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Lisman, T.; de Groot, P.G.; Meijers, J.C.M.; Rosendaal, F.R.

Link to publication

Reduced plasma fibrinolytic potential is a risk factor for venous thrombosis

Ton Lisman, Philip G. de Groot, Joost C.M. Meijers, and Frits R. Rosendaal

The role of the fibrinolytic system in the development of deep vein thrombosis (DVT) is unclear. We determined the plasma fibrinolytic potential of patients enrolled in the Leiden Thrombophilia Study (LETS), a population-based case-control study on risk factors for DVT. Plasma fibrinolytic potential was determined in 421 patients and 469 control subjects by means of a tissue factor–induced and tissue-type plasminogen activator (tPA)–induced clot lysis assay. Using clot lysis times above the 70th, 80th, 90th, 95th, and 99th percentiles of the values found in control subjects as cut-off levels, we found a dose-dependent increase in risk for DVT in patients with hypofibrinolysis (odds ratios of 1.4, 1.6, 1.9, 2.1, and 2.2, respectively). This indicates a 2-fold increased risk of DVT in subjects with clot lysis times above the 90th percentile. The risk increase was not affected by age or sex (adjusted odds ratio for 90th percentile, 2.0), and after correction for all possible confounders (age, sex, and levels of procoagulant pro-

teins shown to associate with clot lysis times in the control population), the risk estimate was marginally reduced (odds ratio, 1.6 for 90th percentile). Taken together, these results indicate that plasma hypofibrinolysis constitutes a risk factor for venous thrombosis, with a doubling of the risk at clot lysis times that are present in 10% of the population. (Blood. 2005; 105:1102-1105)

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Introduction

Evidence for a role of fibrinolysis, the dissolution of a fibrin clot, in the etiology of venous thrombosis is scarce. Genetic evidence has been limited to reports on patients with hereditary plasminogen deficiency,[1] although the relationship between heterozygous plasminogen deficiency and thrombosis has been questioned (for a review, see Brandt[2]). Moreover, homozygous plasminogen deficiency is not associated with deep vein thrombosis (DVT), but rather presents as ligneous conjunctivitis caused by vascular deposition of fibrin within the eye.[3]

Elevated levels of plasminogen activator inhibitor type 1 (PAI-1), and the PAI-1 4G/5G polymorphism have been suggested as a risk factor for venous thrombosis, but data are highly conflicting and unconvincing (for a review, see Francis[4]). Baseline plasma levels of tissue-type plasminogen activator (tPA) did not predict a first episode of venous thrombosis[5] nor did they predict the risk of recurrence.[6] Elevated plasma levels of thrombin activatable fibrinolysis inhibitor (TAFI), however, were a mild risk factor for the development of venous thrombosis.[7] Next to levels of proteins involved in fibrinolysis, levels of markers of fibrinolytic activity were studied in relation to the development of venous thrombosis. Levels of tPA/PAI-1 complex and plasmin-antiplasmin (PAP) complex were not associated with an increased risk for venous thrombosis.[8]

Studies on a relationship between fibrinolysis and venous thrombosis have focused on plasma levels of proteins or protease-inhibitor complexes. Global tests of fibrinolytic activity such as the euglobulin clot lysis time (ECLT) and the dilute whole-blood clot lysis time (DWBCLT) have been used to study the association of thrombosis and hypofibrinolysis, but these studies gave inconclusive results (summarized by Prins and Hirsh[9]). Moreover, the outcome of these tests do not reflect a true global fibrinolytic potential, because these tests are either performed with plasma fractions (ECLT) or in the presence of citrate (DWBCLT), which excludes the interplay between coagulation and fibrinolysis through TAFI and factor XIII.

