

# Lysis Onset Time as Diagnostic Rotational Thromboelastometry Parameter for Fast Detection of Hyperfibrinolysis

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## ABSTRACT

**Background:** Rotational thromboelastometry is increasingly used to detect hyperfibrinolysis, which is a predictor of unfavorable outcome in patients with coagulation disturbances. In an *in vitro* study, the authors investigated which thromboelastometric hemostatic parameters could be valuable for fast diagnosis of the severity of hyperfibrinolysis and confirmed their findings in a patient population with hyperfibrinolysis.

**Methods:** Thromboelastometry was performed after adding increasing concentrations of tissue plasminogen activator (0 to 400 ng/ml) to citrated blood samples of 15 healthy volunteers. Lysis parameters included the clotting time, maximum clot firmness, maximum lysis, and lysis onset time (LOT). The relation of tissue plasminogen activator with the LOT was further investigated in a patient population with out-of-hospital cardiac arrest and hyperfibrinolysis.

**Results:** The LOT showed a dose-dependent association with increasing tissue plasminogen activator concentrations. Late, intermediate, or fulminant hyperfibrinolysis was associated with an average LOT (mean  $\pm$  SD) of  $42.7 \pm 13.8$ ,  $23.2 \pm 8.2$ , and  $17.5 \pm 4.6$  min in the *in vitro* study and estimated  $42.2 \pm 8.3$ ,  $29.1 \pm 1.2$ , and  $14.6 \pm 7.7$  min in patients, respectively. The authors found a moderately negative correlation between patient plasma tissue plasminogen activator levels and the LOT ( $r = -0.67$ ;  $P = 0.01$ ).

**Conclusion:** This study shows that the LOT may be used for fast detection of severe hyperfibrinolysis, with a better resolution than the maximum lysis, and should be further evaluated for optimization of therapeutic strategies in patients with severe clot breakdown. (*ANESTHESIOLOGY* 2014; 121:89-97)

UNDER physiological conditions, blood clot lysis is mainly driven by tissue plasminogen activator (t-PA), which converts plasminogen into plasmin and leads to breakdown of fibrin networks.<sup>1</sup> In case of major surgery, trauma,<sup>2,3</sup> or shock,<sup>4,5</sup> the rate of clot breakdown may exceed clot formation, which is reflected by increased t-PA and D-dimer levels and hyperfibrinolysis.<sup>1,6,7</sup> In addition, previous research showed that t-PA could reduce platelet aggregation and induce platelet-related plasmin release, suggesting that platelets might have a profibrinolytic function through t-PA release.<sup>7-9</sup>

Fast detection of excessive clot breakdown may play a crucial role in patients at risk for hyperfibrinolysis. Except for the time-consuming D-dimer test, excessive clot lysis is currently not well detected by routine laboratory coagulation tests. Rotational thromboelastometry is a point-of-care hemostasis monitoring device that determines the properties of clot initiation, formation, and breakdown over time in

### What We Already Know about This Topic

- Rotational thromboelastometry is a point-of-care hemostasis monitoring device that determines the properties of clot initiation, formation, and breakdown over time in whole blood
- In an *in vitro* assay, this study investigated which thromboelastometric hemostatic parameters could be valuable for fast diagnosis of the severity of hyperfibrinolysis and then confirmed the findings in a patient population with hyperfibrinolysis

### What This Article Tells Us That Is New

- This study shows that the continuous parameter lysis onset time may be used for fast detection of severe hyperfibrinolysis, with a better resolution than the maximum lysis index

whole blood.<sup>2-5,10</sup> Previously, the maximum lysis (ML) index was the most commonly used marker to qualify hyperfibrinolysis using rotational thromboelastometry. Alternatively, we recently showed that the continuous parameter lysis onset time (LOT), which represents the time to initiation of fibrinolysis

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during thromboelastometry, may provide more detailed information regarding lysis severity than that by the ML.<sup>4</sup> Whether the LOT permits a faster detection of hyperfibrinolysis than the ML has however never been investigated.

It has previously been shown that increasing concentrations of t-PA affect the level of fibrinolysis in an *in vitro* set-up using thromboelastography.<sup>11,12</sup> The current study investigated the relation between incremental concentrations of t-PA with the level of hyperfibrinolysis defined by the ML and LOT using rotational thromboelastometry in an *in vitro* study and investigated the effects of t-PA on platelet aggregation. Moreover, we evaluated whether the use of the onset time of lysis may reduce the time to diagnose hyperfibrinolysis when compared with other severity markers for excessive clot breakdown and expanded our findings to a patient population with out-of-hospital cardiac arrest and hyperfibrinolysis.

## Materials and Methods

### Healthy Volunteers

The Human Subjects Committee of VU University Medical Center, Amsterdam, The Netherlands (NL40763.029.12), approved the observational, single-center study in healthy volunteers, and written informed consent was obtained from each participant before blood sampling. Blood samples were collected from 15 adult subjects with ages between 18 and 40 yr. Exclusion criteria were known hemostatic deficiencies, use of anticoagulant medication, and pregnancy.

