Assessment of Fibrinolytic Activity by Measuring the Lysis Time of a Tissue Factor-induced Clot: A Feasibility Evaluation

Anna Paola Cellai, Donatella Lami, Alberto Magi, Agatina Alessandrello Liotta, Angela Rogolino, Emilia Antonucci, Brunella Bandinelli, Rosanna Abbate and Domenico Prisco

CLIN APPL THROMB HEMOST 2010 16: 337 originally published online 30 December 2008
DOI: 10.1177/1076029608325542

Clinical and Applied Thrombosis/Hemostasis
Volume 16 Number 3
May/June 2010 337-344

10.1177/1076029608325542
http://cath.sagepub.com

Assessment of Fibrinolytic Activity by Measuring the Lysis Time of a Tissue Factor–induced Clot: A Feasibility Evaluation

Anna Paola Cellai, BSc, Donatella Lami, BSc, PhD, Alberto Magi, PhD, Agatina Alessandrello Liotta, BSc, Angela Rogolino, BSc, Emilia Antonucci, MD, Brunella Bandinelli, BSc, Rosanna Abbate, MD, and Domenico Prisco, MD

A clot lysis time assay in which a tissue factor–induced fibrin clot is lysed by exogenously added tissue plasminogen activator has been recently reported. We evaluated the feasibility of clot lysis time in a routine hemostasis laboratory, and its correlation with thrombin activatable fibrinolysis inhibitor and plasminogen activator inhibitor-1 levels and changes with aging in 185 healthy participants. Clot lysis time was assessed by monitoring changes in turbidity during clot formation and subsequent lysis using a computerized kinetic spectrophotometric microtiter plate. After preliminary experiments, 100 and 160 ng/mL tissue plasminogen activator concentrations were chosen for the study. Clot lysis time was calculated by a new mathematical analysis of the lysis curve based on discrete derivative. Clot lysis time, thrombin activatable fibrinolysis inhibitor, and plasminogen activator inhibitor-1 plasma levels showed a normal distribution. For both concentrations of tissue plasminogen activator, clot lysis time progressively increased with increase in age ($P < .0001$) and was significantly correlated with thrombin activatable fibrinolysis inhibitor antigen, thrombin activatable fibrinolysis inhibitor activity, and plasminogen activator inhibitor-1 antigen (at least $P < .01$). During linear regression analysis, thrombin activatable fibrinolysis inhibitor and plasminogen activator inhibitor-1 antigen were found to significantly influence clot lysis time (at least $P < .01$). Clot lysis time determination has a good laboratory performance. Our new method of calculation is independent of the time of reading and allows a more accurate and consistent detection of both short and prolonged lysis times. Our data suggest the feasibility of the use of this test in the work of routine hemostasis laboratory.

Keywords: clot lysis time; fibrinolysis; t-Pa; TAFI; PAI-1 antigen

Introduction

Coagulation and fibrinolysis are regulated by coordinated molecular events in which substrates, activators, and inhibitors are involved in the formation and degradation of fibrin. Plasminogen activator inhibitor-1 (PAI-1) and α2-antiplasmin (α2AP) are the key inhibitors of the fibrinolytic system and their target proteases are tissue plasminogen activator (t-PA), urokinase (u-PA), and plasmin. Thrombin

From the Department of Heart and Vessels, Azienda Ospedaliero-Universitaria Careggi (APC, AAL, AR) and Department of Medical and Surgical Critical Care, Thrombosis Center (DL, AM, EA, BB, RA, DP), University of Florence, Florence, Italy.

Address correspondence to: Anna Paola Cellai, Centro Trombosi – Malattie Aterotrombotiche, Piastra dei Servizi Azienda Ospedaliero-Universitaria Careggi, Viale Morgagni 85, 50134 Firenze, Italia; e-mail: annapaolacellai@tiscali.it.

activatable fibrinolysis inhibitor (TAFI), a plasma zymogen, is activated to yield an active carboxypeptidase (TAFIa). TAFIa regulates fibrinolysis by removing carboxyterminal lysine residues from fibrin. This enzyme acts as a modulator of fibrinolysis, whereas PAI-1 interacts directly with its targets, forming stable equimolar complexes. Recent observations suggest that, despite the differences in concentrations and activities of inhibitors and the different modes of action, the roles of fibrinolysis inhibitors are complementary in both plasma clot lysis and thrombus lysis.[1]