We have developed a plasma-based, tissue factor-induced clot lysis assay, in which a fibrin clot is lysed by exogenously added tPA. Although this assay was initially developed to study TAFI-related processes, we have shown that the clot lysis time (CLT) in this assay is also influenced by levels of other proteins involved in fibrinolysis and thrombin generation (which is required for TAFI activation), including plasminogen, α_2-antiplasmin, PAI-1, and antithrombin.[10] The outcome of this assay therefore reflects an overall plasma fibrinolytic potential. This test has been used to study fibrinolysis in a number of pathologies. We demonstrated a hyperfibrinolytic state in hemophilia,[11,12] factor XI deficiency,[13] and after use of heparin and heparin-like anticoagulants[14,15] as a consequence of reduced thrombin generation resulting in defective TAFI activation. In patients with liver disease, plasma fibrinolytic potential was similar to that in controls, despite severely reduced TAFI levels, which could be explained by a concomitant decrease in profibrinolytic proteins.[10] During oral contraceptive use, overall plasma fibrinolytic potential did not change, but factor XI–independent CLTs were prolonged.[16]

Here, we assessed the contribution of the fibrinolytic system to the development of venous thrombosis. For this, our CLT assay, which measures global plasma fibrinolytic potential, taking the interplay between coagulation and fibrinolysis (eg, through TAFI) into account, was used. This study was part of the Leiden Thrombophilia Study (LETS), a large population-based case-control study designed to estimate the contributions of genetic and acquired factors to the risk of venous thrombosis.

Patients, materials, and methods

Patients

The design of the LETS has been extensively described previously.[17,18] In short, 474 consecutive patients with an objectively confirmed first episode of DVT that occurred between January 1988 and December 1992 were selected from 3 anticoagulation clinics in The Netherlands. Patients were younger than 70 years and had no known malignancy. Controls were acquaintances or partners, and they were matched for age and sex.

Blood was collected in tubes containing 0.106 M trisodium citrate. Plasma was prepared by centrifugation at 2000*g* for 10 minutes at room temperature and stored at −70°C. Samples were obtained at least 6 months after the thrombotic event and at least 3 months after discontinuation of oral anticoagulant treatment. For this study, plasma samples from 469 controls and 469 patients were available. However, 48 patients were excluded from the analysis because they were still using oral anticoagulation at the time of venipuncture. Samples, which were kept at −70°C at all times and were not previously thawed, were used for this study. Approval for this study was obtained from the Institutional Review Board of the Leiden University Medical Center. Informed consent was provided according to the Declaration of Helsinki.

Clot lysis assay

Lysis of a tissue factor–induced clot by exogenous tPA was studied by monitoring changes in turbidity during clot formation and subsequent lysis essentially as described previously, except for a change in tissue factor concentration.[10] Plasma (50 μL) was pipetted into a microtiter plate, after which 50 μL of a mixture containing tissue factor (diluted Innovin; Dade Behring, Marburg, Germany; final dilution 1000 times), $CaCl_2$ (final concentration 17 mM), tPA (Chromogenix, Mölndal, Sweden; final concentration 30 U/mL; 56 ng/mL), and phospholipid vesicles (consisting of 40% L-α-dioleoylphosphatidylcholine, 20% L-α-dioleoylphosphatidylserine, and 40% L-α-dioleoylphosphatidylethanolamine, all from Sigma, St Louis, MO, prepared according to Brunner et al[19] with minor modifications as described by van Wijnen et al[20]; final concentration 10 μM), diluted in HEPES buffer (25 mM HEPES [*N*-2-hydroxyethylpiperazine-*N′*-2-ethanesulfonic acid], 137 mM NaCl, 3.5 mM KCl, 3 mM $CaCl_2$, 0.1% bovine serum albumin [BSA], pH 7.4) was added. After thorough mixing, turbidity at 405 nm was measured in time at 37°C in a Spectramax 340 kinetic microplate reader (Molecular Devices Corporation, Menlo Park, CA). CLT was defined as the time from the midpoint of the clear to maximum turbid transition, which characterizes clot formation, to the midpoint of the maximum turbid to clear transition, which represents clot lysis. The intra-assay and interassay coefficients of variation were 3.4% (n = 16) and 4.0% (n = 32), respectively. All assays were performed without knowledge of whether the sample was from a patient or a control.

Statistical analysis

Data were analyzed with 2 objectives. First, determinants of CLT were assessed in the control subjects as reflecting the general population. Determinants were established by comparing means and using linear regression. Second, it was investigated whether elevated CLT is a risk factor for DVT by calculating odds ratios (ORs) and 95% confidence intervals (CIs). As cut-off points we used percentiles (70, 80, 90, 95, and 99) of CLTs measured in the control subjects. We used multivariate modeling by unconditional logistic regression to adjust for sex, age, and other putative confounding variables.

