was less than 0.1 The wash procedure was continued with 300 mL TEA–NaCl buffer. This wash, which contained approximately 100 U TAFI, was passed through a glu–plasminogen–Sepharose column, equilibrated in TEA–NaCl (20 mL bed volume). The column was washed with 2 vol TEA–NaCl. A 0- to 200-mmol/L linear gradient of ϵ-amino-n-caproic acid (ϵ-ACA) in TEA–NaCl was applied, and TAFI was eluted at approximately 35 mmol/L ϵ-ACA. These TAFI-containing fractions were stored at −20°C. The final yield of TAFI was approximately 10%.

After SDS-PAGE purified TAFI shows a single band of 60 kd. SDS-PAGE was performed under nonreducing conditions, using the Fast System (Amersham Pharmacia Biotech, Uppsala, Sweden) and a 5% to 15% gradient gel. Protein bands were visualized by silver staining. The concentration of isolated TAFI was calculated from the absorbance at 280 nm (after correction for the absorbance at 320 nm) using a molar absorption coefficient of 1.28×10^5 mol/L^{-1}cm^{-1} (see [10]).

Preparation of TAFI-deficient plasma

Rabbits were immunized with isolated human TAFI using standard procedures. IgG was isolated from the antiserum with protein-A Sepharose 4B and coupled to CNBr-activated Sepharose-4B (Amersham Pharmacia Biotech) as described by the manufacturer. Citrated plasma was passed over

From the Departments of Hematology and Clinical Epidemiology, Hemostasis and Thrombosis Research Center, Leiden University Medical Center, Leiden, The Netherlands.

Submitted August 16, 1999; accepted January 4, 2000.

The Leiden Thrombophilia Study was originally supported by grant 89.063 from The Netherlands Heart Foundation.

Reprints: Rogier M. Bertina, Department of Hematology, Hemostasis and Thrombosis Research Center, Leiden University Medical Center, Building 1 C2-R, PO Box 9600, 2300 RC Leiden, The Netherlands.

The publication costs of this article were defrayed in part by page charge payment. Therefore, and solely to indicate this fact, this article is hereby marked "advertisement" in accordance with 18 U.S.C. section 1734.

anti-TAFI–IgG Sepharose (10 mg IgG/mL Sepharose), and fall-through fractions were tested for the presence of TAFI with an electroimmunoassay using rabbit polyclonal antibodies against TAFI. TAFI-deficient fractions (less than 1.6 U/dL) were pooled and frozen at −20°C.

Preparation of specific polyclonal anti-TAFI antibodies

The crude rabbit anti-TAFI serum was tested in crossed immunoelectrophoresis. Pooled normal plasma showed 1 major precipitation arc with some minor contaminations. To remove these contaminating antibodies, the antiserum was mixed with one-tenth vol TAFI deficient plasma (prepared as described above), stored overnight at 4°C, heated at 56°C for 30 minutes, and centrifuged (10 000*g*, 10 minutes, 4°C). IgG was isolated using protein A–Sepharose-4B and stored in TEA–NaCl, at −20°C at a concentration of 6 mg/mL.

Glu–plasminogen–Sepharose

Glu-plasminogen was isolated by affinity chromatography on lysine–Sepharose as described previously[20] and coupled to CNBr-activated Sepharose (0.25 mg/mL) according to the instructions of the manufacturer.

Electroimmunoassay

Electroimmunoassay was carried out following standard procedures. Briefly, a suitable percentage (0.5% vol/vol) of anti-TAFI IgG was added to 1.0% agarose (SeaKem LE, cat. No. 50 004; FMC BioProducts, Rockland, ME) in 31.6 mmol/L tricine, 91.5 mmol/L Trizma base, 1 mmol/L EDTA, pH 8.8. TAFI standards were prepared by dilution of purified TAFI in TAFI-deficient plasma. These standards (20.5 to 164 U/dL) had been calibrated against pooled normal plasma. Standards and undiluted 5-μL samples were applied in wells 2.5 mm in diameter. Plates were electrophoresed at 2 to 3 V/cm for 18 hours at 10°C to 15°C. After drying and staining, the length of the precipitation peak was measured and the amount of TAFI was calculated by intrapolation on the standard curve as the mean of duplicate tests. Serial dilutions of isolated TAFI (0 to 95 nmol/L) in buffer, TAFI-deficient plasma, or plasminogen- and TAFI-deficient plasma gave identical precipitation peaks in the Laurell assay. From these data it could be calculated that with the Laurell assay we recovered 100% of the TAFI, which was added to the deficient plasmas. The same recovery was obtained when different amounts of purified TAFI were added to pooled normal plasma.