### Patient Population

From two prospective observational studies in adult patients with nontraumatic out-of-hospital cardiac arrest undergoing cardiopulmonary resuscitation by the emergency medical service on hospital admission, we selected 33 patients with hyperfibrinolysis to examine whether the LOT may be faster in the diagnosis of hyperfibrinolysis compared with other markers of excessive clot breakdown.<sup>4</sup> The first study included 30 patients with 16 patients suffering from hyperfibrinolysis, and part of these data were previously published by Viersen *et al.*<sup>4</sup> The second study included 57 patients, and 17 patients had hyperfibrinolysis (study number NL39831.029.12). Both studies were approved by the local Human Subjects Committee of the VU University Medical Center, Amsterdam, The Netherlands. Relatives of patients provided written informed consent. Exclusion criteria were the absence of a peripheral intravenous catheter, pregnancy, the use of anticoagulation medication (vitamin K antagonists, clopidogrel, or dabigatran), and a traumatic origin of cardiac arrest. The study procedure included blood sampling on arrival of the patient at the shock room of the VU University Medical Center in Amsterdam, The Netherlands.

### Study Procedures

In healthy subjects, blood was sampled from a single venous puncture through a vacutainer system using six 4.5-ml sodium citrate-containing tubes (total volume of 27 ml).

Blood samples were divided for the distinct laboratory and point-of-care measurements. Recombinant t-PA was added to untreated blood samples in the following concentrations: 0, 50, 100, 200, 300, and 400 ng/ml (Actilyse®; Boehringer Ingelheim Pharma, Ingelheim, Germany) to induce fibrinolysis. The t-PA concentrations were based on previous research showing that a concentration of 100 ng/ml causes a ML of approximately 15%,<sup>11</sup> which corresponds with the category of late hyperfibrinolysis as described by Schöchel *et al.*<sup>2</sup>

In patients, venous blood samples were drawn on admission to the emergency department. Patient coagulation parameters included the international normalized ratio of the prothrombin time, activated partial thromboplastin time, platelet count, fibrinogen (Clauss test), and D-dimers (normal D-dimer levels are less than 0.5 mg/l), which were performed by the central laboratory for clinical chemistry.

### Rotational Thromboelastometry

Rotational thromboelastometry (ROTEM®; TEM International GmbH, Munich, Germany) is a reagent-supported point-of-care device that provides information about the viscoelastic properties of the clot in citrated whole blood during formation and lysis. The methodology of ROTEM® has previously been described in detail.<sup>13</sup> Thromboelastometry was performed at 37°C and a normal pH range. The Star-TEM reagent was used to neutralize citrate. The effect of t-PA on *in vitro* clot lysis and lysis characteristics in patients were evaluated using the EXTEM test for the extrinsic coagulation, which is primed by rabbit brain tissue factor. Moreover, the FIBTEM test was used to analyze the effect of t-PA on the fibrin part of clot formation. The FIBTEM test is an EXTEM-based assay that uses cytochalasin D to inhibit the platelet contribution to clot formation. The APTEM test, which consists of the EXTEM test in the presence of the antifibrinolytic aprotinin, was used to evaluate clot formation under inhibition of plasminogen. Improvement of clot formation in the APTEM test when compared with the EXTEM test allows the detection of excessive clot lysis. The platelet component of the clot strength is measured as EXTEM MCE minus FIBTEM MCE, in which maximal clot elasticity (MCE) was calculated using the following formula: (maximum clot firmness [MCF] × 100)/(100 – MCF).<sup>14</sup>

### Definition of Hyperfibrinolysis in Out-of-hospital Patients with Cardiac Arrest

Hyperfibrinolysis in patients with out-of-hospital cardiac arrest was diagnosed using rotational thromboelastometry. Hyperfibrinolysis was defined as a ML exceeding 15% within 60 min after the initiation of the thromboelastometric measurement or complete absence of clot formation.<sup>4</sup>

### Fibrinolytic Parameters

Fibrinolytic parameters included the lysis index (LI), ML, and LOT. The ROTEM® software automatically provides the fibrinolytic parameters. The LI is the percentage

of remaining clot stability in relation to the MCF value at 30 or 45 min after the clotting time (CT), whereas the ML describes the relative reduction in MCF due to clot lysis. The LI and ML are expressed as percentages. The LOT is the time from the start of the coagulation reaction to the point that a clot lysis of 15% or more is reached.

### Platelet Aggregation

Platelet aggregation was assessed using whole blood electrical impedance aggregometry (Chrono-log® CH592A whole blood aggregometry; Stago BNL, Leiden, The Netherlands). Whole blood aggregometry tests were performed with the aforementioned concentrations of t-PA. The Chrono-log device measures platelet aggregation by determining the electrical impedance reflected as the slope of the aggregometry curve, which is influenced by platelet adhesion to the electrodes. Electrical impedance aggregometry was performed after a 5-min incubation period of 1 ml of 1:1 diluted whole blood (500 µl blood plus 500 µl 0.9% NaCl) in the presence of 10 µl of 1 mmol/l adenosine diphosphate. The level of platelet aggregation was expressed as the area under the curve (AUC).

### Plasma t-PA Determinations

To determine t-PA in patient blood samples, whole blood of patients was centrifuged and platelet-free plasma was extracted. Plasma samples were stored at  $-80^{\circ}\text{C}$  until further analysis. The t-PA levels were determined by a tissue-type plasminogen activator human enzyme-linked immunosorbent assay in 10 times diluted plasma samples (Abcam, Cambridge, United Kingdom).