Few global tests of fibrinolytic activity, such as the euglobulin lysis time (ELT) and the dilute whole-blood clot lysis time (WBCLT), were used to assess the association of thrombosis and hypofibrinolysis, with inconclusive results.[2] Commonly used global fibrinolysis assays have major drawbacks. For example, the dilute WBCLT is performed in the absence of calcium, and a clot is formed by the addition of thrombin, therefore the lysis time is independent of coagulation process and, as a consequence, independent of TAFI. However, the ELT and the fibrin plate method use the euglobulin fraction (which includes only limited amounts of TAFI, PAI-1, and α2AP) so that the test is not representative of plasma concentration of fibrinolysis inhibitors.[3]

In patients with deep vein thrombosis enrolled in the Leiden Thrombophilia Study, low plasma fibrinolytic potential has been demonstrated to be a risk factor for venous thrombosis.[4] Very recently, these results were confirmed in a large population-based case-control study on venous thrombosis, the Multiple Environmental and Genetic Assessment—MEGA—trial,[5] in which it was also reported that the combination of hypofibrinolysis with oral contraceptive use, immobilization or factor V Leiden, results in a risk of venous thrombosis exceeding the sum of the individual risks. The test used in these studies is a plasma-based test, Clot Lysis Time (CLT), in which a tissue factor–induced fibrin clot is lysed by exogenously added t-PA. Although this assay was initially developed to study TAFI-related processes, it is also influenced by the levels of other proteins involved in fibrinolysis and thrombin generation (which is required for TAFI activation), including plasminogen, α2-AP, PAI-1, and antithrombin.[6] The outcome of this assay therefore reflects the plasma fibrinolytic potential. In addition to its use to assess hypofibrinolysis,[4] this test has been used to study hyperfibrinolysis in a number of conditions such as liver cirrhosis, hemophilia, and factor XI deficiency.[7-10]

Table 1. Clinical and Laboratory Characteristics of Participants

N	185
M/F, n	98/87
Age, years	44.5 ± 13.9
Smoking habit, %	28.2
BMI	22.5 ± 4.3
Erythrocytes, $\times 10^{12}$/L	4.3 ± 1.2
Leukocytes, $\times 10^{9}$/L	8.4 ± 2.8
Hemoglobin, mg/dL	13.6 ± 2.0
Hematocrit, %	40.6 ± 6.7
Platelet, $\times 10^{9}$/L	255 ± 85
Total cholesterol, mg/dL	207 ± 28
HDL cholesterol, mg/dL	53 ± 14
LDL cholesterol, mg/dL	135 ± 27
Triglycerides, mg/dL	113 ± 56

NOTES: BMI = body mass index; HDL = high density lipoprotein; LDL = low density lipoprotein.

In the light of its possible extensive clinical use, we aimed at evaluating the feasibility of CLT determination in a routine hemostasis laboratory and propose a new method for calculating CLT.

Materials and Methods

Participants Investigated

We studied 185 clinically healthy participants who were recruited from the members of Hospital and University staff and their relatives. In Table 1, clinical and laboratory characteristics of participants are reported. Exclusion criteria were pregnancy, use of estrogen pills, or hormone replacement therapy and diabetes. Approval for this study was obtained from the local Institutional Review Board, and informed consent was provided according to the Declaration of Helsinki.

The participants were divided into 5 classes based on their age: 24 years or younger (class 0), 25 to 34 years (class 1), 35 to 44 years (class 2), 45 to 54 years (class 3), and ≥55 years (class 4).

Blood samples from the antecubital vein were collected after overnight fasting between 8 AM and 10 AM, in vacutainer tubes containing 0.109 M trisodium citrate (1:10, v/v, anticoagulant: blood; Becton Dickinson, Plymouth, United Kingdom). Tubes were immediately centrifuged at 1500*g* for 10 minutes, and plasma was separated, snap frozen in small portions, and stored at −70°C until the assays were performed.