Measurement of TAFI antigen in plasma

Using the electroimmunoassay, no TAFI (less than 1.6 U/dL) was detected in TAFI-depleted plasma, prepared by immuno-depletion with a monoclonal antibody against TAFI (Nik-9H10; see Mosnier et al[27]). At a TAFI antigen level of approximately 100 U/dL, the intra-assay and inter-assay coefficients of variation were 6% (n = 20) and 6% (n = 107), respectively. In the 64 healthy control subjects who contributed to the pooled normal plasma, the mean TAFI level was 100 U/dL (SD, 9 U/dL; range, 53 to 139 U/dL).

Pooled normal plasma

Blood was collected by venipuncture in plastic tubes containing one-tenth vol 0.106 mol/L trisodium citrate. The blood was centrifuged at 2000*g* for 20 minutes at 20°C. The platelet-poor plasma of 64 healthy volunteers (women on oral contraceptives were excluded) was pooled and stored at −70°C in aliquots of 0.5 mL. This pooled normal plasma was considered to contain 100 U/dL TAFI; in this pooled normal plasma, 100 U/dL TAFI corresponded with 106 nmol/L TAFI or 6.4 g/mL TAFI (using a molecular weight of 60 000).

Leiden thrombophilia study

The design of this population-based case–control study (LETS) has been described in detail.[18] Briefly, consecutive patients with an objectively diagnosed first episode of deep vein thrombosis were selected from the files of 3 anticoagulation clinics in The Netherlands. All patients were younger than 70 years and were not diagnosed with malignant disorders. Control subjects were acquaintances of patients or partners of other patients, matched for age and sex with the patients. The study included 474 patients and 474 control subjects. Blood was collected into 0.1 vol of 0.106 mol/L trisodium citrate. Plasma was prepared by centrifugation for 10 minutes at 2000*g* at room temperature and was stored at −70°C.

Statistical analysis

Determinants of TAFI were investigated by linear regression. Odds ratios (ORs) were calculated as estimates of relative risk for thrombosis in the unmatched fashion adjusted for age and sex and possible confounders by logistic regression; 95% confidence intervals (95% CI) were constructed according to Woolf[21] or were derived from the model estimates.

Results

TAFI antigen levels

The mean TAFI antigen concentration in all 948 patients and controls was 107 (± 13 SD) U/dL. TAFI antigen levels were normally distributed. Mean TAFI levels were similar in patients (mean, 107 U/dL; SD, ± 14) and control subjects (mean, 107 U/dL; SD ± 12), but there were more patients than controls with high TAFI levels; 14% of patients had levels exceeding the 90th percentile compared with 9% in control subjects.

Determinants of TAFI were studied in the healthy control subjects. There was no difference in the mean TAFI levels in men (mean, 107 U/dL; SD ± 13) and women (mean, 106 U/dL; SD ±12). Univariate analysis by linear regression showed an increase of TAFI concentration of 0.11 U/dL per year (95% CI, 0.03 to 0.19). In men TAFI levels were not affected by age (0.06 U/dL per year; 95% CI, −0.08 to 0.20), whereas in women the TAFI concentration increased (0.14 U/dL per year; 95% CI, 0.03 to 0.25). TAFI concentrations in women using oral contraceptives were slightly higher than in women not using them (111 U/dL; 95% CI, 108 to 114 and 105 U/dL; 95% CI, 103 to 106, respectively). After correction for the effect of oral contraceptive use (at the time of blood collection), the effect of age on TAFI concentration in women became even more pronounced (0.28 U/dL per year; 95% CI, 0.17 to 0.39).

TAFI concentration and the risk for venous thrombosis

Table 1 shows stratification into 2 groups of the TAFI levels of patients and control subjects, above and below the 90th percentile (as observed in control subjects). Crude odds ratios were calculated for patients with TAFI concentrations above the 90th percentile, with the group below the 90th percentile as the reference category. Higher TAFI concentrations (more than 122 U/dL) were associated with an increased risk for thrombosis (OR, 1.7; 95% CI, 1.1 to 2.5). The age- and sex-adjusted odds ratio was 1.7 (95% CI, 1.1 to 2.5). In men, TAFI levels exceeding 122 U/dL were associated with an odds ratio of 1.3 (95% CI, 0.7 to 2.5), whereas in women the odds ratio was 2.0 (95% CI, 1.1 to 3.4). An additional increase in the cutoff point to the 95th or 99th percentile did not result in a further increase of the odds ratios (OR, 1.5; 95% CI, 0.8 to 2.7 and OR, 2.0; 95% CI, 0.5 to 8.1, respectively).

