Potential Use of Rotational Thromboelastometry to Explore Hemostatic Abnormalities in Patients with Acute Liver Failure or Acute Liver Injury

Protocol Number:	ALFSG-ROTEM
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Version:	2
Version Date:	April 20, 2017

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Abbreviations

ALI	acute liver injury
ALF	acute liver failure
ALFSG	Acute Liver Failure Study Group
APAP	acetaminophen
CRF	case report form
ICF	informed consent form
ICP	intracranial pressure
IL	Instrumentation Laboratory
INR	international normalized ratio
LAR	legally authorized representative
MCF	Maximum Clot Firmness
MOSF	multi-organ system failure
NAC	*N*-acetylcysteine
PI	principal investigator
rFVIIa	pro-hemostatic factors
RBC	red blood cells
ROTEM	rotational thromboelastometry
RRT	renal replacement therapy
TEG	thromboelastography
UTSW	UT Southwestern Medical Center
VCU	Virginia Commonwealth University
vWF	von Willebrand factor

1. Background

Patients with acute liver failure (ALF) are commonly viewed as having a bleeding tendency due to elevated international normalized ratio (INR) and thrombocytopenia. Indeed, practitioners often administer pro-hemostatic factors (rFVIIa) and/or blood component transfusions (plasma, platelets) for prophylaxis against bleeding before invasive procedures, or for the expectation that a severely elevated INR connotes increased risk of spontaneous (non-procedure-related) bleeding. In early studies of ALF, non-procedure-related bleeding leading to death was reported frequently (in 27%).[1] However, the magnitude of the bleeding risk in contemporary series is poorly defined, and probably much lower. A recent analysis of the ALF Study Group (ALFSG) Registry of nearly 1600 patients enrolled between 1998 and 2011 revealed a 10% incidence of spontaneous bleeding complications that were rarely the proximate cause of death.[2] Even in ALF patients undergoing invasive procedures such as intracranial pressure (ICP) monitoring, the incidence of bleeding complications possibly contributing to death was <3%.[3] The practice of administering pro-hemostatic factors or transfusions to patients with acute liver injury (ALI) or ALF has considerable potential for adverse effect complications that were rarely the proximate cause of death.[2]s, such as transfusion-related acute lung injury, thromboembolism, and volume overload. Moreover, the correction of the INR obscures the all-important trend in INR as a measure of spontaneous liver recovery. Finally, the administration of pro-hemostatic factors may exacerbate a pro-thrombotic local environment within the liver, potentially increasing liver injury.

Recent experimental data have, in fact, suggested that this patient population has a normal or pro-thrombotic global state of hemostasis.[4] The ALF Study Group has published a series of 4 manuscripts within the last 2 years to attempt to define the state of global hemostasis in patients with ALF and to identify mechanisms of compensation for low pro-hemostatic coagulation factor concentrations and thrombocytopenia. In a single-center cohort of 51 patients[5], thromboelastography (TEG), a measure of the dynamics and firmness of overall clot

formation, was normal or in the hypercoagulable range in ~80% (Figure 1), findings confirmed later by a second group. In contrast to the INR, which measures only the activity of the extrinsic coagulation cascade, TEG accounts for contributions from the coagulation cascades, platelets, and fibrin to blood clot formation, and is, therefore, a more physiologic assay of hemostasis. In the same study, we offered one possible explanation for re-balanced hemostasis in patients with ALF, the fact that deficiency of liver-derived pro-coagulant proteins is balanced by commensurate deficiency of liver-derived anti-coagulant proteins.