### Categorization LOT

The LOT was further categorized according to the definition of late, intermediate, and fulminant hyperfibrinolysis as described by Jámbor and Schöchl.<sup>2,15</sup> This classification is based on the clot lysis index at 30 and 60 min after the start of clot formation, which is automatically provided by the thromboelastometric device. Late, intermediate, and fulminant hyperfibrinolysis was defined as significant clot breakdown (>15% lysis of the clot) in the first 30 min (fulminant lysis), between 30 and 60 min (intermediate lysis), or after 60 min (late lysis) after initiation of thromboelastometry.

### Statistical Analysis

Data analysis was performed using the SPSS statistical software package 17.0 (IBM, New York, NY). Data represent mean  $\pm$  SD, median with interquartile range, or frequencies. A two-sided *P* value of less than 0.05 was considered as statistical significant. The effects of incremental concentrations of t-PA on EXTEM and FIBTEM values in the absence or presence of aprotinin (APTEM) were analyzed using linear mixed models. Models included the concentration of t-PA and presence of aprotinin as categorical-independent variables and their two-way interaction. Differences between

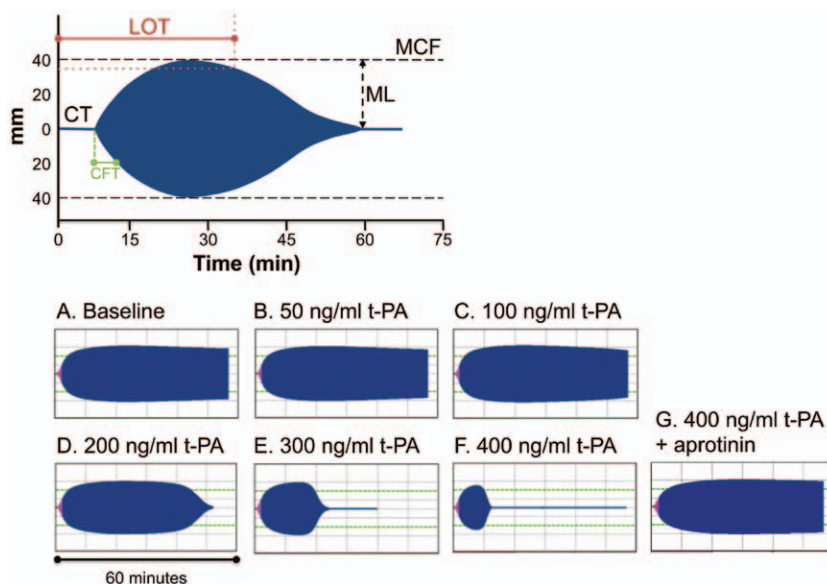
subjects were taken into account by including a random effect for subject. The same model but with concentration as a continuous variable was also estimated. These two nested models were compared using a likelihood ratio (LR) test to elaborate whether the association between the EXTEM value and concentration of t-PA was linear. In case of a linear relation and significant interaction between concentration and presence of aprotinin, the changes in mean EXTEM value per ng/ml increase in concentration of t-PA in the absence or presence of aprotinin are reported as effect sizes together with their 95% CIs. In case of a nonlinear relation and a significant interaction between the concentration of t-PA and presence of aprotinin, differences between the average EXTEM values in presence or absence of aprotinin were tested for each concentration separately using *post hoc* tests with Bonferroni correction. In addition to this, comparisons between mean EXTEM values between concentrations were also made separately for presence and absence of aprotinin using *post hoc* tests.

To test whether the association between Chrono-log AUC and EXTEM MCE was dependent on t-PA concentration, we considered a mixed linear model with Chrono-log AUC as dependent variable and EXTEM MCE, t-PA concentration, and their interaction as independent variables. LOT values were compared between concentrations using the nonparametric Friedman test where for samples for which LOT could not be determined (as clot lysis of 15% or more was not reached) was set at a fixed value (9,999) exceeding the maximum value observed over all samples. Pearson correlations between Chrono-log AUC and EXTEM MCE are computed separately for each concentration of t-PA together with their 95% bias-corrected and accelerated CIs.

## Results

### Effects of t-PA on Thromboelastometric Coagulation Parameters and Platelet Aggregation

Fifteen healthy subjects (nine women, six males, age  $25 \pm 5$  yr) were included in the study. Five percent (9 of 180) of all lysis indices were missing and 18.9% (17 of 90) of platelet-aggregation AUC measurements were missing due to a technical error during rotational thromboelastometry or aggregometry, respectively, and mixed models were used assuming that these data were missing at random. There were no missing values for the other coagulation and lysis parameters. Figure 1 shows a graphical explanation of distinct thromboelastometric parameters as used in the current study and a typical example of the effect of different concentrations of t-PA on the rotational thromboelastometry EXTEM test (fig. 1, A–G). t-PA concentrations were incremented from 0 ng/ml (fig. 1A) to 400 ng/ml (fig. 1F). Higher concentrations of t-PA were associated with a stronger fibrinolytic response and a shorter LOT. Figure 1G shows that the addition of aprotinin in the presence of 400 ng/ml t-PA (APTEM test) prohibited the development of hyperfibrinolysis.