Table 2 shows patients and control subjects stratified into 5 groups according to the TAFI concentration in the controls. Crude odds ratios were calculated using the lowest quintile as the

Table 1. Thrombosis risk for TAFI antigen

TAFI Antigen (U/dL)	Patients n (%)	Controls n (%)	OR	95% CI
<123	408 (86)	432 (91)	1*	
>122 (P90)	66 (14)	42 (9)	1.7	1.1-2.5

*Reference category.

Table 2. Thrombosis risk for TAFI antigen

TAFI Antigen (U/dL)	Patients n (%)	Controls n (%)	OR	95% CI
< 96	94 (20)	86 (18)	1*	
96-103	105 (22)	98 (21)	1	0.7-1.5
104-109	86 (18)	94 (20)	0.8	0.6-1.3
110-116	74 (16)	96 (20)	0.7	0.5-1.1
> 116	115 (24)	100 (21)	1.1	0.7-1.6

*Reference category.

reference category. The odds ratios did not increase with higher TAFI concentrations over these quintiles.

Oral contraceptive use is associated with an increased risk for venous thrombosis.[22,23] Hence, we adjusted for oral contraceptive use (either at the time of thrombosis or at the time of blood collection) by logistic regression analysis. This resulted only in mild changes in the odds ratio for levels exceeding the 90th percentile (OR, 1.9; 95% CI, 1.1 to 3.3 and OR, 2.3; 95% CI, 1.3 to 4.1, respectively).

Association of TAFI with other coagulation factors

Univariate regression analysis demonstrated that in the controls (n = 474) TAFI levels were dependent on several other coagulation factors tested. Table 3 summarizes the parameters of the regression lines that define the relation between TAFI levels and the levels of other clotting factors. When the influence of the concentration of the same coagulation proteins on the TAFI level was analyzed in multiple regression, fibrinogen and antithrombin disappeared as independent variables. Therefore, it is not surprising that compared with persons with TAFI levels lower than the 90th percentile, those with TAFI levels higher than the 90th percentile also have elevated levels of protein C and factor II (Table 4).

When we adjusted for all factors associated with TAFI levels (sex, age, use of oral contraceptives, fibrinogen, antithrombin, protein C, and factor II levels, excluding persons using oral anticoagulant drugs), there remained an increased risk for thrombosis in persons whose TAFI levels exceeded the 90th percentile (OR, 1.5).

Elevated TAFI and other common risk factors for thrombosis

Tables 5 and 6 summarize the effect of elevated TAFI levels (greater than 90th percentile) on the risk for thrombosis of factor V Leiden and elevated factor VIII (more than 150 IU/dL). There are no indications that an elevated TAFI level will enhance the effect of factor V Leiden on risk for thrombosis,[24] whereas there may be some synergy with high levels of factor VIII with regard to risk for thrombosis.

Discussion

TAFI (or procarboxypeptidase B) is a plasma zymogen that, when converted to an enzyme, potently inhibits fibrinolysis.[11,17] This indicates that increased levels of TAFI may be associated with an increased risk for thrombosis.[25,26] To investigate this, we measured TAFI antigen levels in patients and control subjects of the Leiden Thrombophilia Study. In the first part of this study, we analyzed the effects of sex, age, and oral contraceptive use on TAFI levels. In the second part, we studied the possibility of an association of increased TAFI concentrations and thrombosis.

No difference in TAFI concentration was found between men and women. Analysis of the effect of age showed no increase of TAFI concentration in men and an age-dependent increase in women. The latter effect was partially masked by the use of oral contraceptives. Recently Schatteman et al[25] reported an effect of age on TAFI levels in men, using a 2-stage functional assay for procarboxypeptidase U (or TAFI).

Stratification of patients and control subjects, with the 90th percentile in the controls as the cutoff, resulted in a mildly increased odds ratio (1.7) for those with TAFI levels greater than 122 U/dL. Use of the 95th and 99th percentiles did not result in a further increase of the odds ratio. There is no support for a gradual relationship between mildly increased TAFI levels and the risk for thrombosis (Table 2). After adjustment for age, elevated TAFI antigen level remained a risk factor for thrombosis. Adjustment for oral contraceptive use during blood collection did not affect the association between TAFI and the risk for thrombosis. Further, elevated TAFI levels did not enhance the risk for thrombosis associated with factor V Leiden but may have interacted with high factor VIII levels (Tables 5 and 6).

