Evaluation of rotation thrombelastography for the diagnosis of hyperfibrinolysis in trauma patients

A. Levrat², A. Gros², L. Rugeri³, K. Inaba⁴, B. Floccard², C. Negrier³ and J.-S. David¹*

¹Department of Anesthesiology and Critical Care, Lyon-Sud Hospital, Hospices Civils de Lyon and University Lyon 1, Pierre-Bénite F-69495, France. ²Department of Anesthesiology and Critical Care and ³Division of Haemostasis, Edouard Herriot Hospital, Hospices Civils de Lyon and Université Lyon 1, Lyon F-69437, France. ⁴Division of Trauma and Critical Care, Department of Surgery, LAC+USC Medical Center, University of Southern California, Los Angeles, CA 90033, USA

*Corresponding author. E-mail: js-david@univ-lyon1.fr

Background. Blood loss and uncontrollable bleeding are major factors affecting survival in trauma patients. Because treatment with antifibrinolytic drugs may be effective, early detection of hyperfibrinolysis with rotation thrombelastography (ROTEM®) may be beneficial.

Methods. Eighty-seven trauma patients were included in this prospective observational study. Blood samples were collected at admission. After in vitro activation with tissue factor (EXTEM) and inhibition with aprotinin (APTEM), ROTEM® parameters including maximal clot firmness (MCF) and clot lysis index at 30 min (CLI₃₀) were determined. Hyperfibrinolysis was defined as a euglobulin lysis time (ELT) < 90 min. Threshold for ROTEM® parameters were determined with receiver-operating characteristic curves (ROC) analysis according to the ELT results.

Results. ELT was determined in a subgroup of 23 patients. In this group of patients, ROC analysis showed that for a threshold of 18 mm (MCF-EXTEM), 71% (CLI₃₀) and 7% (increase of MCF-APTEM), sensitivity was, respectively, 100%, 75%, and 80% with a specificity of 100%. With the application of these thresholds to the whole trauma cohort, ROTEM® analysis detected hyperfibrinolysis in five patients [6%, 95% confidence interval (CI): 2–13%]. As expected, patients with hyperfibrinolysis were more severely injured (median Injury Severity Score: 75 vs 20, P<0.05), had greater coagulation abnormalities [international normalized ratio (INR): 8.2 vs 1.3, P<0.05; fibrinogen: 0.0 vs 2.2 g litre⁻¹, P<0.05], and a higher mortality rate (100%, CI: 48–100% vs 11% CI: 5–20%, P<0.05).

Conclusions. ROTEM® provided rapid and accurate detection of hyperfibrinolysis in severely injured trauma patients.

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Blood loss and uncontrollable bleeding are major factors affecting survival in trauma patients¹-³ and haemorrhage continues to be one of the leading causes of death after trauma.⁴ Coagulopathy that is frequently encountered in haemorrhagic shock has been shown to be an independent risk factor for death after trauma.⁵ Critically ill trauma patients suffer from a complex coagulopathy with multiple aetiologies, including overwhelming activation of tissue factor, consumption of coagulation factors and platelets, haemodilution, hypothermia, metabolic acidosis, massive transfusions, and hyperfibrinolysis.⁵-⁷ The ability to detect and treat coagulopathy early appears to be a key component of the multi-factorial approach to haemorrhage control in the trauma patient.⁵,⁸

We have recently shown that rotation thrombelastography (ROTEM®) is a point-of-care device that rapidly detects systemic changes in in vivo coagulation and that a coagulopathy can be observed in almost 30% of trauma patients.⁴ Moreover, these patients may also develop disseminated intravascular coagulation which includes both
activation of coagulation factors and fibrinolysis. Because early treatment with antifibrinolytic drugs such as aprotinin or tranexamic acid may be effective, early detection of hyperfibrinolysis during the initial management of bleeding trauma patients may be beneficial.9

The goal of this study was to evaluate the accuracy of ROTEM® in detecting hyperfibrinolysis in trauma patients and to describe the incidence of hyperfibrinolysis in a prospective trauma cohort.

Methods

Design, setting, and inclusion criteria

This prospective observational study was carried out between July 4 and October 30, 2004 in the trauma resuscitation unit (TRU) of a 1000-bed university hospital. The study protocol was approved by the institutional review board (IRB) of the Hospices Civils de Lyon. Waived informed consent was authorized by the IRB as this study did not impact patient care. All trauma patients admitted during the study period were included in the study.

Protocol

The characteristics of each patient and their injury, and Injury Severity Score (ISS) were recorded.10 Blood samples were collected immediately after the patient’s arrival at the TRU.

