NAME OF PROJECT: The assessment of fibrinolytic resistance in septic patients using the ROTEM velocity curves of clot formation

Person responsible (Chair / Principal Investigator): Paul Y. Kim/ Ecaterina Scarlatescu

Description Abstract

Sepsis is frequently associated with dysfunction of coagulation pathways with inflammation-induced coagulation activation and decreased activity of anticoagulant systems leading to increased fibrin formation combined with impaired fibrin removal due to decreased fibrinolytic activity[1]. Detection of subtle changes in fibrinolysis may be important to the clinical management of critically-ill septic patients as increased resistance to fibrinolysis is associated with increased mortality[2]. Rotational thromboelastometry (ROTEM) and thrombelastography (TEG) have proved useful for the real-time detection of hyperfibrinolysis diagnosed by the decrease of clot firmness related to the maximum clot amplitude. This method of measurement is useful when overt changes are present, though the overall sensitivity to detect any fibrinolysis is quite limited[3]. Resistance to fibrinolysis is even more difficult to diagnose using clot lysis indices, as in most clinical situations characterized by low-grade fibrinolysis the decrease in clot firmness after reaching the maximum amplitude is not visible during the limited measurement time.

Coagulation and fibrinolysis are two processes that overlap in time, meaning that fibrinolysis begins before the clot reaches the maximal firmness and fibrinolytic activation is also reflected by the kinetics of clot formation before reaching the point of maximal amplitude. To better capture dynamic changes in clot formation using ROTEM, we identified a new early kinetic parameter represented by the portion of time required to reach maximal clot amplitude after maximal clot formation velocity has been reached. The new parameter is calculated using the formula: \( t\text{-AUC}= CT+(t\text{-MCF})-(t\text{-MaxVel}) \). According to previous publications, the clot maximal amplitude is reached earlier when fibrinolytic activity is increased[4]. In a retrospective observational study on septic patients, the new parameter was increased in patients with decreased clot lysis and was correlated with worse outcome[5].

The objective of the project is to evaluate the correlation between the degree of fibrinolytic activation and the newly calculated parameter (t-AUCi) reflecting the latter part of the clot amplitude velocity curve obtained from ROTEM analysis. Our hypothesis is that the value of t-AUCi parameter decreases with increasing fibrinolytic activity. Compared to current viscoelastic-based lysis indices expressed as percentages, thus being semi-quantitative measures of fibrinolysis, the t-AUCi value is a more parametric evaluation that might be useful for a better characterization of subjects having similar clot lysis indices.
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Design and methodology (Data expected to collect, sample size and statistical analysis):

**Research design**: prospective observational study. **Sample size** was calculated using G Power program aiming for a large correlation between t-AUCi parameter and lysis indices (Cohen’s effect size 0.5), with a type I α error=0.05 and study power (1-β)=0.8 resulting in a total number of 58 subjects.

**Data collection** will include demographic data, ICU diagnosis and associated pathology, survival at ICU discharge and 30-day survival (for the ICU patients) and the results of the hemostasis assessment. Blood samples (2 EDTA, 4 citrate tubes) will be collected from each subject included and used for the following tests: Standard coagulation tests (PT/INR, aPTT, Fibrinogen level), Complete Blood Count, Rotational Thromboelastometry (ROTEM). Other plasmatic markers such as the D-dimer, plasmin-α2-antiplasmin (PAP) complex, and plasminogen activator inhibitor 1 (PAI-1) levels will be measured in the samples obtained from the septic patients.

The ROTEM analysis will be performed on ROTEM Delta using standard reagents from manufacturer with runtime 90 minutes. The ROTEM standard, derived and the newly calculated parameter t-AUCi will be collected from the baseline assays (NATEM, EXTEM, FIBTEM, APTEM) and from modified assays (EXTEM, APTEM). For the modified assays the ROTEM will be performed after adding rtPA (Actylise) to the tests in order to obtain final concentrations of 100 ng/ml and 175 ng/ml.

Study population (Inclusion, exclusion, eligibility) (patient population; recruitment of participating institutions/physicians and subjects; minimum number needed; expected number):

**Patient selection**:

- Study group: patients with sepsis (according to Sepsis-3)
- Control group: healthy volunteers (recruited from the medical staff)

**Exclusion criteria**: congenital or acquired conditions that could influence the hemostasis assessment results (such as liver or chronic kidney diseases, thrombolytic or anticoagulant/antiplatelet therapy including heparin in anticoagulant dosage, recent therapy with blood derivatives or pro-coagulant treatment within the last 7 days prior to enrolment).

**Minimum number of subjects** needed is 29 for each group with an expected number of patients to be recruited of 30 subjects in each group (60 subjects in total). Informed consent will be signed by all subjects recruited to participate in the study (or by the next-of-kin in the case of ICU patients).

**Participating institution**: Fundeni Clinical Institute, Department of Anaesthesia and Intensive Care III. **Physicians involved**: Ecaterina Scarlatescu, MD, PhD; Dana R. Tomescu, MD, PhD, Head of Department of Anaesthesia and Intensive Care III
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Expected timeline:

- Project stage/set up: Planning stage
- Launch: 2019
- Duration: 12 months
- Finalization/analysis: 2020
- Reporting: 2020

Expected outcomes (ie. publications): The results will be published in a specialized journal.

Publication type (SSC Communication, Guidance document or original article): Original article

Description of project set/up and management, needed infrastructure and resources (summary):

In this project the subjects will be recruited in Fundeni Clinical Institute, Bucharest, Romania. The septic patients will be recruited in the Department of Anaesthesia and Intensive Care III and the controls will be recruited from the medical staff working in the department. The blood samples will be collected by specialized staff working in the ICU and the standard coagulation tests and complete blood count will be performed in the central hospital laboratory as a part of the routine tests for ICU patients and as a part of the regular health check-up for the controls recruited from the medical staff. The study project is approved and supported by the Chief of the Department of Anaesthesia and Intensive Care III, Dr. Dana R. Tomescu. The ROTEM analysis will be performed by the Principal Investigator (Dr. Ecaterina Scarlatescu). The study benefits from the infrastructure and fixed resources assured in the research institute affiliated to Fundeni Clinical Institute (http://cemt.icfundeni.ro/index.php/acasa) providing adequate conditions for the research activity and for the storage of samples.

References:

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