In this study we used the TAFI antigen as a measure of plasma TAFI concentration. Recently Mosnier et al[27] and Schatteman et al[25] reported methods for the measurement of TAFI activity in plasma. Mosnier et al[27] found a linear relationship between TAFI activity and TAFI antigen in plasma in 20 healthy control subjects. Therefore, we may conclude that there is no indication for the common presence of molecular variants of TAFI with enhanced specific activity in the general population.

The molecular basis of elevated TAFI levels is not yet clear. There are no known polymorphisms in the TAFI gene that are associated with plasma TAFI. Recently, Zhao et al[28] described 2 isoforms of TAFI. Detailed functional studies of the recombinant proteins did not reveal a difference between the 2 proteins (TAFI Ala 147 and TAFI Thr 147). The authors proposed to investigate the isoform distribution in healthy volunteers and patients with thrombotic disorders. A recent report[26] on the characterization of

Table 3. Association of TAFI and other coagulation factors

Factor	b*	95% CI
Fibrinogen (g/L)	3.04	1.36-4.72
Antithrombin (U/dL)	0.20	0.09-0.30
Protein C (U/dL)	0.21	0.15-0.27
Prothrombin (U/dL)	0.23	0.16-0.30

*Regression coefficient b shows the increase of TAFI (U/dL) per U/dL or g/L increase of the coagulation factor studied.

Table 4. Levels of other coagulation factors in persons with elevated TAFI levels

Factor	TAFI < P90* n = 432 (431†)	TAFI > P90* n = 42
Fibrinogen (g/L)	3.24 (0.64)	3.50 (0.83)
Antithrombin (U/dL)	99.0 (10.4)	99.9 (10.5)
Protein C (U/dL)†	101.0 (17.7)	116.2 (17.9)
Prothrombin (U/dL)†	102.9 (14.6)	112.1 (13.7)

*Mean (±SD) levels of coagulation proteins in persons with TAFI levels <P90 and >P90.

†Number of persons for analysis of protein C and prothrombin, i.e. not using oral anticoagulants.

Table 5. Elevated TAFI and factor V Leiden

TAFI	F V Leiden	Patients (n)	Control Subjects (n)	OR	95% CI
<P90	−	326	420	1	
<P90	+	82	12	8.8	4.7-16.4
>P90	−	56	40	1.8	1.1-2.8
>P90	+	10	2	6.4	1.4-29.6

Table 6. Elevated TAFI and high factor VIII:C

TAFI	F VIII:C (IU/dL)	Patients (n)	Control Subjects (n)	OR	95% CI
<P90	<150	267	358	1	
<P90	>150	141	74	2.6	1.9-3.5
>P90	<150	27	32	1.1	0.7-1.9
>P90	>150	39	10	5.2	2.6-10.7

the gene encoding human TAFI will aid in identifying sequence variations associated with plasma TAFI levels.[26] Most persons with TAFI antigen levels exceeding the 90th percentile also have elevated plasma levels of some other coagulation factors (Table 4). However, none of these is responsible for the risk for thrombosis associated with elevated TAFI levels.

Disturbances of the balance between coagulation and fibrinolysis may result in a bleeding disorder or in a thrombotic tendency. In the fibrinolytic pathways, both genetic defects (plasminogen deficiencies and dysplasminogenemias)[29,30] and abnormal laboratory phenotypes (increased levels of PAI-1,[31] decreased levels of t-PA[32]) have been reported in patients with venous thrombosis. However, there is still serious doubt whether heterozygosity for a plasminogen deficiency is associated with an increased risk for venous thrombosis.[29,30,33] Homozygous plasminogen deficiency has been reported in children.[34,35] Surprisingly these children suffer from ligneous conjunctivitis and show no signs of excessive fibrin formation in the vascular compartments, indicating that plasminogen-dependent fibrinolysis is not important for the removal of intravascular fibrin in these children. With respect to the elevated PAI-1 levels that have been observed in patients with venous thrombosis,[36-39] it still must be established that this is not a postthrombotic phenomenon. On the other hand, elevated levels of PAI-1 and t-PA antigen seem to be good markers for predicting a second thrombotic event.[32] Overall, (genetic) abnormalities in the fibrinolytic system seem not to contribute to the risk for venous thrombosis. Excessive fibrin formation seems to depend mainly on the balance between procoagulant and anticoagulant reactions.