Blood samples were collected by venipuncture into vacutainer tubes (Becton Dickinson, Plymouth, UK) containing ethylenediaminetetraacetic acid (EDTA) for platelet and haemoglobin counts (SE-9500®, Sysmex, Japan) or citrate (0.129 M trisodium citrate) for standard tests: prothrombin time (PT) and international normalized ratio (INR) (Neoplastin® CI plus, Diagnostica Stago, Asnieres, France), activated partial thromboplastin time (aPTT, MDA Platelin® LS, Biomérieux, France), fibrinogen (Clauss technique, Fibriquick®, reagent, Biomérieux), and ROTEM® measurements. Standard coagulation tests and ROTEM® measurements were performed within 2 h of blood sample collection and at least 15 min after venipuncture.11

ROTEM® measurements

The ROTEM® coagulation analyzer (Pentapharm, Munich, Germany) and the parameters of thrombelastography have been described previously in detail.11–13 Briefly, thrombelastography measures shear elastic modulus during clot formation and subsequent fibrinolysis. The ROTEM® uses a ball-bearing system for power transduction, which makes it less susceptible to mechanical stress, movement, and vibration. In the ROTEM® analyzer, coagulation is mildly activated either with ellagic acid (INTEM test) or tissue factor from rabbit brain (EXTEM test). This is performed in order to standardize the in vitro coagulation process and also to speed up the analysis that typically lasts 15–20 min.11 In addition to the INTEM and EXTEM screening tests, additional reagents such as aprotinin (APTEM) or cytochalasin D (FIBTEM) can be used in order to study the EXTEM with in vitro inhibition of fibrinolytic activity and the EXTEM with inhibition of platelets, respectively. All pipetting steps and mixing are standardized by utilizing an automated electronic program. The ROTEM® analysis was performed at 37°C, in parallel, on the four channels (INTEM, EXTEM, FIBTEM, and APTEM). The following ROTEM® parameters were analysed: clotting time (CT); clot formation time (CFT); maximum clot firmness (MCF); and the amplitude of clot at 10 and 15 min (CA10, CA15). The clot lysis index (CLI30–CLI60) describes the ratio between MCF and CA 30–60 min after CT and thus describes the progress of fibrinolysis at that particular time. In Figure 1, four patterns of coagulation that may be encountered with ROTEM® are illustrated: normal tracing (Fig. 1A), hyperfibrinolysis (Fig. 1B), severe coagulopathy (Fig. 1C) and severe coagulopathy with the complete absence of any clotting (‘ultimate coagulopathy’, Fig. 1D).

Reference ranges for ROTEM® parameters were obtained from a control group (healthy volunteers) previously described,4 and were determined according to National Committee for Clinical Laboratory Standards (NCCLS) Guidelines calculating the 2.5 and 97.5% percentiles (Table 3).14 These values were very similar to those previously published by Lang and colleagues.11

Assessment of fibrinolysis

Plasma fibrinolytic activity was evaluated by measuring D-Dimer concentrations (Asserachrom® D-DI, Diagnostica Stago, Asnieres, France), fibrin degradation products (FDP) (FDP Plasma, Diagnostica Stago, Asnieres, France) and euglobulin lysis time (ELT). ELT represents a test of global fibrinolysis and has been described in 1948 by McFarlane and Biggs.15 Briefly, the citrated plasma was precipitated with cold acetic acid. The precipitate contained fibrinogen, plasminogen, and plasminogen activators, with fibrinolytic inhibitors theoretically removed. The precipitate was redissolved and the euglobulin solution was clotted with 0.025 M CaCl2. The clotted sample was then incubated at 37°C and was observed at 10-min intervals for clot lysis. ELT was scored from 0 to 5 as follows: 0 (ELT >90 min), 1 (60–90 min), 2 (30–59 min), 3 (15–29 min), 4 (5–14 min), and 5 (<5 min). ELT was utilized in our study as the reference diagnostic method for the assessment of fibrinolysis with a threshold value of <90 min for the diagnosis of hyperfibrinolysis (i.e. ELT score from 1 to 5).17 18

Because of logistic limitations (manpower and time), ELT could not be tested in all trauma patients and was only performed in the subgroup of patients that was considered to have severe injuries by the physician in charge. All patients who did not have an ELT determination had an assessment of their fibrinogen concentrations as a
surrogate marker of hyperfibrinolysis. None of these patients had a fibrinogen concentration <0.9 g litre\(^{-1}\).