**Fig. 1.** The upper panel shows a graphical representation of thromboelastometric parameters that are most relevant for the current article. A–G represent typical examples of distinct concentrations of tissue plasminogen activator (t-PA) on the rotational thromboelastometry EXTEM test for the extrinsic coagulation over a time period of 60 min. Baseline (A), 50 ng/ml t-PA (B), 100 ng/ml t-PA (C), 200 ng/ml t-PA (D), 300 ng/ml t-PA (E), 400 ng/ml t-PA (F), and 400 ng/ml t-PA in the presence of aprotinin (G). CFT = clot formation time (indicated in green); CT = clotting time; LOT = lysis onset time (indicated in red); MCF = maximum clot firmness; ML = maximum lysis.

Figure 2 displays the relation between the concentration of t-PA and distinct coagulation parameters in the absence (control) or presence (APTEM) of aprotinin and platelet aggregation. The relation between the concentration of t-PA and coagulation parameters was found to be linear for the EXTEM clot formation time (CFT; fig. 2B;  $P = 0.11$  for LR test) and EXTEM MCF (fig. 2C;  $P = 0.11$  for LR test), but not for the EXTEM CT (fig. 2A;  $P < 0.001$  for LR test). The association between the concentration of t-PA and coagulation parameters did not depend on the presence or absence of aprotinin for the CT (fig. 2A;  $P = 0.18$ ) and CFT (fig. 2B;  $P = 0.69$ ). The mean decrease in EXTEM CFT and MCF and FIBTEM MCF in the absence of aprotinin per ng/ml increase in the concentration of t-PA is shown in table 1. The relation between the concentration of t-PA and MCF was found to be different in the presence of aprotinin ( $P < 0.001$ ). In the presence of aprotinin, the EXTEM CFT ( $-0.03$  s per 100 ng/ml t-PA; 95% CI,  $-1.14$  to  $-1.20$ ;  $P = 0.96$ ) and EXTEM MCF ( $0.11$  mm per 100 ng/ml t-PA; 95% CI,  $-0.24$  to  $-0.46$ ;  $P = 0.54$ ) were not found to depend on the t-PA concentration. The association between the concentration of t-PA and FIBTEM MCF was found to be linear (fig. 2D;  $P = 0.84$  for LR test).

The effect of t-PA on platelet function was assessed using Chrono-log whole blood impedance aggregometry. Platelet function is represented as AUC. The relation between the concentration of t-PA and platelet function was found to be linear (Chrono-log AUC; fig. 2E;  $P = 0.09$  for LR test), but no significant change was found as a result of increasing concentration of t-PA (table 1). There was no statistical

significant association between the AUC and EXTEM MCE at each t-PA concentration ( $P = 0.33$  for interaction between concentration of t-PA and EXTEM MCE). Correlations between Chrono-log AUC and EXTEM MCE were 0.67 (95% CI, 0.13 to 0.96), 0.58 (95% CI,  $-0.09$  to 0.95), 0.31 (95% CI,  $-0.27$  to 0.68), 0.33 (95% CI,  $-0.35$  to 0.84),  $-0.03$  (95% CI,  $-0.57$  to 0.61), and 0.37 (95% CI,  $-0.18$  to 0.79) for concentrations of t-PA of 0, 50, 100, 200, 300, and 400 ng/ml, respectively.

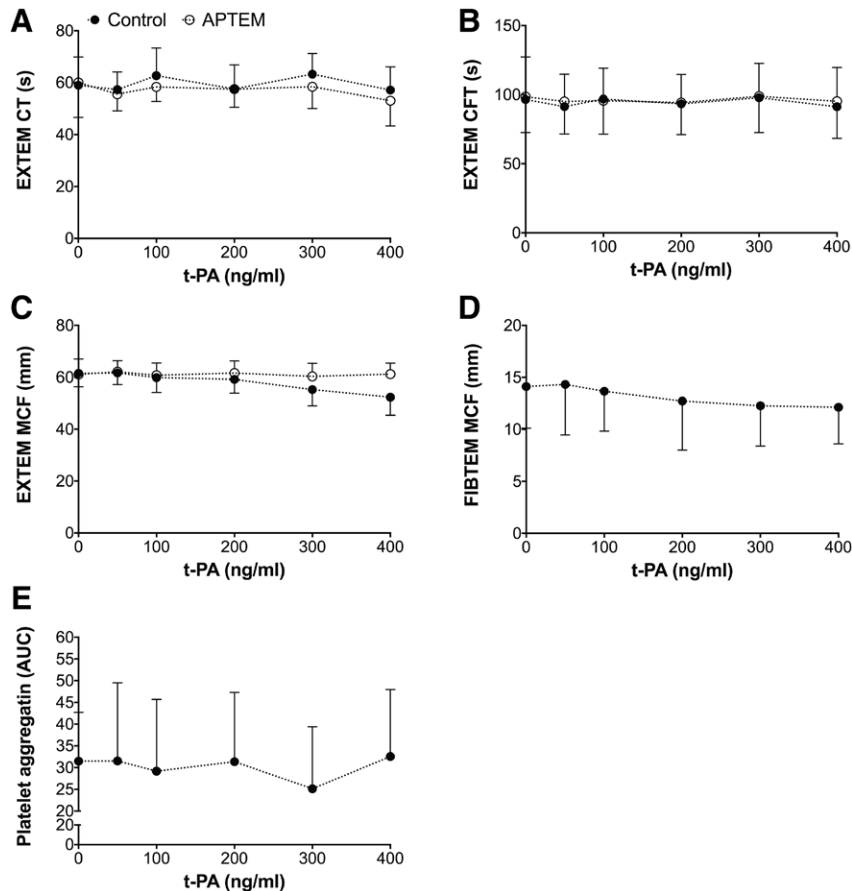
#### Effects of t-PA on Thromboelastometric Lysis Parameters