Clot Lysis Time

The lysis of a tissue factor–induced clot by exogenous t-PA was studied by monitoring changes in turbidity during clot formation and subsequent lysis according to Lisman et al,[4] with some changes, in particular, in t-PA concentrations used and CLT calculation model. Briefly, plasma (100 µL) was pipetted into a microtiter plate, after which 100 µL of a mixture containing tissue factor (diluted Innovin; Dade Behring, Marburg, Germany; 1/1000 final dilution), $CaCl_2$ (final concentration 17 mmol/L), t-PA (Actilyse; Boehringer Ingelheim, Germany; 50-180 ng/mL, final concentration, see below), and phospholipids (lyophilized bovine brain phospholipids from chloroform extract resuspended in distilled water; Cabru, Arcore, Italy; final concentration 10 µM) diluted in HEPES (*N*-2-hydroxytethylpiperazine-*N*'-2-ethanesulfonic acid) buffer (25 mmol/L HEPES, 137 mmol/L NaCl, 3.5 mmol/L KCl, 3 mmol/L $CaCl_2$, 0.1% bovine serum albumin, pH 7.4) was added. After thorough mixing, the plate was incubated at 37°C in TECAN Infinite M 200, Grodig, Austria, UE, and the changes in the optical density (OD) at 405 nm were monitored every minute for the first 15 minutes (to evaluate clotting time) and every 5 minutes thereafter up to 3 hours (to evaluate lysis time). For each sample, CLT was determined in duplicate. Clot lysis time 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 the clear transition, which represents clot lysis. Fifty percent CLT was defined as the time between 50% clotting and 50% lysis, that is, the time between the half-peak heights.

Preliminary experiments were conducted on 6 healthy participants to choose the optimal t-PA concentration for use in our studies. For this purpose, concentrations ranging from 50 to 180 ng/mL of t-PA in HEPES buffer were used. In addition, preliminary experiments were performed to evaluate if different centrifugation rates might influence CLT results. Five samples were evaluated and for each of them 3 aliquots of plasma were obtained and centrifuged: (*a*) once at 1500*g* for 10 min; (*b*) once at 2000*g* for 10 min; and (*c*) first at 1500*g* for 10 min and then at 6000*g* for 3 minutes. Finally, to evaluate the effect of temperature of centrifugation on CLT results, 5 samples were centrifuged at different rates (1500*g*, 2000*g*, 1500*g* + 6000*g*) either at +4°C or +22°C.

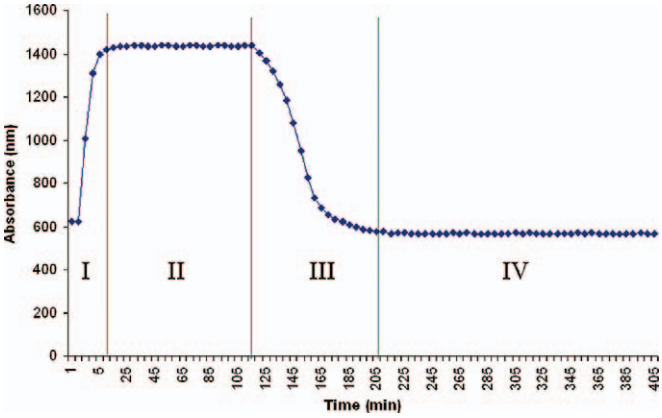

Figure 1. Typical example of a clot lysis profile (CLP) in normal plasma. The curve was generated by plotting absorbance at 405 nm (*y*-axis) as a function of time (*x*-axis). Coagulation was initiated by adding tissue factor, phospholipids, and calcium chloride. Fibrinolysis was initiated by the addition of tissue plasminogen activator. Clot lysis profile has 4 parts: clot formation (I), latency (II), clot dissolution (III), and latency again (IV). See text for details.

Clot Lysis Profile Analysis

As mentioned above, clot lysis profile (CLP) was generated by plotting A405 as a function of time. Different authors[4,11-13] suggest to calculate the CLT as a function of the maximum (maximum turbid transition) and the minimum (the clear transition) of the absorbance of the CLP (Minimum/Maximum method). A typical model of CLP (reported in Figure 1) can be divided into 4 distinct parts: clot formation (increase in turbidity), latency (when the OD is steady at maximum), clot dissolution (decrease in turbidity), and latency again (when the OD is steady at minimum).