In the group of patients with ELT determination, the accuracy of ROTEM\(^{\text{w}}\) parameters from the EXTEM channel (CA\(_{10}\), CA\(_{15}\), MCF, CLI\(_{30}\) and CLI\(_{60}\)) was assessed for the diagnosis of hyperfibrinolysis with receiver-operating characteristic curve (ROC) analysis and subsequent thresholds were defined.\(^{19,20}\) Moreover, we studied the ability of aprotinin to reverse the decrease of the following EXTEM parameters: MCF, CA\(_{10}\), CA\(_{15}\), CLI\(_{30}\) and CLI\(_{60}\) by calculating \(\Delta\) parameter \(=\) [(parameters-APTEM - parameter-EXTEM)/ parameter-EXTEM] \(\times\) 100. Utilizing the diagnostic criteria previously defined with ROTEM\(^{\text{w}}\), the incidence of hyperfibrinolysis in the whole trauma cohort was calculated.

**Statistical analysis**

Results are expressed as median [inter-quartile range (IQR)], mean (SD), or \% [95% confidence interval (CI)]. Normality of the distribution was tested using the Kolmogorov-Smirnov test and the Mann–Whitney U-test; the Student’s \(t\)-test was used for continuous variables as appropriate. Statistical differences between groups were evaluated by \(\chi^2\) test or by Fisher’s exact test when appropriate. A two-tailed \(P\)-value <0.05 was considered significant. ROC and the area under the curves (AUCs) were constructed for ROTEM\(^{\text{w}}\) parameters (EXTEM) for the diagnosis of hyperfibrinolysis. Sensitivity, specificity, and positive and negative predictive values were calculated for the best cut-off value (corresponding to the highest accuracy, i.e. minimal false-negative and false-positive results).\(^{21}\) Statistical comparison of the areas under two ROC curves was done with Wilcoxon rank-sum test as previously described by Hanley and McNeil.\(^{22}\) All statistical tests were performed using commercially available statistical software (NCSS 2004, Kaysville, UT, USA; Medcalc 9.3.6.0, Mariakerke, Belgium).

**Results**

During the study period, 89 consecutive trauma patients were admitted to the TRU. Two of these were excluded from analysis owing to missing data. Baseline characteristics of this trauma cohort have been previously reported.\(^{4}\)

**Definition of threshold for the detection of hyperfibrinolysis by ROTEM\(^{\text{w}}\)**

The reliability of ROTEM\(^{\text{w}}\) for the diagnosis of hyperfibrinolysis was tested in a subgroup of 23 patients who had an ELT [age: 25 (21–47); sex male: 78% (95% CI: 56–93%);...
blunt trauma: 87% (95% CI: 66–97%); ISS: 38 (24–75), haemoglobin: 106 g litre\(^{-1}\) (82–128), platelet count: \(160 \times 10^9\) litre\(^{-1}\) (110–209); INR: 1.6 (1.4–4.0); aPTT: 35 s (28–126); fibrinogen: 0.9 g litre\(^{-1}\) (0.5–1.7).\]

According to the ELT score, this group consisted of five patients with hyperfibrinolysis (Fig. 1B and D; respectively, with ELT score of 1, 2, 3, 4, and 5) and 18 patients without hyperfibrinolysis (Fig. 1A and C, ELT score of 0).

Linear regression analysis showed that among EXTEM parameters, MCF showed the best correlation with the ELT score (\(r^2: 0.63\); slope: \(P = 0.001\), CLI\(_{10}\) (\(r^2: 0.57\); slope: \(P < 0.001\), CLI\(_{30}\) (\(r^2: 0.15\); slope: \(P = 0.126\), and CLI\(_{60}\) (\(r^2: 0.65\); slope: \(-0.03\); \(P = 0.002\)).

Results of ROC analysis are presented in Table 1. Pairwise comparisons of the areas under ROC curves did not show any significant difference between ROTEM\(^{\text{®}}\) parameters.

**Incidences of hyperfibrinolysis in the trauma cohort according to the ROTEM\(^{\text{®}}\)**

By using previously defined threshold (EXTEM), hyperfibrinolysis was observed in five out of the whole trauma patient cohort (6%, 95% CI: 2–13%).

As expected, patients with hyperfibrinolysis were more severely injured, had lower haemoglobin and platelet counts, more severe coagulation abnormalities, higher D-Dimers and FDP concentrations, and a greater mortality rate than patients of the control group (Table 2). Hyperfibrinolysis patients also had greater ROTEM\(^{\text{®}}\) abnormalities than control patients (Table 3).