Acknowledgments

We thank Dr J. C. M. Meijers (Department of Haematology, University Hospital, Utrecht) for providing us with TAFI-deficient plasma, prepared by immuno-depletion with a monoclonal antibody against TAFI. We thank Dr Ted Koster, Mrs T. Visser, and Mrs A. Schreijer for contacting the patients and processing the blood samples. We thank Dr F. J. M. van der Meer (Anticoagulation Clinic, Leiden), Dr L. P. Colly (Anticoagulation Clinic, Amsterdam), and Dr P. H. Trienekens (Anticoagulation Clinic, Rotterdam) for their assistance.

References

1. Esmon CT, Schwarz HP. An update on clinical and basic aspects of the protein C anticoagulant pathway. Trends Cardiovasc Med. 1995;5:141-148.
2. Dahlbäck B. The protein C anticoagulant system: inherited defects as basis for venous thrombosis. Thromb Res. 1995;77:1-43.
3. Kisiel W, Fujikawa K, Davie EW. Activation of bovine factor VII (proconvertin) by factor XIIa (activated Hageman factor). Biochemistry. 1977;16:4189-4194.
4. Vehar GA, Davie EW. Preparation and properties of bovine factor VIII (antihemophilic factor). Biochemistry. 1980;19:401-410.
5. de Fouw NJ, Haverkate F, Bertina RM, Koopman J, van Wijngaarden A, van Hinsbergh VW. The cofactor role of protein S in the acceleration of whole blood clot lysis by activated protein C in vitro. Blood. 1986;67:1189-1192.
6. Bajzar L, Nesheim M. The effect of activated protein C on fibrinolysis in cell-free plasma can be attributed specifically to attenuation of prothrombin activation. J Biol Chem. 1993;268:8608-8616.
7. van Hinsbergh VW, Bertina RM, van Wijngaarden A, van Tilburg NH, Emeis JJ, Haverkate F. Activated protein C decreases plasminogen activator–inhibitor activity in endothelial cell-conditioned medium. Blood. 1985;65:444-451.
8. Taylor FB Jr, Lockhart MS. A new function for activated protein C: activated protein C prevents inhibition of plasminogen activators by releasate from mononuclear leukocytes—platelet suspensions stimulated by phorbol diester. Thromb Res. 1985;37:155-164.
9. de Fouw NJ, Haverkate F, Bertina RM. Protein C and fibrinolysis: a link between coagulation and fibrinolysis. Adv Exp Med Biol. 1990;281:235-243.
10. Bajzar L, Manuel R, Nesheim ME. Purification and characterization of TAFI, a thrombin-activable fibrinolysis inhibitor. J Biol Chem. 1995;270:14,477-14,484.
11. 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;266:21,833-21,838.
12. Hendriks D, Wang W, van Sande M, Scharpe S. Human serum carboxypeptidase U: a new kininase? Agents Actions Suppl. 1992;38:407-413.
13. Bajzar L, Morser J, Nesheim M. TAFI, or plasma procarboxypeptidase B, couples the coagulation and fibrinolytic cascades through the thrombin-thrombomodulin complex. J Biol Chem. 1996;271:16,603-16,608.
14. Nesheim M, 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;78:386-391.
15. Wang W, Boffa PB, Bajzar L, Walker JB, Nesheim ME. A study of the mechanism of inhibition of fibrinolysis by activated thrombin-activable fibrinolysis inhibitor. J Biol Chem. 1998;273:27,176-27,181.
16. Plow EF, Allampallam K, Redlitz A. The plasma carboxypeptidases and the regulation of the plasminogen system. Trends Cardiovasc Med. 1997;7:71-75.
17. Nesheim M. Fibrinolysis and the plasma carboxypeptidase. Curr Opin Hematol. 1998;5:309-313.
18. Koster T, Rosendaal FR, de Ronde H, Briët E, Vandenbroucke JP, Bertina RM. Venous thrombosis due to poor anticoagulant response to activated protein C: Leiden Thrombophilia Study. Lancet. 1993;342:1503-1506.
19. van der Meer FJ, Koster T, Vandenbroucke JP, Briët E, Rosendaal FR. The Leiden Thrombophilia Study (LETS). Thromb Haemost. 1997;78:631-635.
20. de Fouw NJ, de Jong YF, Haverkate F, Bertina RM. The influence of thrombin and platelets on fibrin clot lysis rates in vitro: a study of using a clot lysis system consisting of purified proteins. Fibrinolysis. 1988;2:235-244.
21. Woolf B. On estimating the relation between blood group and disease. Am J Human Genet. 1955;19:251-253.
22. Vandenbroucke JP, Helmerhorst FM, Bloemenkamp KW, Rosendaal FR. Third-generation oral contraceptive and deep venous thrombosis: from epidemiologic controversy to new insight in coagulation. Am J Obstet Gynecol. 1997;177:887-891.
23. Vessey M, Mant D, Smith A, Yeates D. Oral contraceptives and venous thromboembolism: findings in a large prospective study. Br Med J (Clin Res Ed). 1986;292:526.
24. Bajzar L, Kalafatis M, Simioni P, Tracy PB. An antifibrinolytic mechanism describing the prothrombotic effect associated with factor V Leiden. J Biol Chem. 1996;271:22,949-22,952.
25. Schatteman KA, Goossens FJ, Scharpé SS, Neels HM, Hendriks DF. Assay of procarboxypeptidase U, a novel determinant of the fibrinolytic cascade, in human plasma. Clin Chem. 1999;45:807-813.
26. Boffa MB, Reid TS, Joo E, Nesheim ME, Koschinsky mL. Characterization of the gene encoding human TAFI (thrombin-activable fibrinolysis inhibitor; plasma procarboxypeptidase B). Biochemistry. 1999;38:6547-6558.
27. Mosnier LO, Von dem Borne PAK, Meijers JCM, Bouma BN. Plasma TAFI levels influence the clot lysis time in healthy individuals in the presence of an intact intrinsic pathway of coagulation. Thromb Haemost. 1998;80:829-835.
28. Zhao L, Morser J, Bajzar L, Nesheim M, Nagashima M. Identification and characterization of two thrombin-activatable fibrinolysis inhibitor isoforms. Thromb Haemost. 1998;80:949-955.
29. Demarmels BF, Sulzer I, Stucki B, Wuillemin WA, Furlan M, Lammle B. Is plasminogen deficiency a thrombotic risk factor? a study on 23 thrombophilic patients and their family members. Thromb Haemost. 1998;80:167-170.
30. Sartori MT, Patrassi GM, Theodoridis P, Perin A, Pietrogrande F, Girolami A. Heterozygous type I plasminogen deficiency is associated with an increased risk for thrombosis: a statistical analysis