Results

In this study, 421 patients and 469 controls were used. The mean age of the patients and controls was 45 years (range, 14-69 years in patients; 14-72 years in controls). In the control population 47% were male, whereas 43% of the patients were male.

Determinants of CLTs

Determinants of CLTs in the control population are shown in Table 1. The mean of the CLTs in the 469 control subjects was 61.0 minutes (range, 38.8-135.2 minutes). CLTs were slightly higher in men than in women (mean difference, 3.3 minutes; 95% CI, 1.3-5.3). CLTs progressively increased with age and were reduced by oral contraceptive use, although this effect was small.

To investigate the relationship between levels of clotting factors and the CLT, univariate regression analysis was performed. Table 2 shows regression coefficients (b) and their 95% CIs for all the tested variables. Although the levels of several coagulation factors were associated with the CLTs, all associations were weak, indicating that levels of these factors only to a small extent determined the CLT in our assay. A weak association between CLT and the endogenous thrombin potential was present; however, this association disappeared after correction for age, indicating that CLTs were not influenced by the thrombin-generating capacity of the plasma. No association between CLT and TFPI activity, total TFPI, factor XIII activity, and factor XIII a-subunit could be demonstrated.

CLT as a risk factor for DVT

For determination of the contribution of elevated CLTs to the risk of DVT, cut-off levels of CLTs in the control group were set at the 70th, 80th, 90th, 95th, and 99th percentiles. The ORs for DVT in subjects with CLTs above these cut-off values, with values below the cut-off as reference group, progressively increased over the range of cut-off levels (Table 3). For a cut-off point at the 90th percentile, 18% of the individuals with DVT had a CLT above this cut-off, compared to 10% (per definition) in the control group. This implies that CLTs above 74.1 minutes were associated with an almost 2-fold increased risk of DVT (OR, 1.9; 95% CI, 1.3-2.9).

After adjustment for sex and age, the OR for the 90th percentile was 2.0 (95% CI, 1.3-3.0). After adjustment for all factors that,

Table 1. CLTs in control subjects

	No. of subjects	CLT, mean min
Sex		
Male	198	63.0
Female	271	59.7
Use of oral contraceptives in premenopausal women		
Yes	67	53.4
No	114	56.9
Age, y		
24 or younger	42	53.9
25-34	55	54.2
35-44	104	59.1
44-54	133	62.7
55 and older	135	66.0

Table 2. Association of CLTs with levels of coagulation factors and the ETP

	b (95% CI)	b (95% CI) adjusted for age
Fibrinogen, g/L	3.251 (1.758-4.744)	1.963 (0.540-3.387)
Factor II, %	0.160 (0.092-0.227)	0.163 (0.102-0.225)
Factor VII, %	0.121 (0.076-0.166)	0.090 (0.050-0.135)
Factor VIII, %	0.004 (−0.026-0.034)	−0.016 (−0.044-0.013)
Factor IX, %	0.058 (0.049-0.111)	0.033 (−0.017-0.083)
Factor X, %	0.064 (0.001-0.129)	0.105 (0.044-0.166)
Factor V, %	0.070 (0.041-0.100)	0.038 (0.010-0.068)
Factor XI, %	0.091 (0.041-0.141)	0.074 (0.028-0.122)
TAFI, %	0.228 (0.149-0.306)	0.188 (0.115-0.261)
Factor XIII, b-subunit, %	0.182 (0.109-0.255)	0.145 (0.074-0.214)
Free TFPI, ng/mL	0.953 (0.715-1.192)	0.762 (0.519-1.004)
Antithrombin III, %	0.050 (−0.047-0.146)	0.107 (0.017-0.196)
C-reactive protein, μg/mL	0.095 (−0.107-0.297)	0.076 (−0.110-0.263)
ETP, nM IIa · min	−0.001 (−0.002-0.000)	0.000 (−0.001-0.000)

The regression coefficient b shows the increase in clot lysis time (in minutes) per unit increase in the variable studied. Also shown are regression coefficients after correction for age. Levels of coagulation factors are expressed as percentage of pooled normal plasma (ie, U/dL), except for TFPI and fibrinogen and other variables as indicated.

after correction for age, had shown an association with CLTs in the controls (levels of fibrinogen, factors II, VII, X, V, XI, TAFI factor XIII b subunit, TFPI [free], and antithrombin III), the risk remained elevated at an OR of 1.6 (95% CI, 1.0-2.5).