After addition of aprotinin in the test reaction cup, EXTEM parameters remained unchanged in one patient in the hyperfibrinolysis group (Fig. 1D), whereas one patient in the control group had a significant increase of MCF-EXTEM (9%).

**Discussion**

In the current study, ROTEM\(^{\text{®}}\) was able to quickly (\(\leq 15\) min) and accurately detect hyperfibrinolysis. This was observed in 6% of our cohort of trauma patients. As previously described,\(^2\) we also observed a close relation between injury severity, coagulation abnormalities, and outcome (Table 2).

In our study, ELT was utilized as the gold standard test for the diagnosis of hyperfibrinolysis. ELT is the most commonly used test and it has been demonstrated to be a useful indicator of in vivo fibrinolysis in many physiologic as well as pathologic conditions.\(^{16–26}\) Nevertheless, because ELT remains a complex and time-consuming procedure that can take more than 180 min,\(^27\) practically, it is not a feasible test for the diagnosis of hyperfibrinolysis in bleeding patients. For the same reasons, the other techniques used to diagnose hyperfibrinolysis (plasmin–antiplasmin complex, plasminogen activator inhibitor 1, thrombin activatable fibrinolytic inhibitor, D-Dimers/plasmin–antiplasmin)\(^{18–28}\) are also not relevant in the context of haemorrhagic shock. Furthermore, these tests
have to be performed in series and therefore cannot be
done routinely for logistic and economic reasons.

Clinically, the treatment of coagulopathic bleeding is
critically compromised by current coagulation monitoring
technologies that can take up to 45–60 min. The entire
blood volume of the bleeding trauma patient may have been
exchanged several times during that time interval, making
the results of the laboratory tests obsolete. In a previous
study, we have recently demonstrated that ROTEM® can
rapidly detect systemic changes in in vitro coagulation in
trauma patients and may help to guide transfusion.4

In this study, we demonstrated the ability of ROTEM®
to diagnose hyperfibrinolysis with a decrease in EXTEM
parameters such as MCF or CA15 and a significant
increase of these parameters after the addition of aprotinin
in the test reaction cup (Table 3). This was observed in all
patients except for one, likely because the patient having
reached the ‘ultimate’ state of fibrinolysis (Fig. 1A). At the
opposite end of the spectrum, one patient from the control
group had an increase of MCF-EXTEM exceeding the
threshold value (9%) after addition of aprotinin.

The use of antifibrinolytic agents is a management
option for bleeding in trauma patients with hyperfibrinolysis.9 29 This treatment has been used successfully to reduce
blood loss in various non-traumatic surgical settings, such
as cardiac and orthopaedic surgery or hepatic transplantation.30 – 32 However, owing to the cost and because
adverse events have been reported with their use,33 – 35
medical treatment with antifibrinolytics should be guided by lab-
oratory testing or a point-of-care device. ROTEM® pro-
vides a rapid and accurate diagnosis of hyperfibrinolysis,
which may guide therapy with antifibrinolytic agents. It is
still unknown whether all patients with hyperfibrinolysis
require antifibrinolytic therapy or if only those with a sig-
nificant increase in EXTEM parameters as depicted in
Figure 1A will benefit. Randomized prospective evaluation
of the safety and efficacy of these treatments in bleeding
injured patients with hyperfibrinolysis is warranted, such
as the Crash 2 trial (http://www.crash2.lshtm.ac.uk).

This study has several limitations. First, the power of the
study is limited by the small number of patients with
hyperfibrinolysis (five patients). Secondly, the diagnosis of
hyperfibrinolysis which was made by the ELT was not con-
firmed by another diagnostic tool, such as plasminogen
activator inhibitor 1. However, there is to date no data
showing that any of the other test parameters are superior
to ELT.28 Thirdly, vascular disorders and adhesion of plate-
lets to collagenous fibres still cannot be estimated by
thrombelastography. Fourth, because this study was done
on admission, it did not take into account the effect of
couagulation therapy on ROTEM®
parameters and standard
coeagulation tests. Fifth, ROTEM® requires a fair degree of
technical expertise that necessitates training of the phys-
icians who will be utilizing it. However, we recently
demonstrated that there was a very good correlation
between results of ROTEM® done by trained physicians
and haemostasis technicians.36 Savry and colleagues also
showed that ROTEM® sample processing by trained phys-
icians took only a mean of 171 s.37

In summary, ROTEM® accurately and quickly diag-
nosed hyperfibrinolysis in trauma patients. This may allow
for the early identification of this subset of severely
injured patients and are continuously bleeding who may benefit from treatment with antifibrinolytic agents.

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**References**