Figure 3 shows the effects of increasing concentrations of t-PA on the LI at 30 min (LI30; fig. 3A) and at 45 min (LI45; fig. 3B), the ML (fig. 3C), and LOT (fig. 3D) in the absence (control) or presence (APTEM) of aprotinin. The LOT cannot be determined in the presence of aprotinin.

We found a significant interaction between the concentration of t-PA and presence of aprotinin for LI30, LI45, and ML ( $P < 0.001$  for all three lysis parameters). *Post hoc* tests revealed significant differences in all lysis parameters between presence and absence of aprotinin for concentrations of 200 ng/ml and higher (Bonferroni corrected  $P < 0.001$  for all comparisons). For the ML, a difference was also found for the lower concentration of 100 ng/ml (Bonferroni corrected  $P < 0.001$ ). In the presence of aprotinin, no differences in mean lysis parameters were found between all pairs of concentrations (Bonferroni corrected  $P = 1.000$  for all comparisons).

In the absence of aprotinin, the LI30 decreased significantly when the concentration of t-PA increased from





**Fig. 2.** Effect of different concentrations of tissue plasminogen activator (t-PA) on the EXTEM clotting time (CT; A), EXTEM clot formation time (CFT; B), and EXTEM maximum clot firmness (MCF; C) in the absence (control) or presence of the antifibrinolytic aprotinin (APTEM). D shows a dose-dependent effect of t-PA on the FIBTEM MCF. The effects of incremental concentrations of t-PA on the level of platelet aggregation expressed as area under the curve (AUC) are depicted in E. Data represent mean  $\pm$  SD.

100 to 200 ng/ml, from 200 to 300 ng/ml, and from 300 to 400 ng/ml (table 1). In the absence of aprotinin, the LI45 decreased significantly when the concentration t-PA increased from 100 to 200 ng/ml and from 200 to 300 ng/ml, whereas the ML increased when the concentration of t-PA increased from 50 to 100 ng/ml and from 100 to 200 ng/ml (table 1). The LOT was found to differ between t-PA doses ( $P < 0.001$  for Friedman test with mean ranks 4.5, 4.3, 3.9, 3.3, 2.8, and 2.3 for concentrations 0, 50, 100, 200, 300, and 400 ng/ml, respectively).

Figure 4 shows the categorization of 114 LOT values as measured in the *in vitro* study according to the definition by Jámor and Schöchl<sup>15</sup> and Schöchl *et al.*<sup>2</sup> for late ( $n = 34$ ), intermediate ( $n = 32$ ), or fulminant hyperfibrinolysis ( $n = 10$ ; fig. 4A). In 38 cases, there was no detectable hyperfibrinolysis (ML  $> 15\%$ ). LOT values corresponding with late, intermediate, or fulminant hyperfibrinolysis estimated  $42.7 \pm 13.8$ ,  $23.2 \pm 8.2$ , and  $17.5 \pm 4.6$  min, respectively ( $P < 0.001$ ).

#### LOT in Patients with Hyperfibrinolysis

This study included 33 of 87 patients with out-of-hospital cardiac arrest who were diagnosed with hyperfibrinolysis on

emergency department admission. Patient characteristics of the 33 patients with out-of-hospital cardiac arrest and hyperfibrinolysis are shown in table 2. The LOT was measured in 32 patients and was categorized according to the definition by Jámor and Schöchl<sup>15</sup> and Schöchl *et al.*<sup>2</sup> LOT values corresponding with late ( $n = 19$ ), intermediate ( $n = 4$ ), or fulminant ( $n = 10$ ) hyperfibrinolysis estimated  $42.2 \pm 8.3$ ,  $29.1 \pm 1.2$ , and  $14.6 \pm 7.7$  min, respectively ( $P < 0.001$ ; fig. 3B). In a subgroup of 13 patients with available plasma t-PA levels, the relation between t-PA and the EXTEM LOT was determined, showing a moderate negative correlation between t-PA and the time to onset of clot lysis (fig. 5;  $r = -0.67$ ;  $P = 0.01$ ).