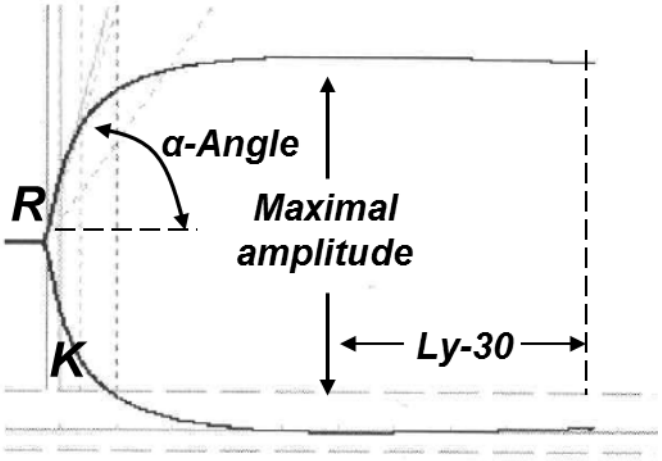

Figure 1. Thromboelastogram from a patient with APAP-induced ALF. This patient had the following laboratories at the time the above TEG was obtained: INR 4.2, Factor VII 4% of normal, platelets 163 x 10^9/L, Factor VIII 558% of normal. Despite standard laboratories suggest that this patient had a bleeding tendency, all of the parameters in this TEG were in the ***hyper*coagulable** range for the VCU laboratory.

The above is a typical TEG indicating a hypercoagulable state despite individual studies suggesting deficient coagulation factors. The R-time represents the latency of clot formation, and corresponds most closely to the PTT and INR in patients with ALF. The K-time represents the kinetics of fibrin formation, which reflects the fibrinogen concentration and platelet count. The maximal amplitude reflects the maximal strength of clot formation (a product of platelets, fibrin, and the coagulation cascades in whole blood). The LY-30 reflects the fibrinolysis for 30 minutes beyond the maximal amplitude.

Thus, the state of global hemostasis has been shown to be normal or hypercoagulable in approximately 80% of patients with ALF/ALI by TEG, but populations in these studies were small. Overall clot strength appears to increase by SIRS-driven compensatory mechanisms in proportion to the severity of ALF, despite higher INR in the setting of more severe ALF. A more

robust analysis of clot formation is needed in order to define (1) the proportion of patients with normal, hyper-, and hypo-coagulable hemostasis; (2) the clinical factors which have the potential to upset re-balance in favor of bleeding or thrombosis; (3) the relationship of global hemostasis to systemic complications and outcomes of ALF. In addition, the potential effects of *N*-acetylcysteine (NAC) on global hemostasis will be analyzed, as NAC has been shown to decrease the size and activity of von Willebrand factor (vWF), a possible mechanism by which NAC improves outcome in non-APAP ALF, by reducing thrombosis in sinusoidal and peripheral microvasculature.[6]

Rotational thromboelastometry (ROTEM) is an assay of global hemostasis almost identical to TEG.[7] ROTEM can be performed within 1 hour at the bedside with minimal training and maintenance. We anticipate that ROTEM will provide an assessment of overall hemostasis in whole blood, and will identify deficiencies in plasmatic and cellular components of hemostasis. Moreover, we anticipate ROTEM to confirm that global hemostasis remains generally rebalanced in patients with ALI or ALF, and that transfusion of pro-hemostatic factors (predominantly plasma and platelets) may tip the rebalanced state toward hypercoagulability, with possible adverse consequences.

2. Rationale

Why might an improved understanding of hemostasis in patients with ALI or ALF improve outcome? The observations above suggest that this patient population maintains normal global hemostasis under most conditions and offer mechanisms whereby the “bleeding tendency” may actually be a tendency to clot. Furthermore, these findings offer a possible explanation for the occurrence and persistence of microvascular thrombosis within the liver and in peripheral tissues, which have been suggested to participate in the liver injury and the multi-organ system failure (MOSF) of ALF. We believe it is imperative to collect information about global hemostasis in the real world situation, where outside physicians may be exacerbating the

hypercoagulable state of ALF unknowingly; we need to know whether this hypercoagulable state is spontaneous or induced.