Discussion

This study shows that individuals with reduced fibrinolytic potential, as measured by a plasma-based assay, have an increased risk of developing a first venous thrombosis. The risk of thrombosis increased with increasing plasma CLT. Individuals with a plasma CLT above the 90th percentile of the control subjects have an almost 2-fold increased risk for development of a first DVT. The risk remained elevated even after extensive adjustment for other factors, and therefore is likely to be indicative of a real effect. The relative risk of 1.9 associated with elevated CLTs, which are present in 10% of the population, suggests that plasma hypofibrinolysis is an important contributor to the overall burden of venous thrombosis. Based on these data, the population-attributable risk of thrombosis is 9% (ie, 9% of all cases of thrombosis in the general population may be attributable to plasma hypofibrinolysis). The relative risk of 1.9 at the 90th percentile of CLTs in control subjects indicates that plasma hypofibrinolysis forms a risk for venous thrombosis comparable to the risk associated with other risk factors such as high levels of factor XI[21] (relative risk, 2.2 at levels above the 90th percentile of the controls), high levels of factor IX[22] (relative risk, 2.3 at levels above the 90th percentile of the controls), and heterozygotes for the prothrombin G20210A mutation (relative risk, 2.8).[23]

In this study we show for the first time a clear association between plasma hypofibrinolysis and the risk of thrombosis. The role of the fibrinolytic system in DVT has been debated.[24] Previous studies have either examined single fibrinolytic parameters (tPA and PAI-1 activity or antigen, PAI-1 4G/5G polymorphism), plasma markers of fibrinolysis (PAP complexes, tPA/PAI-1 complexes), or clot lysis assays in which fibrinolytic potential was only assessed partially, that is, in a plasma fraction, or in the absence of calcium (ELT or DWBCLT, respectively), and were often in small

or selected groups of patients.[4-6,8,9] Here, we have used a tissue factor–induced clotting assay, in which fibrinolysis is initiated by exogenous tPA in a large population-based case-control study. The outcome of the assay presumably represents an overall measure for the plasma fibrinolytic potential resulting from a combination of plasma levels of proteins important for clot dissolution. Indeed, we have shown previously that this clot lysis assay is influenced by levels of plasminogen, α_2-antiplasmin, PAI-1, and TAFI.[10] In accordance, in this study, in which individuals have been characterized very extensively, no strong associations of proteins involved in clot formation were observed.

Surprisingly, TAFI levels were only weakly associated with CLTs. Previously, it has been demonstrated that addition of purified TAFI to TAFI-depleted plasma dose-dependently prolongs CLT.[25] Moreover, an association between TAFI antigen levels and CLTs were found in plasma samples from 20 healthy volunteers.[25] In this study we show that although an association between TAFI antigen levels and CLTs exists, the contribution of TAFI levels to CLTs is small. In other words, the CLT in a given plasma sample is determined by the total fibrinolytic capacity of the plasma, which is determined by the balance of the levels of all fibrinolytic proteins.

No association between the thrombin-generating capacity of the plasma (endogenous thrombin potential [ETP]) and CLT could be demonstrated, which is in contrast with earlier observations in which the extent of TAFI activation was linked to the amount of thrombin generated after clot formation via the factor XI feedback loop.[13] Also, both in the present study and in literature,[26] CLTs were associated with prothrombin plasma levels, which were shown to be accompanied by an increased generation of activated TAFI. Because it was previously demonstrated that ETP values are strongly linked to prothrombin plasma levels,[27] it is surprising that no association between ETP and CLTs times was found now.