#### Discussion

Although the ML is the most frequently used thromboelastometric parameter to describe the severity level of hyperfibrinolysis, the current study shows that this index is limited due to a ceiling effect. Alternatively, we found a good association between increasing doses of t-PA with the LOT, which represents the time to onset of hyperfibrinolysis. The LOT is a continuous scale and exerts a higher discriminative level

**Table 1.** The Effects of Increasing Concentrations of t-PA on Coagulation and Lysis Parameters

| ROTEM Parameter  | Mean  | 95% CI Lower Limit | 95% CI Upper Limit | P Value |
|--|-------|--------------------|--------------------|---------|
| Change in EXTEM CFT (s) per 100 ng/ml increase in t-PA           | -0.37 | -1.53              | -0.80              | 0.54    |
| Change in EXTEM MCF (mm) per 100 ng/ml increase in t-PA          | -2.32 | -2.67              | -1.97              | <0.001  |
| Change in FIBTEM MCF (mm) per 100 ng/ml increase in t-PA         | -0.58 | -0.85              | -0.32              | <0.001  |
| Change in platelet function (AUC) per 100 ng/ml increase in t-PA | -0.49 | -2.22              | -1.24              | 0.57    |
| EXTEM LI30 (%)   |       |                    |                    |         |
| t-PA increase: 100–200 ng/ml                                     | -34.5 | -49.8              | -19.1              | <0.001  |
| t-PA increase: 200–300 ng/ml                                     | -35.9 | -51.3              | -20.6              | <0.001  |
| t-PA increase: 300–400 ng/ml                                     | -22.9 | -38.2              | -7.5               | <0.001  |
| EXTEM LI45 (%)   |       |                    |                    |         |
| t-PA increase: 100–200 ng/ml                                     | -67.5 | -77.7              | -57.3              | <0.001  |
| t-PA increase: 200–300 ng/ml                                     | -18.8 | -30.3              | -7.4               | <0.001  |
| ML (%)   |       |                    |                    |         |
| t-PA increase: 50–100 ng/ml                                      | 21.5  | 11.8               | 31.2               | <0.001  |
| t-PA increase: 100–200 ng/ml                                     | 58.7  | 49                 | 68.4               | <0.001  |

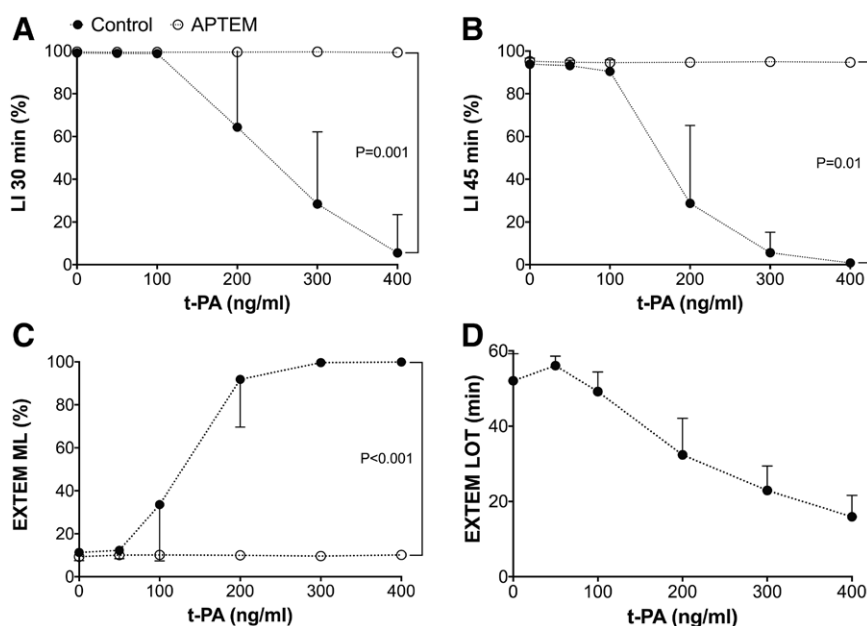
Changes in mean EXTEM value per ng/ml increase in concentration of t-PA in the absence of aprotinin reported as effect sizes together with their 95% CIs. Data represent mean change and lower and upper limits of the 95% CI. P values after Bonferroni correction.

AUC = area under the curve; CFT = clot formation time; EXTEM = test for extrinsic coagulation; FIBTEM = test for fibrin part of clot formation; LI30 = lysis index at 30 min; LI45 = lysis index at 45 min; MCF = maximum clot firmness; ML = maximum t-PA = tissue plasminogen activator.

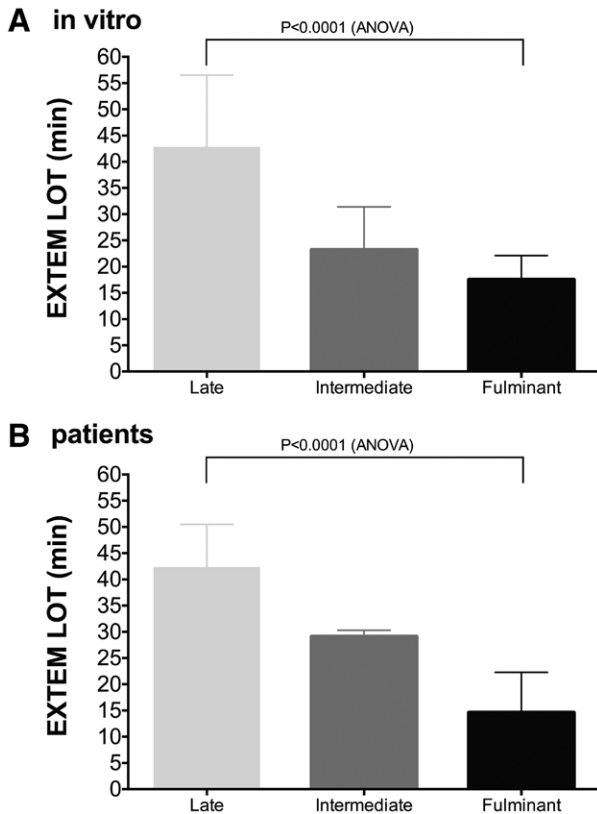
in the presence of high t-PA concentrations than the ML. t-PA had no effect on the ML and LOT in the presence of aprotinin during thromboelastometric measurements. We found no association between t-PA concentrations and the level of platelet aggregation. In the *in vitro* study design, we found that, according to the classification of the hyperfibrinolysis severity level by Jámboř *et al.*<sup>15</sup> and Schöchel *et al.*,<sup>2</sup> the start of hyperfibrinolysis within 20 min after initiation of the thromboelastometric test may be indicative of severe clot breakdown. This finding was confirmed in a patient population with out-of-hospital cardiac arrest and hyperfibrinolysis,