However, CLP of some samples are a noisy version of the model described above, and the identification of both the maximum turbid transition point and clear transition point may be difficult. In particular, the identification of the maximum turbid transition point is dependent on the shape of the latency part of the signal. Actually, maximum turbid transition might be located either at the beginning or at the end of the latency part of the CLP, although CLPs may have a similar shape (Figure 2). Similarly, the identification of the clear transition point of the CLP after the clot dissolution might give similar problems

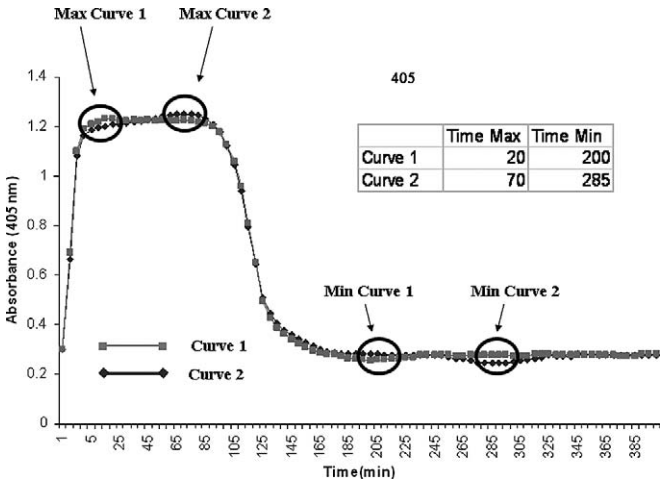

Figure 2. An example of 2 clot lysis profiles (CLP) that have a very similar shape but different time points of maximum turbid transition and clear transition. These differences are due to small variations of the 2 latency parts of the signals (see circles). In particular, the small variations of absorbance in the first part of the pink curve (Max curve 1) led to identify the maximum turbid transition 26 minutes before vs. the blue curve (Max curve 2). Similar differences were found for the identification of the time of clear transition (Min curve1 and Min curve 2). A table reporting the maximum turbid transition and clear transition for the 2 curves is also reported.

(Figure 2). Despite the similar shape of 2 curves, the detection of the maximum and minimum turbid transition points could affect the measurement of the CLT.

For these reasons, we decided to study the time interval between the maximum rate of clot formation and the maximum rate of clot lysis of the CLP. Because the absorbance is a direct measure of fibrin formation and lysis, the rate of these reactions can be calculated by means of the discrete derivative of the CLP: the maximum rate of clot formation corresponds to the maximum of the discrete derivative, whereas the maximum rate of clot lysis corresponds to the minimum of the discrete derivative. To calculate CLT from CLP, we have built a simple algorithm in R language.[14]

Clot lysis profile analysis based on the discrete derivative was applied on 36 participants and compared with the Minimum/Maximum method of measurement across the time of 3, 4, and 6 hours of reading.

Other Laboratory Assays

Plasma TAFI antigen (ag) level was measured with a commercially available ELISA kit (Asserachrom TAFI, Diagnostica Stago, Asnieres, France) that uses 2 specially selected monoclonal antibodies (MAb)-F(ab')2 of 1B1 as a capturing MAb and horse radish peroxidase (HRP)–labeled P4C2 as a detecting MAb. This makes this assay specific only for TAFI with little or no reactivity with other TAFI isoforms. The assay is calibrated with a preparation of freeze-dried TAFI of a known concentration and is insensitive to the Ala147Thr and Thr325Ile polymorphisms.[15] The intraassay and interassay coefficients of variability were 3.2% and 6.2%, respectively.

Plasma TAFI activity (act) was measured by a chromogenic assay (Actichrome TAFI activity, American Diagnostica, Greenwich, Connecticut). Platelet-poor plasma was incubated with TAFI activation reagent (thrombin–thrombomodulin complex) and TAFI act was then determined using a highly specific TAFI substrate. Similar measurements performed without the addition of thrombin–thrombomodulin were used to calculate plasma carboxypeptidase *N* activity. The values obtained from nonactivated plasma were subtracted from values obtained after activation with the thrombin–thrombomodulin complex to quantify the specific TAFI act levels. TAFI act concentration was determined by extrapolation of the absorbance taken from a TAFI act standard curve. Results are expressed as microgrammi/mL. The intraassay and interassay coefficients of variability were 6.0% and 9.0 %, respectively.

Plasma PAI-1 antigen (ag) levels were determined using a commercially available ELISA kit (Asserachrom PAI-1). The intraassay and interassay coefficients of variability were 5.5% and 6.5%, respectively.