The risk for DVT associated with plasma hypofibrinolysis did not disappear after adjustment for all variables shown to influence CLTs in the control population. This again is an indication that the risk of DVT associated with prolonged CLTs reflects abnormalities in the fibrinolytic system. Unfortunately, levels of fibrinolytic proteins, such as plasminogen, α_2-antiplasmin, and PAI-1, as well as the PAI-1 4G/5G polymorphism, have not been measured in the LETS population. Consequently, potential denominators of the CLT in terms of proteins known to be important in fibrinolysis could not be elucidated from this study.

Our clot lysis assay is presumably not sensitive for variations in plasma levels of tPA or urokinase, because a fixed amount of exogenous tPA is added in the assay to induce clot lysis. Also, a contributory role of factor XIII in determining CLTs was excluded from the present study. No association between CLT and factor XIII activity or the a-subunit antigen level could be demonstrated. An association between the catalytically inactive factor XIII b-subunit and CLT was present, which may indicate that the b-subunit has an

Table 3. Risk of venous thrombosis according to CLTs

Cut-off, percentile	Lysis time at cut-off, min	No. of patients	No. of controls	OR (95% CI)
70	65.4	158	141	1.4 (1.1-1.8)
80	68.6	119	94	1.6 (1.2-2.1)
90	74.1	75	47	1.9 (1.3-2.9)
95	79.3	42	23	2.1 (1.3-3.6)
99	97.8	8	4	2.2 (0.7-7.5)

Cut-off levels of CLTs in the control group were set at the 70th, 80th, 90th, 95th, and 99th percentiles, and ORs for the development of DVT were calculated.

until now incompletely understood role in modulation of fibrinolysis. Thus, the outcome of our assay represents a tPA- and factor XIII-independent measure of the plasma fibrinolytic potential.

Our findings that hypofibrinolysis plays a role in the development of a first venous thrombosis is supported by a recent publication in which an asymptomatic venous thrombus was detected in 1% of a healthy population (n = 1213).[28] The fact that small, nonsymptomatic thrombi occur this frequently may explain why a defective fibrinolytic system, that is, the incapacity to remove these asymptomatic thrombi, is associated with a higher risk for thrombosis.

In conclusion, hypofibrinolysis, as measured by a tissue factor– and tPA-induced plasma-based clot lysis assay is a risk factor for the development of a first DVT. Whether this hypofibrinolytic state is determined by genetic or acquired factors, or a combination of these, and which proteins are involved is at present unknown.

Acknowledgments

References

1. Aoki N, Moroi M, Sakata Y, Yoshida N, Matsuda M. Abnormal plasminogen. A hereditary molecular abnormality found in a patient with recurrent thrombosis. J Clin Invest. 1978;61:1186-1195.

2. Brandt JT. Plasminogen and tissue-type plasminogen activator deficiency as risk factors for thromboembolic disease. Arch Pathol Lab Med. 2002;126:1376-1381.

3. Schuster V, Zeitler P, Seregard S, et al. Homozygous and compound-heterozygous type I plasminogen deficiency is a common cause of ligneous conjunctivitis. Thromb Haemost. 2001;85:1004-1010.

4. Francis CW. Plasminogen activator inhibitor-1 levels and polymorphisms. Arch Pathol Lab Med. 2002;126:1401-1404.

5. Ridker PM, Vaughan DE, Stampfer MJ, et al. Baseline fibrinolytic state and the risk of future venous thrombosis. A prospective study of endogenous tissue-type plasminogen activator and plasminogen activator inhibitor. Circulation. 1992; 85:1822-1827.

6. Crowther MA, Roberts J, Roberts R, et al. Fibrinolytic variables in patients with recurrent venous thrombosis: a prospective cohort study. Thromb Haemost. 2001;85:390-394.

7. van Tilburg NH, Rosendaal FR, Bertina RM. Thrombin activatable fibrinolysis inhibitor and the risk for deep vein thrombosis. Blood. 2000;95: 2855-2859.

8. Folsom AR, Cushman M, Heckbert SR, Rosamond WD, Aleksic N. Prospective study of fibrinolytic markers and venous thromboembolism. J Clin Epidemiol. 2003;56:598-603.

9. Prins MH, Hirsh J. A critical review of the evidence supporting a relationship between impaired fibrinolytic activity and venous thromboembolism. Arch Intern Med. 1991;151:1721-1731.