in 20 kindreds. Blood Coagul Fibrinolysis. 1994;5: 889-893.

31. Wiman B. Plasminogen activator inhibitor 1 (PAI-1) in plasma: its role in thrombotic disease. Thromb Haemost. 1995;74:71-76.
32. Wiman B, Ljungberg B, Chmielewska J, Urden G, Blomback M, Johnsson H. The role of the fibrinolytic system in deep vein thrombosis. J Lab Clin Med. 1985;105:265-270.
33. Tait RC, Walker ID, Conkie JA, Islam SI, McCall F. Isolated familial plasminogen deficiency may not be a risk factor for thrombosis. Thromb Haemost. 1996;76:1004-1008.
34. Mingers AM, Philapitsch A, Schwarz HP, Zeitler P, Kreth HW. Polymorphonuclear elastase in patients with homozygous type I plasminogen deficiency and ligneous conjunctivitis. Semin Thromb Hemost. 1998;24:605-612.
35. Schuster V, Mingers AM, Seidenspinner S, Nussgens Z, Pukrop T, Kreth HW. Homozygous mutations in the plasminogen gene of two unrelated girls with ligneous conjunctivitis. Blood. 1997;90: 958-966.
36. Schulman S, Wiman B. The significance of hypofibrinolysis for the risk for recurrence of venous thromboembolism: Duration of Anticoagulation (DURAC) Trial Study Group. Thromb Haemost. 1996;75:607-611.
37. Engesser L, Brommer EJ, Kluft C, Briët E. Elevated plasminogen activator inhibitor (PAI), a cause of thrombophilia? a study in 203 patients with familial or sporadic venous thrombophilia. Thromb Haemost. 1989;62:673-680.
38. Juhan-Vague I, Valadier J, Alessi MC, et al. Deficient t-PA release and elevated PA inhibitor levels in patients with spontaneous or recurrent deep venous thrombosis. Thromb Haemost. 1987;57: 67-72.
39. Nilsson IM, Ljungner H, Tengborn L. Two different mechanisms in patients with venous thrombosis and defective fibrinolysis: low concentration of plasminogen activator or increased concentration of plasminogen activator inhibitor. Br Med J. 1985;290:1453-1456.