We anticipate that approximately 30-40% of this patient population will have received transfusions prior to enrollment into the ROTEM Protocol based upon the incidence of plasma transfusion prior to enrollment into the ALFSG Registry, a field collected by the ALF Study Group since its inception. The data suggest that the administration of pro-hemostatic factors to this patient population may be unnecessary and potentially harmful. However, to change clinical practice will require additional clinical studies to show that global hemostasis in patients with ALI or ALF remains re-balanced under most conditions. These studies have the potential to show that administering pro-hemostatic factors does not decrease post-procedural bleeding complications, but may adversely affect outcome, contrary to common belief. Since prior transfusions will be collected, we also will be able to examine such patients separately from those who were not transfused before entry into the ROTEM Protocol.

3. Hypotheses

i. Global hemostasis as determined by ROTEM is normal or hypercoagulable in most patients with ALF/ALI.

ii. Factor transfusion increases the hypercoagulability of patients with ALF/ALI.

iii. The administration of NAC results in a more hypocoagulable state of global hemostasis.

4. Inclusion Criteria

i. All patients enrolled in the ALFSG Registry with hepatic encephalopathy (ALF) will be eligible.

ii. ALI patients enrolled into the ALFSG Registry may also be enrolled (INR ≥ 2.0 and no evidence of encephalopathy).

5. Exclusion Criterion

i. Patients not enrolled into the ALFSG Registry.

6. Informed Consent

Patients who meet criteria for ALI are, by definition, not encephalopathic and will be able to consent for enrollment without additional consents being obtained from a legally authorized representative or family member, although both consents may be obtained. In some cases, patients with ALF may have already returned to their baseline mental status and will be able to provide consent for themselves. In all other cases, the legally authorized representative (LAR) of eligible patients will provide written informed consent prior to study participation. Informed consent will be obtained by either the site Principal Investigator (PI) or by individuals approved by the PI to do so. Each patient or LAR will be given a copy of the informed consent form and the original signed consent form will be maintained within the study files.

Patients will be informed that they may still participate in the ALFSG Registry if they do not provide consent for the ROTEM study. However, patients cannot participate in the ROTEM study if they are not first enrolled in the ALFSG Registry.

7. Methods

A. Preamble

i. A maximum of 12 sites participating in the ALF Study Group located in the United States and Canada will participate. We anticipate that approximately 200 subjects will be enrolled through August 2019.

ii. Instrumentation Laboratory (IL), manufacturer of the ROTEM device, will provide each site with a device, the training for the research coordinator at each site, and instructions for maintenance of the device. The ALFSG will be responsible for purchasing the disposable reagents. Sites which already have ROTEM on-line at their hospital will also

be supplied with a separate ROTEM device and should use the device earmarked for this research protocol.

iii. The ROTEM device will be maintained according to institutional laboratory standards.

iv. ROTEM will be performed by the Study Coordinator, and results will be blinded to the intensive care team.

v. We recognize that some sites already use ROTEM (or TEG) as part of their routine practice. The data output for patients enrolled into the ROTEM study will not be used for clinical decision making during the study.

B. Reagents

i. ROTEM reagents will be sent to each study site by UT Southwestern (UTSW) and stored in the laboratory refrigerator until used, or until specified expirations are met (as stated in each reagent's package insert).

ii. On each day of use, the reagents will be brought to room temperature prior to performing ROTEM and returned to the refrigerator after use. Opened reagents are good until specified expirations are met, as stated in each reagent's package insert).

iii. Expired reagents should be destroyed according to standard operating procedures (SOPs) established by the study site or governing institution.

C. Blood Collection

i. Blood will be collected from patients in 1 "Carolina Blue" citrated 1.8 ml Vacutainer tube provided to each study site by UTSW. There will be no method of blood collection specified; samples may be obtained through routine phlebotomy, or through central venous catheters (as long as they have been purged of heparin), or arterial lines.