showing that fulminant hyperfibrinolysis could be detected within 15 min if the LOT was used. Moreover, in a small subgroup of patients with available t-PA plasma levels, we found a good correlation between t-PA and the LOT. Our findings suggest that the LOT may be a faster marker for the severity level of blood clot breakdown than the ML.

We previously showed that patients with out-of-hospital cardiac arrest may suffer from fulminant hyperfibrinolysis.<sup>4</sup> Our study showed an average LOT that was longer in survivors (approximately 50 min) than in nonsurvivors (approximately 23 min).<sup>4</sup> Hyperfibrinolysis is not detectable



**Fig. 3.** Effect of different concentrations of tissue plasminogen activator (t-PA) on the lysis index at 30 min after the clotting time (LI30; A), the lysis index at 45 min (LI45; B), the maximum lysis index (ML; C), and lysis onset time (LOT; D), respectively. The LI30, LI45, and ML were determined in the absence (control) or presence of the antifibrinolytic aprotinin (APTEM). Data represent mean  $\pm$  SD.

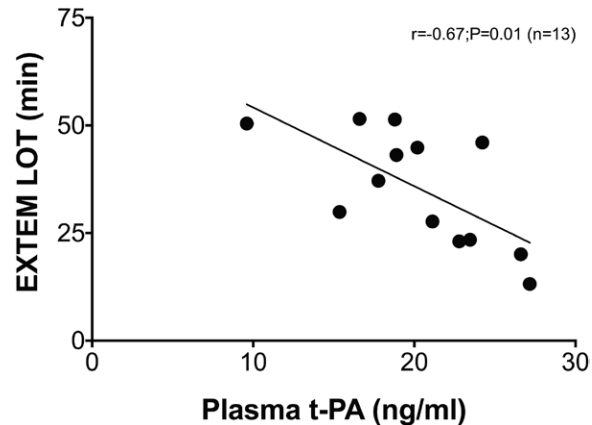


**Fig. 4.** Categorization of the lysis onset time (LOT) according to the level of hyperfibrinolysis (HF) as defined by Jámbor *et al.*<sup>15</sup> and Schöchl *et al.*<sup>2</sup> A shows the categorization of the LOT based on the administration of different concentrations of tissue plasminogen activator in an *in vitro* study, whereas B represents the findings in our patient population (n = 32). Data represent mean ± SD and were analyzed by a one-way ANOVA.

**Table 2.** Characteristics of Patients with Out-of-hospital Cardiac Arrest and Hyperfibrinolysis on Emergency Department Admission (n = 33)

| Variable                             | Value       |
|--------------------------------------|-------------|
| Men/women                            | 20/13       |
| Age                                  | 65 ± 14     |
| Reason for OHCA, %                   |             |
| Cardiac                              | 77          |
| Pulmonary                            | 10          |
| Other                                | 13          |
| Mortality                            | 65          |
| INR in prothrombin time              | 1.72 ± 1.02 |
| aPTT (s)                             | 65 ± 52     |
| Platelet count (×10 <sup>9</sup> /l) | 196 ± 78    |
| Fibrinogen (g/l)                     | 3.0 ± 1.48  |
| D-dimers (µg/ml)                     | 16.8 ± 20.6 |

Coagulation parameters were determined in the first blood sample drawn on emergency department arrival. Data represent mean ± SD. aPTT = activated partial thromboplastin time; INR = international normalized ratio; OHCA = out-of-hospital cardiac arrest.



**Fig. 5.** Association of plasma levels of tissue plasminogen activator (t-PA) and the lysis onset time (LOT) in a subgroup of patients with hyperfibrinolysis ( $r = -0.67$ ;  $P = 0.01$ ;  $n = 13$ ).

by classical hemostatic test methods, such as the activated partial thromboplastin time or prothrombin time. Moreover, D-dimers determinations are time consuming. Novel measurement methods for rapid detection of the degree of fibrinolysis are therefore essential to optimize therapeutic strategies in patients with severe clot breakdown. Jámbor *et al.*<sup>15</sup> were the first to categorize the severity level of hyperfibrinolysis based on the time to full clot breakdown by associating the clot LI at 30 and 60 min with the LOT. The classification was further used by Schöchl *et al.*,<sup>2</sup> showing that the severity level of fibrinolysis based on the clot LI was associated with outcome in patients with trauma. Despite the artificial categorization of the LOT values into three categories, our data suggest a clear distinction in the time to onset of lysis between fulminant, intermediate, and late hyperfibrinolysis based on these categories. Whether the three categories for the severity level of fibrinolysis are useful in the clinical setting warrants further investigation.