Statistical Analysis

All analyses were performed by SPSS (Statistical Package for Social Sciences; Chicago, Illinois, software for Windows; version 11.0). Unless otherwise stated, data are expressed as mean $\pm$ SD. Analysis of variance (ANOVA) test was performed through different concentrations of t-PA. Kruskall-Wallis test was performed to study the variations of CLT, TAFI ag, TAFI act, and PAI-1 ag with age. Correlations between continuous variables were calculated with Pearson's product–moment correlation coefficients. A 2-sided P value $< .05$ was considered statistically significant. Log-transformed values for CLT, TAFI ag, TAFI act, and PAI-1 ag were used to asses linear

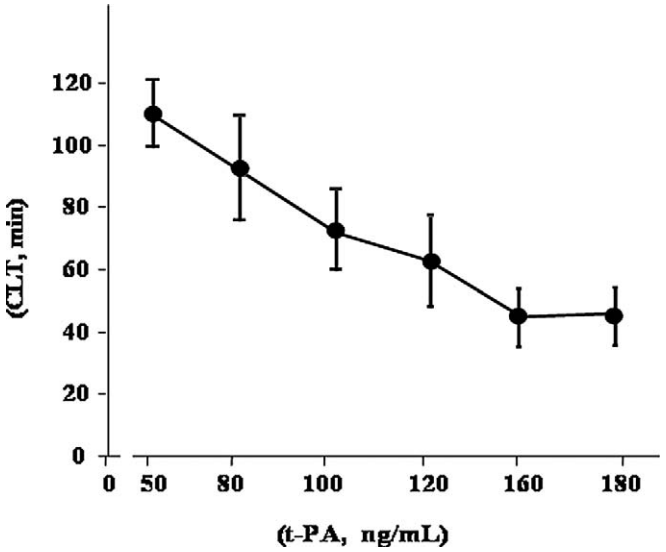

Figure 3. Tissue plasminogen activator dose–response curve for CLT. Plasma obtained from the same samples was titrated in vitro with the indicated t-PA concentration. Data are expressed as mean ± (error bars). Clot lysis time values progressively decrease for increasing t-PA concentrations (ANOVA: $P < .001$). CLT, clot lysis time; t-PA, tissue plasminogen activator.

regression analyses to evaluate the association of CLT with TAFI ag, TAFI act, and PAI-1 ag. Results were expressed as regression coefficient (β) ± standard error (SE).

Results

Clot lysis time Assay Conditions

Tissue plasminogen activator concentrations of 50 to 180 ng/mL in HEPES buffer were used, and mean CLTs ranging from 109.9 to 46.3 minutes were obtained with a progressive decrease in relation to t-PA concentration ($F = 37.8$, $P < .001$; Figure 3). The t-PA concentrations of 100 and 160 ng/mL were chosen for further studies.

In relation to the effect of centrifugation either at 4°C or 22°C and at different rates (1500*g*, 2000*g*, 6000*g*) on CLT (t-PA, 100 ng/mL), no differences were found (data not shown) so that further studies were performed by centrifuging samples at 1500*g* at +22°C.

Precision analysis of CLT was tested by using normal plasmas pooled from 20 healthy volunteers. The intraassay and interassay coefficients of variations were 3.0% (71.2 ± 2.2, n = 14) and 4.0% (71.8 ± 2.9, n = 7), respectively, with t-PA concentration of 100 ng/mL, and 2.8% (44.3 ± 1.2, n = 14) and 3.9% (42.0 ± 1.6, n = 7), respectively, with t-PA concentration of 160 ng/mL.

Clot lysis time values calculated by using the maximum and the minimum of the CLP (see "Materials and Methods" section for details) were significantly different (P at least <.0001) across the 3 reading times, whereas no difference was observed by using derivative method (Table 2). Significant relationships between the 2 calculation methods were observed (at least $r = .96$, $P < .0001$) at all reading times considered.

Influence of gender, age, TAFI ag, TAFI act, and PAI-1 ag on CLT and other fibrinolysis-related parameters.

In participants investigated, CLT, TAFI ag, TAFI act, and PAI-1 ag showed a normal distribution of values (Table 3A). No differences were found between men and women in both t-PA concentrations (t-PA, 100 ng/mL: 72.1 ± 15.4 and 71.4 ± 15.2 minutes respectively; t-PA, 160 ng/mL: 46.3 ± 10.3 and 45.3 ± 11.3 minutes, respectively). Clot lysis time progressively increased with increase in age (t-PA, 100 ng/mL: $r = .37$, $P < .0001$; t-PA, 160 ng/mL: $r = .35$, $P < .0001$ (Table 3B).