D. Performance of ROTEM

i. ROTEM will be performed on admission to the study, as close as possible to the time of admission (Day 1) laboratories and blood sample collection for the ALFSG Registry. If it is not possible to collect a sample on Day 1, ROTEM may begin on any subsequent day (Days 2, 3, 4 or 5) of the study.

ii. ROTEM will be performed daily for up to 5 days after admission to the study (*ie.*, Days 2,3,4,5). Daily samples will be collected with routine morning labs, if possible.

iii. If an enrolled ALI patient converts to ALF, the ROTEM collection will stop at the time of ALF enrollment into the ALFSG Registry (i.e. regardless of whether 5 days of collection has been achieved). Additional ROTEM collections will not be performed once this patient has converted to ALF nor may the patient be re-consented into the ROTEM study as an ALF patient.

iv. If an enrolled patient receives a liver transplant or is discharged from the hospital, ROTEM collection will stop at the time of transplant or discharge.

v. Daily ROTEM determinations are required and will be performed on whole blood within 2 hours of blood draw. Samples that are not processed within this 2 hour window will be documented on the sample deviation log. If 4 hours have passed since the blood draw, then the sample should not be run and should be discarded.

vi. ROTEM blood sample should be collected within 1 hour of completion of transfusion/infusion of pro-hemostatic factors (plasma, platelets, cryoprecipitate, rFVIIa), if such transfusion occurs within usual working hours of the site PI and/or study coordinator. The ROTEM should be performed within 2 hours of the blood draw. Post-transfusion ROTEM determinations are *optional, but strongly encouraged*. The “optional” status of these tests is necessary in order to account for times of day where coordinators are not in the hospital, or are unable to perform the ROTEM because of other obligations, such as

performing the methacetin breath test. Reasons for inability to collect post-transfusion samples will be documented, though these missed optional assessments will not be recorded as deviations.

a. Obtaining blood for ROTEM after completion of one type of blood product is preferred.

b. Obtaining blood for ROTEM after completion of transfusion of multiple types of blood products is also recommended if unable to obtain after transfusion after one type of product.

vii. ROTEM will be run for 90 minutes, which will allow capture of clot lysis up to 60 minutes after Maximum Clot Firmness (MCF), and will be performed by a ROTEM-trained site study coordinator.

8. Data to be Collected

a. **ROTEM Data**

i. INTEM (intrinsic coagulation pathway)

ii. EXTEM (extrinsic coagulation pathway)

iii. FIBTEM (test to isolate the effects of coagulation pathways from the effects of platelets on hemostasis)

For each of the above, the following data will be recorded by the ROTEM analyzer:

CT: clotting time

CFT: clot formation time

alpha-angle

A10, A20: amplitude 10 and 20 minutes after CT

MCF: Maximum Clot Firmness

LI30 and LI60: Lysis Index 30 and 60 min after CT - % of clot remaining

ML: Maximum Lysis - % of clot lost relative to MCF at any time during the test run

LOT: lysis onset time

b. **Patient Data**

i. *Laboratories*. The standard-of-care laboratories will be collected as per the usual practice of the ALF Study Group.

ii. *Events*. The ALF Study Group case report forms (CRFs) will be modified to collect the following in addition to the standard events:

-- Bleeding events (day and approximate time of occurrence, location of bleed, relationship to invasive procedure or spontaneous, units of factors/blood components and RBC transfused within 24 hours of the bleeding episode).

-- Thrombotic events (time and day of occurrence, location of thrombotic complication (renal replacement catheter, central venous catheter, native blood vessel)).

iii. *All transfusions, infusions, and medications* which may have an impact on ROTEM (plasma, rFVIIa, cryoprecipitate, RBC, NAC, gastric acid suppression medications, heparin).

- Information on pre-enrollment transfusions that were administered to the enrolled patient will be collected regardless of the timing of the transfusion. Date, type and units will be collected if available.
- Ideally, the clinicians caring for the patient should notify the study coordinator of an impending transfusion post study enrollment, so that plans for the ROTEM can be made ahead of time. The request for advance notice of impending transfusion should be made to our intensivist colleagues during the pre-study In-Service.
- The laboratory information leading to the clinical decision to transfuse should be recorded. In most instances, the decision will be made by clinicians considering

results of standard hemostatic tests (INR, platelet count, fibrinogen). In some centers where TEG or ROTEM is already available and used as local standard-of-care, the results of these tests may be considered in deciding to transfuse.