10. Lisman T, Leebeek FW, Mosnier LO, et al. Thrombin-activatable fibrinolysis inhibitor deficiency in cirrhosis is not associated with increased plasma fibrinolysis. Gastroenterology. 2001;121:131-139.

11. Mosnier LO, Lisman T, van den Berg HM, Nieuwenhuis HK, Meijers JCM, Bouma BN. The defective down regulation of fibrinolysis in haemophilia A can be restored by increasing the TAFI plasma concentration. Thromb Haemost. 2001; 86:1035-1039.

12. Lisman T, Mosnier LO, Lambert T, et al. Inhibition of fibrinolysis by recombinant factor VIIa in plasma from patients with severe haemophilia A. Blood. 2002;99:175-179.

13. von dem Borne PAKr, Bajzar L, Meijers JCM, Nesheim ME, Bouma BN. Thrombin-mediated activation of factor XI results in a thrombin-activatable fibrinolysis inhibitor-dependent inhibition of fibrinolysis. J Clin Invest. 1997;99:2323-2327.

14. Lisman T, Bijsterveld NR, Adelmeijer J, et al. Recombinant factor VIIa reverses the in vitro and ex vivo anticoagulant and profibrinolytic effects of fondaparinux. J Thromb Haemost. 2003;1:2368-2373.

15. Schutgens REG, Esseboom EU, Snijder RJ, et al. Low molecular weight heparin (dalteparin) is equally effective as unfractionated heparin in reducing coagulation activity and perfusion abnormalities during early treatment of pulmonary embolism. J Lab Clin Med. 2004;144:100-107.

16. Meijers JC, Middeldorp S, Tekelenburg W, et al. Increased fibrinolytic activity during use of oral contraceptives is counteracted by an enhanced factor XI-independent down regulation of fibrinolysis: a randomized cross-over study of two low-dose oral contraceptives. Thromb Haemost. 2000;84:9-14.

17. 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.

18. van der Meer FJ, Koster T, Vandenbroucke JP, Briët E, Rosendaal FR. The Leiden Thrombophilia Study (LETS). Thromb Haemost. 1997;78: 631-635.

19. Brunner J, Skrabal P, Hauser H. Single bilayer vesicles prepared without sonication. Physicochemical properties. Biochim Biophys Acta. 1976; 455:322-331.

20. Van Wijnen M, Stam JG, Chang GTG, et al. Characterization of mini-protein S, a recombinant variant of protein S that lacks the sex hormone binding globulin-like domain. Biochem J. 1998;330: 389-396.

21. Meijers JC, Tekelenburg WL, Bouma BN, Bertina RM, Rosendaal FR. High levels of coagulation factor XI as a risk factor for venous thrombosis. N Engl J Med. 2000;342:696-701.

22. van Hylckama Vlieg A, van der Linden IK, Bertina RM, Rosendaal FR. High levels of factor IX increase the risk of venous thrombosis. Blood. 2000;95:3678-3682.

23. Poort SR, Rosendaal FR, Reitsma PH, Bertina RM. A common genetic variation in the 3′-untranslated region of the prothrombin gene is associated with elevated plasma prothrombin levels and an increase in venous thrombosis. Blood. 1996; 88:3698-3703.

24. Bauer KA. Conventional fibrinolytic assays for the evaluation of patients with venous thrombosis: don't bother. Thromb Haemost. 2001;85:377-378.

25. Mosnier LO, von dem Borne PAKr, 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.

26. Colucci M, Binetti BM, Tripodi A, Chantarangkul V, Semeraro N. Hyperprothrombinemia associated with prothrombin G20210A mutation inhibits plasma fibrinolysis through a TAFI-mediated mechanism. Blood. 2004;103:2157-2161.

27. Al Dieri R, Peyvandi F, Santagostino E, et al. The thrombogram in rare inherited coagulation disorders: its relation to clinical bleeding. Thromb Haemost. 2002;88:576-582.

28. Schwarz T, Siegert G, Oettler W, et al. Venous thrombosis after long-haul flights. Arch Intern Med. 2003;163:2759-2764.