Clot breakdown takes time, and this classification may therefore delay the diagnosis of severe hyperfibrinolysis. In the current study, we show that fulminant hyperfibrinolysis, defined as complete clot breakdown within 30 min after initiation of the thromboelastometric test, was associated with an average LOT between 12 to 22 min in our *in vitro* experiments and between 7 and 22 min in the patient setting. These data suggest that initiation of hyperfibrinolysis within this time frame may be indicative of severe clot breakdown and may contribute to a reduction in the time to the administration of antifibrinolytics.

Nielsen *et al.*<sup>11</sup> previously showed that increasing concentrations of t-PA, ranging from 0 to 300 U/ml, were associated with dose-dependent decrease in LOT and increased rate and extent of hyperfibrinolysis using thromboelastography. Likewise, Kupesziz *et al.*<sup>12</sup> found a higher inhibitory effect on clotting activity and faster completion of fibrinolysis of higher t-PA concentrations using thromboelastography. As thromboelastography and thromboelastometry exert different measurement characteristics, here we studied the

effects of t-PA on fibrinolytic parameters using rotational thromboelastometry. In agreement with the thromboelastographic findings, we show a dose-dependent association of t-PA concentrations with the ML and LOT using rotational thromboelastometry, starting at a concentration of 50 pg/ml of t-PA. In addition to the effect of t-PA on fibrinolytic parameters during thromboelastometry, we found that t-PA induced a slight reduction in the EXTEM and FIBTEM MCF, whereas there was no influence of t-PA on the CT and CFT. Although the CT and CFT are indicative for changes in coagulation factor concentrations, the MCF represents the clot firmness based on the cross-link of fibrin, platelets, and factor XIII. In parallel, fibrinolytic processes can reduce the MCF, as clot breakdown leads to alterations in clot strength and consumption of coagulation factors such as fibrinogen. The observed reduction in FIBTEM MCF with increasing concentrations of t-PA may hint toward two processes. First, the FIBTEM MCF may reduce to concomitant clot lysis.<sup>16</sup> Second, the reduction in FIBTEM MCF may result from a reduction in fibrinogen availability and fibrin polymerization. Although the FIBTEM MCF values remained above normal reference values in our investigation ( $\geq 9$  mm), the observed reduction in clot firmness on t-PA administration warrants attention for impairment in clot formation in the presence of fibrinolysis and low fibrinogen levels in the clinical setting, that is, in case of severe hemorrhage. The association between t-PA and the LOT was further confirmed in our patient population.

Our data do not show a relation between t-PA and platelet aggregation using impedance aggregometry. It has previously been shown in platelet-rich plasma that t-PA inhibits collagen or adrenaline-induced platelet aggregation, and this effect could be potentiated by plasminogen.<sup>7,17,18</sup> The absence of t-PA-induced inhibition of platelet aggregation in our study may be explained by the 2.5- to 250-fold lower concentration of t-PA and the use of whole blood instead of platelet-rich plasma when compared with the study by Chen and Mehta.<sup>7</sup> A second explanation might be that whole blood aggregometry based on an impedance method might reveal different effects than aggregometry based on light transmission. In the presence of adenosine diphosphate, other studies showed a slight but insignificant increase in platelet activity in whole blood of healthy volunteers.<sup>17</sup> Interestingly, others have shown that the process of clot lysis is slowed down in the presence of platelets, whereas the inhibitory effects of the antifibrinolytic tranexamic acid on clot lysis are more profound in the presence of platelets.<sup>19</sup> From these findings, it was suggested that platelets may exert profibrinolytic effects. As it should be excluded whether the effects of t-PA on platelet function are influenced by the use of whole blood or platelet-free plasma and the type of measurement device, further research is required to understand the relation between platelets and fibrinolytic processes.

Several limitations of this study require discussion. The *in vitro* part of our study was limited by the use of t-PA

concentrations exceeding 50 ng/ml, whereas normal t-PA levels range from 3 to 13 ng/ml.<sup>8</sup> However, previous investigations showed that plasma t-PA levels in patients with trauma range from less than 12.3 to 95 ng/ml,<sup>20</sup> whereas our patient study revealed t-PA concentrations ranging from 10 to 30 ng/ml, which are in accordance with the lowest two concentrations in our study. As most patients with cardiac arrest show hyperfibrinolysis in combination with acidosis, this may additionally enhance clot breakdown through a t-PA-independent route. Dirkmann *et al.*<sup>21</sup> recently showed that acidosis potentiates t-PA-induced fibrinolysis, whereas hypothermia inhibited t-PA-mediated clot breakdown.

According to the manufacturer (TEM International), an ML exceeding 15% is indicative of hyperfibrinolysis, but the time to 15% ML may be highly variable. The *in vitro* and patient observations presented in this study suggest that hyperfibrinolysis may be earlier diagnosed when the LOT is used instead of the ML. Our results are therefore supportive for standard monitoring of the LOT in patients who are prone to develop hyperfibrinolysis and require early antifibrinolytic therapy. Further studies should reveal whether not only the occurrence but also the onset time of hyperfibrinolysis holds predictive value for patient outcome.<sup>2-4</sup>

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## Competing Interests

The authors declare no competing interests.

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