Clot lysis time was significantly correlated with TAFI ag and TAFI act using t-PA at both 100 and 160 ng/mL concentrations (TAFI ag: $r = .35$, $P < .0001$ and $r = .40$ $P < .001$, respectively; TAFI act: $r = .19$, $P = .04$ and $r = .31$ $P = .001$, respectively). Clot lysis time was also significantly correlated with PAI ag at t-PA concentrations of both 100 and 160 ng/mL ($r = .40$, $P < .0001$ and $r = .27$ $P = .007$, respectively).

During linear regression analysis, TAFI ag and PAI ag were found to significantly influence CLT (β ± SE: 0.29 ± 0.07; $P < .001$ and 0.11 ± 0.03; $P < .0001$), whereas no significant association was found between CLT and TAFI act levels (Table 4). As a whole, PAI-1 and TAFI levels account for one thirds of CLT variability. No correlation was found between PAI ag and both TAFI ag and TAFI act. TAFI ag and TAFI act progressively increased with increase in age ($P < .001$ for both); PAI-1 ag levels showed only a trend to increase with age (Table 3B).

Table 2. Comparison of Mean (±SD) Values of CLT Obtained Using Maximum/Minimum Method with the Derivative Method Proposed in This Article at 3, 4, and 6 hours of Clot Lysis Curve

	3 hours	4 hours	6 hours
Derivative method	77.6 ± 26.7	77.6 ± 26.7	77.6 ± 26.6
Minimum/Maximum method	79.8 ± 19.9[a,b]	85.5 ± 24.18[a,b]	93.3 ± 30.3[a,b]

P values for the differences between the means are also reported.
Minimum/Maximum method: [a] 3 hours vs. 4 hours, $P < .0001$; [a] 3 hours vs. 6 hours, $P < .0001$; [a] 6 hours vs. 4 hours, $P < .0001$.
Minimum/Maximum vs. Derivative method: [b] 4 hours vs. 4 hours, $P < .0001$; [b] 6 hours vs. 6 hours, $P < .0001$; [b] 3 hours vs. 3 hours, $P < .0001$.

Table 3a. Fibrinolytic Parameters of Participants Investigated

	Mean ± SD	Median (Range)
CLT (t-PA, 100 ng/mL), min	70.0 ± 15.6	70.0 (27.2-127.0)
CLT (t-PA, 160 ng/mL), min	44.5 ± 13.9	42.0 (18.1-84.5)
TAFI act, μg/mL	18.3 ± 5.8	18.1 (8.0-34.9)
TAFI ag, μg/mL	10.2 ± 2.0	10.3 (6.1-14.6)
PAI-1 ag, ng/mL	39.1 ± 17.41	38.0 (10.4-77.3)

NOTES: CLT = clot lysis time; t-PA = tissue plasminogen activator; TAFI act = thrombin activatable fibrinolysis inhibitor activity; TAFI ag = thrombin activatable fibrinolysis inhibitor antigen; PAI-1 ag = plasminogen activator inhibitor antigen.

Discussion

In this study, we evaluated a number of laboratory variables to establish the optimal conditions to use CLT in assessing fibrinolytic activity in a routine hemostasis laboratory. The relevance of fibrinolytic system in thrombosis has long been reported, but global fibrinolytic tests have been difficult to standardize. Lisman et al[4] demonstrated a clear association between plasma hypofibrinolysis and the risk of venous thrombosis using an assay in which a tissue factor–induced clot is lysed after the addition of exogenous t-PA, and very recently, the results of MEGA study are the background for a future possible extensive use of CLT in the clinical approach to thrombophilia. Because of the potential application of these results in the study of thrombophilia, we evaluated for the first time the feasibility of such test for clinical application in a routine laboratory of an Italian Thrombosis Center.

A progressive shortening of CLT was found by increasing t-PA from 50 to 160 ng/mL (CLT from 110 to 45 minutes, with a 58% shortening), whereas CLT values at 160 and 180 ng/mL were similar, hence indicating that the concentration of t-PA ≥160 ng/mL allows to reach the maximal extent of lysis (about 45min). Such short values, however, would hamper the capability of the system to detect an activation of fibrinolysis. However, by using the 2 lowest concentrations (50 and 80 ng/mL) as suggested by Lisman et al and Colucci et al,[4-6,13] CLT was longer than previously reported and, probably, in these conditions, the capability of the system to investigate states of hypofibrinolysis would not be appropriate. Because the clinical application of this assay is also the detection of hypofibrinolysis, we evaluated CLT in 185 participants using both 100 and 160 ng/mL t-PA concentrations, and our preliminary data obtained in 6 participants were confirmed.