- These considerations will be captured in the electronic CRF.
    - The time (approximate), day, and route of transfusion administration will be recorded.
    - In patients receiving renal replacement therapy (RRT), the method of circuit anticoagulation (heparin or citrate) will be recorded.

iv. *Invasive Procedures*. All invasive procedures will be recorded, including time, date, prophylactic pre-procedural factor/blood component transfusion, and complications (eg., RRT and central venous catheters, transjugular liver biopsies, intracranial pressure monitors).

9. Statistical Considerations

1. This is an observational study designed to gather data for exploratory analyses and hypothesis-generation for future studies. Clinical data will be captured using case report forms as well as a direct download of the ROTEM parameters. Global hemostasis will be examined using three primary ROTEM parameters for each of the 3 assays: clotting time (CT), maximal clot firmness (MCF), and the maximal lysis (ML). The first test at admission into the study will serve as the baseline against which subsequent daily values will be compared. In addition to the above the following will be examined in the study population: ROTEM parameters obtained routinely on a specific study day will be compared to those parameters obtained within 1 hour of transfusion of blood products or infusion of rFVIIa on the same day.
2. Potential effects on the hypercoagulable state of the patient between those that do and do not receive pre-enrollment transfusions will be examined.

3. Bleeding complications (spontaneous and post-procedural) and thrombotic complications will be assessed in relation to ROTEM parameters from the same day, and whether pro- and anti-hemostatic factors were administered.
4. ROTEM parameters will be compared before and during NAC administration (if available), and compared to those obtained from patients who did not receive NAC.
5. The FIBTEM test will be used to calculate the relative contribution of fibrin and platelets to overall clot firmness.
6. Fibrinolysis will be assessed by the decrease in clot firmness at 60 minutes after reaching maximal clot firmness (MCF).

All data will be presented with two-sided 95% confidence intervals. Associations between variables will be examined using generalized linear regression models with a significance level of 0.05.

10. Data Collection

Data that is collected and generated from the ROTEM device will be electronically transferred to the study database. Sites will enter case report form data into the password protected study database housed in WebDCU™. The WebDCU™ is a validated web-based clinical trials management system that provides the infrastructure for real-time data capture and data sharing and includes designated web servers and supporting database servers. This user-friendly web-based database system will be used for subject enrollment, data entry, data validation, project progress monitoring, user customizable report generation, and secure data transfer. The web-based data capturing system allows for study data to be directly entered into the study specific database by the site via a secure internet connection. Secure Socket Layer (SSL) is used for data encryption. The web system combines all study tools into one system which includes study database, online training and help desk, subject calendar, electronic data clarification request (DCR) process, data entry, case report form (CRF) and participate tracking

system, audit trail, and report generation mechanisms. This is the system used for the currently ongoing ALFSG Registry. Clinical data collected for this study will be manually entered in the ALFSG Registry WebDCU™ for each subject under the identification number assigned when the subject is enrolled into the ALFSG Registry.

11. Institutional Review Board Approval

Prior to beginning subject enrollment in the study, the Institutional Review Board (IRB) for each clinical site must have approved the protocol and informed consent documents for a competent patient and/or a patient's legally authorized representative or family member. IRB renewal of approval of the protocol and informed consent documents must be obtained annually (or more frequently if required by an individual IRB) and for any subsequent protocol amendments. Sites may submit this study as a sub-study to the ALFSG Registry if allowed per local IRB policy.

12. Safety Plan

Given the minimal risk associated with this non-intervention study, a separate Data and Safety Monitoring Board (DSMB) will not convene. Risks to the patient are only those associated with the collection of blood samples. In any hospitalized patient, blood sampling is a routine procedure and complications are limited to minor pain and/or bruising. Thus, adverse events will not be recorded.

13. List of References

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