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 ± SD) of 42.7 ± 13.8, 23.2 ± 8.2, and 17.5 ± 4.6 min in the in vitro study and estimated 42.2 ± 8.3, 29.1 ± 1.2, and 14.6 ± 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)
during thromboelastometry, may provide more detailed information regarding lysis severity than that by the ML. 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. 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. The first study included 30 patients with 16 patients suffering from hyperfibrinolysis, and part of these data were previously published by Viersen et al. 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, 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%, which corresponds with the category of late hyperfibrinolysis as described by Schöchl et al.

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. 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).

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.

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...
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°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.2,15 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 ± SD, median with interquartile range, or frequencies. A two-sided P value of less than 0.05 was considered as statistically 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 ± 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.
Thromboelastometric Parameters for Hyperfibrinolysis

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 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...
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 of 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 be different 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 mg/ml, respectively).

Figure 4 shows the categorization of 114 LOT values as measured in the in vitro study according to the definition by Jámbor et al. $^{15}$ and Schöchl et al. $^{2}$ 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 42.2 ± 8.3, 29.1 ± 1.2, and 14.6 ± 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 in 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 Jámbor and Schöchl $^{15}$ and Schöchl et al. $^{2}$ for late (n = 19), intermediate (n = 4), or fulminant (n = 10) hyperfibrinolysis estimated 42.2 ± 8.3, 29.1 ± 1.2, and 14.6 ± 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; $r = -0.67; P = 0.01$).

**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 Jámbor and Schöchl $^{15}$ and Schöchl et al. $^{2}$ for late (n = 19), intermediate (n = 4), or fulminant (n = 10) hyperfibrinolysis estimated 42.2 ± 8.3, 29.1 ± 1.2, and 14.6 ± 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; $r = -0.67; P = 0.01$).
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 hyperfibri-
nyolysis severity level by Jámbor et al.15 and Schöchl et al.,2
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 popula-
tion 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 sub-
group 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 hyperfibrinoly-
sis.4 Our study showed an average LOT that was longer
in survivors (approximately 50 min) than in nonsurvivors
(approximately 23 min).4 Hyperfibrinolysis is not detectable

| Table 1. The Effects of Increasing Concentrations of t-PA on Coagulation and Lysis Parameters |
|-----------------------------------------------|------------------|------------------|------------------|------------------|
| ROTEI 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 |
| t-PA increase: 300–400 ng/ml                 | 21.5             | 11.8             | 31.2             | <0.001 |
| t-PA increase: 50–100 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.

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 ± SD.

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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.\cite{15} 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.\cite{2} 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.\cite{11} 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, Kupesiz et al.\cite{12} 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

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

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men/women</td>
<td>20/13</td>
</tr>
<tr>
<td>Age</td>
<td>65 ± 14</td>
</tr>
<tr>
<td>Reason for OHCA, %</td>
<td></td>
</tr>
<tr>
<td>Cardiac</td>
<td>77</td>
</tr>
<tr>
<td>Pulmonary</td>
<td>10</td>
</tr>
<tr>
<td>Other</td>
<td>13</td>
</tr>
<tr>
<td>Mortality</td>
<td>65</td>
</tr>
<tr>
<td>INR in prothrombin time</td>
<td>1.72 ± 1.02</td>
</tr>
<tr>
<td>aPTT (s)</td>
<td>65 ± 52</td>
</tr>
<tr>
<td>Platelet count (x10⁹/l)</td>
<td>196 ± 78</td>
</tr>
<tr>
<td>Fibrinogen (g/l)</td>
<td>3.0 ± 1.48</td>
</tr>
<tr>
<td>D-dimers (µg/ml)</td>
<td>16.8 ± 20.6</td>
</tr>
</tbody>
</table>

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 ($r = -0.67; P = 0.01; n = 13$).
effects of t-PA on fibrinolytic parameters using rotational thromboelastometry. In agreement with the thromboelasto-
graphic 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.16
Second, the reduction in FIBTEM MCF may result from a
reduction in fibrinogen availability and fibrin polymeriza-
tion. Although the FIBTEM MCF values remained above
normal reference values in our investigation (29 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 clin-
cal setting, that is, in case of severe hemorrhage. The associa-
tion between t-PA and the LOT was further confirmed in
our patient population.

Our data do not show a relation between t-PA and plate-
et aggregation using impedance aggregometry. It has previ-
ously been shown in platelet-rich plasma that t-PA inhibits
collagen or adrenaline-induced platelet aggregation, and
this effect could be potentiated by plasminogen.7,17,18 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.7 A second explanation might be that whole blood
aggregometry based on an impedance method might reveal
different effects than aggregometry based on light transmis-
ion. In the presence of adenosine diphosphatase, other studies
showed a slight but insignificant increase in platelet activity
in whole blood of healthy volunteers.17 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.19 From these find-
ings, it was suggested that platelets may exert profibrinoly-
tic 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 rela-
tion 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.8 However, previous inves-
tigations showed that plasma t-PA levels in patients with
trauma range from less than 12.3 to 95 ng/ml,20 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.21 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 support-
ive for standard monitoring of the LOT in patients who are
prone to develop hyperfibrinolysis and require early anti-
fibrinolytic therapy. Further studies should reveal whether not
only the occurrence but also the onset time of hyperfibrin-
olysis holds predictive value for patient outcome.2–4

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Competing Interests
The authors declare no competing interests.

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