After these preliminary experiments, we chose the t-PA concentration of 100 ng/mL as the one able to obtain in healthy participants a mean CLT of about 60 to 70 minutes, a reasonably suitable time to detect both hyperfibrinolysis and hypofibrinolysis.

In preliminary tests, various rates of centrifugation performed at various temperatures were assessed to evaluate if different conditions could affect CLT because of the presence of residual platelets in plasma. Because no difference was found, a single centrifugation at 1500*g* at room temperature was chosen to facilitate a routine application of the method.

The test requires about 3½ hours, but up to 48 samples in duplicate can be assayed in a single run. The intraassay and interassay coefficients of variations observed indicate a good reproducibility of the test.

In this study, we also observed the effect of aging on CLT. As expected, there is a progressive increase of CLT from young participants <24 years to those >55 years. The progressive reduction of fibrinolysis activity with aging is well known,[16] and it has been attributed, at least in part, to PAI-1 increase. The expression of PAI-1 is not only elevated in the elderly participants but also induced in a variety of pathologies associated with the process of aging, such as

Table 3b. Fibrinolytic Parameters of Participants Investigated and Their Behavior in Relation to the Different Age-classes

Age, years	n	CLT, min		TAFI ag, μg/mL	TAFI act, μg/mL	PAI-1 ag, ng/mL
		t-PA, 100 ng/mL	t-PA, 160 ng/mL			
<25	12	58.9 ± 7.5	39.4 ± 9.4	9.3 ± 1.9	13.5 ± 4.4	31.3 ± 8.7
25-34	36	64.6 ± 9.4	42.5 ± 6.7	9.7 ± 1.7	17.0 ± 5.3	37.3 ± 21.1
35-44	61	71.6 ± 13.6	43.9 ± 9.2	9.9 ± 2.0	18.7 ± 6.9	39.7 ± 17.5
45-54	32	76.5 ± 18.6	47.6 ± 12.3	10.3 ± 1.7	19.4 ± 5.6	41.5 ± 12.0
≥55	43	78.4 ± 16.3	51.9 ± 12.1	11.4 ± 1.7	19.9 ± 2.7	43.3 ± 16.5

NOTES: CLT = clot lysis time; t-PA = tissue plasminogen activator; TAFI act = thrombin activatable fibrinolysis inhibitor activity; TAFI ag = thrombin activatable fibrinolysis inhibitor antigen, PAI-1 ag = plasminogen activator inhibitor antigen.

Table 4. Multiple Linear Regression Analysis on Variables Which Can Influence Clot Lysis Time[a]

	β ± SE	*P*
TAFI act, μg/mL	−0.002 ± 0.05	.96
TAFI ag, μg/mL	0.29 ± 0.07	<.0001
PAI-1 ag, ng/mL	0.11 ± 0.03	<.002

NOTES: TAFI act = thrombin activatable fibrinolysis inhibitor activity; TAFI ag = thrombin activatable fibrinolysis inhibitor antigen; PAI-1 ag = plasminogen activator inhibitor antigen.

obesity, insulin resistance, emotional stress, immune responses, and vascular sclerosis/remodeling.[17,18] We found a significant correlation between CLT and PAI-1 ag, hence showing the influence of high PAI-1 levels on CLT. On the contrary, the role of aging in determining changes in TAFI levels is less clear.[12,19,20]

In our study, in agreement with Lisman et al,[4] we confirmed a moderate association of both TAFI and PAI-1 levels with CLT. Interestingly, in our model, one thirds of CLT variability was explained by these parameters. Previously, it has been shown that the addition of purified TAFI to TAFI-depleted plasma dose-dependently prolonged CLT.[21] Moreover, an association between TAFI ag levels and CLT was reported in plasma samples from 20 healthy volunteers.[21] Thus, CLT mirrors plasma fibrinolytic capacity, which is determined by the balance of the levels of different fibrinolytic components. However, this clot lysis assay has been shown to be insensitive to variations in plasma levels of t-PA or u-PA, because a fixed amount of exogenous t-PA is added in the assay to induce clot lysis. In addition, a contributory role of factor XIII in determining CLT was excluded by Lisman et al.[4] Thus, this assay supplies a t-PA and factor XIII–independent measure of the plasma fibrinolytic activity.

In this study, we also faced the issue of CLT calculation by introducing a new model based on discrete derivative. Interestingly, our method is independent of the time of reading and allows a more accurate and consistent detection of both short and prolonged lysis times.

This approach may be useful for laboratory routine applications because samples obtained from patients with states of hyperfibrinolysis, normal fibrinolysis, or hypofibrinolysis may need a different time of reading.

In the past, methods for evaluating fibrinolytic activity have been laborious and complex, hence precluding their introduction in clinical practice. This new assay represents an innovative approach to thoroughly investigate different mechanisms of thrombophilia.

In conclusion, these results indicate that CLT determination, as adapted in the present study, has an acceptable performance, which suggests the feasibility of its use for routine diagnostic purpose. Further studies, however, are needed to demonstrate the usefulness of this evaluation in the clinical management of thrombotic disease.

References

1. Mutch NJ, Thomas L, Moore NR, Lisiak KM, Booth NA. TAFIa, PAI-1 and alpha-antiplasmin: complementary roles in regulating lysis of thrombi and plasma clots. *J*. Thromb Haemost. 2007;5:812-817.
2. 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.
3. Smith AA, Jacobson LJ, Miller BI, Hathaway WE, Manco-Johnson MJ. A new euglobulin clot lysis assay for global fibrinolysis. *Thromb Res*. 2003;112:329-337.

4. Lisman T, de Groot PG, Meijers JC, Rosendaal FR. Reduced plasma fibrinolytic potential is a risk factor for venous thrombosis. *Blood*. 2005;105:1102-1105.
5. Meltzer ME, Lisman T, Doggen CJ, de Groot PG, Rosendaal FR. Synergistic effects of hypofibrinolysis and genetic and acquired risk factors on the risk of a first venous Thrombosis. *PLoS*. Med. 2008;5:e97.
6. 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.
7. Von dem Borne PA, Bajzar L, Meijers JC, 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.
8. 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.
9. Lisman T, Mosnier LO, Lambert T, Mauser-Bunschoten EP, Meijers JC, Nieuwenhuis HK, et al. Inhibition of fibrinolysis by recombinant factor VIIa in plasma from patients with severe haemophilia A. *Blood*. 2002;99:175-179.
10. Colucci M, Binetti BM, Branca MG, et al. Deficiency of thrombin activatable fibrinolysis inhibitor in cirrhosis is associated with increased plasma fibrinolysis. *Hepatology*. 2003;38:230-237.
11. Klement P, Liao P, Bajzar L. A novel approach to arterial thrombolysis. *Blood*. 1999;94:2735-2743.
12. Stromqvist M, Schatteman K, Leurs J, et al. Immunological assay for the determination of procarboxypeptidase U antigen levels in human plasma. *Thromb Haemost*. 2001;85:12-17.
13. 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.
14. Gentleman RC, Carey VJ, Bates DM, et al. Bioconductor: open software development for computational biology and bioinformatics. *Genome Biol*. 2004;5:R80.
15. Frère C, Tregouet DA, Morange PE, et al. Fine mapping of quantitative trait nucleotides underlying thrombin-activatable fibrinolysis inhibitor antigen levels by a trans-ethnic study. *Blood*. 2006;108:1562-1568.
16. Franchini M. Hemostasis and aging. *Crit Rev Oncol Hematol*. 2006;60:144-151.
17. Lahlou-Laforet K, Alhenc-Gelas M, Pornin M, et al. Relation of depressive mood to plasminogen activator inhibitor, tissue plasminogen activator, and fibrinogen levels in patients with versus without coronary heart disease. *Am J Cardiol*. 2006;97:1287-1291.
18. Asselbergs FW, Williams SM, Hebert PR, et al. Gender-specific correlations of plasminogen activator inhibitor-1 and tissue plasminogen activator levels with cardiovascular disease-related traits. *J Thromb Haemost*. 2007;5:313-320.
19. Chetaille P, Alessi MC, Kouassi D, Morange PE, Juhan-Vague I. Plasma TAFI antigen variations in healthy subjects. *Thromb*. Haemost. 2000;83:902-905.
20. Juhan-Vague I, Renucci JF, Grimaux M, et al. Thrombin-activatable fibrinolysis inhibitor antigen levels and cardiovascular risk factors. *Arterioscler Thromb Vasc Biol*. 2000;20:2156-2161.
21. Mosnier LO, von dem Borne PA, Meijers JC, Bouma BN. Plasma TAFI levels influence the CLT in healthy individuals in the presence of an intact intrinsic pathway of coagulation. *Thromb Haemost*. 1998;80:829-835.