Altasciences, 6605 Merrill Creek Pkwy, Everett, WA 98203, United States
Altasciences.com | contact@altasciences.com

Use of Thromboelastography (TEG) in Preclinical Studies

N Lalayeva and K Thrall
Altasciences, Seattle WA

Abstract

Aims: Preclinical drug development focused on coagulation will typically include frequent analyses of hematology and coagulation. In addition, thromboelastography (TEG) is useful to monitor clot strength, fibrinolysis, and helping to diagnose platelet dysfunction and hypercoagulability. TEG must be initiated shortly following blood collection, thus, it is essentially a point-of-care technique.

Methods: In practice, whole blood is collected in sodium citrate and an aliquot of citrated blood is added to a vial containing Kaolin. The Kaolin mixture is then pipetted into a disposable TEG cup and analyzed on a calibrated machine. The resulting TEG tracing provides data on the rate of clot formation (alpha-angle), time to achieve a certain clot strength (K), time to initial fibrin formation (R), overall stability of the clot (MA), and decrease in stability of the clot at 30 or 60 min (LY30 or LY60).

Results: TEG analysis has been utilized in preclinical studies focused on bleeding disorders and platelet dysfunction in multiple large animal models and correlated well with conventional hematology data. For example, a TEG tracing illustrating long R and K times, and low alpha-angle and MA suggestive of thrombocytopenia directly correlated with decreased platelet counts. The long R time suggestive of a deficiency in clotting factors was compared to measured levels of individual coagulation factors. Data indicated that coagulation factors VIII, XI, XII, and XIII were markedly altered during the critical point of thrombocytopenia. These data support the continued use of multiple approaches to evaluate the coagulation cascade to provide the most meaningful interpretation.

Introduction

- Thromboelastography is used in the clinic to measure clot strength, diagnose and quantify fibrinolysis, and help diagnose platelet dysfunction and hypercoagulability
  - Two approaches: thromboelastography (TEG) and rotational thromboelastography (ROTEM)
- In the preclinical setting, TEG is rarely encountered, but can add valuable information in drug development focused on hematology and coagulation
- There are challenges associated with TEG
  - Samples must be analyzed within 2 hours from collection
  - The entire analysis can take 30 to 60 minutes to complete; this can be even longer in hemophilia models
  - Samples must be handled carefully, and no vibration can occur once analysis starts

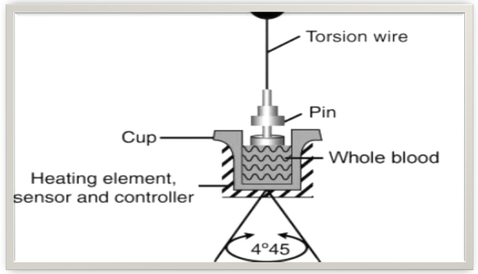

Figure 1: How it works. Citrated whole blood mixed with Kaolin is aliquoted into a heated disposable cup, a pin attached to a wire is lowered into the cup and the apparatus oscillates back and forth. The torsion on the wire measures clot formation.

Methods

- Whole blood is collected in sodium citrate
- An aliquot of citrated blood is transferred to a vial containing Kaolin
- The Kaolin mixture is transferred to a disposable TEG cup and analyzed on a calibrated machine
- For analysis, a pin on a torsion wire is lowered into the blood sample and TEG cup is oscillated back and forth. As the clot develops, measurement on the torsion wire translates into descriptive data.
- The TEG tracing can be interpreted to help understand thrombosis and fibrinolysis changes

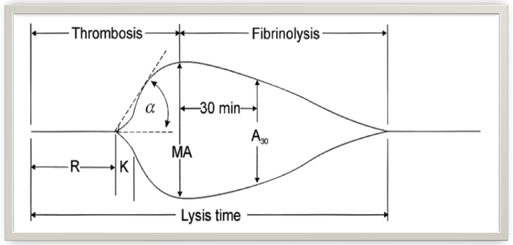

Figure 2: Normal TEG tracing:
- R (reaction time) is the time to initial fibrin formation
- K (kinetics) is the time to achieve a certain level of clot strength
- α angle is the slope of the line between R and K and reflects rate of clot formation
- MA (maximum amplitude) is the overall stability of the clot
- Lysis time (LY30 or LY60) is the decrease in clot stability or fibrinolysis

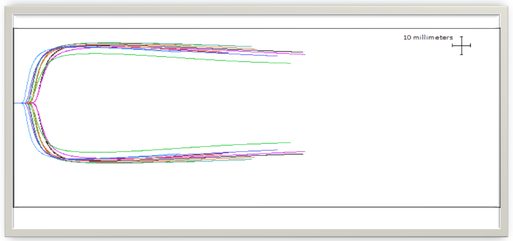

Figure 3: Normal data over multiple time points, with corresponding numeric values given below:

Seq	Channel	R min	K min	Angle deg	MA mm	LY30 %
1	1	4.1	0.9	76.9	74.3	2.1
2	1	2.9	1.0	76.7	77.4	1.3
3	1	5.2	1.1	76.0	76.3	2.6
4	1	3.8	0.9	77.8	76.7	3.5
5	1	4.2	1.0	76.5	71.8	1.2
6	1	2.8	0.8	78.5	78.4	2.3
7	1	4.8	1.0	76.7	71.8	1.7
8	1	4.2	1.0	75.5	69.9	1.6
9	1	4.6	1.0	76.9	66.4	2.4
10	1	5.3	1.1	74.3	71.6	2.5
11	1	2.0	0.9	71.2	73.1	1.5

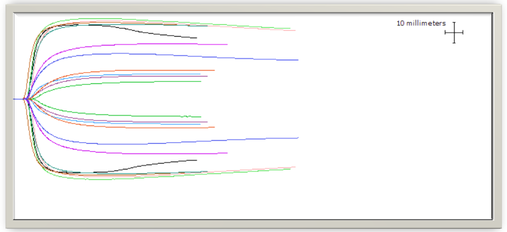

Figure 4: Example of disease state: low α angle and MA consistent with thrombocytopenia

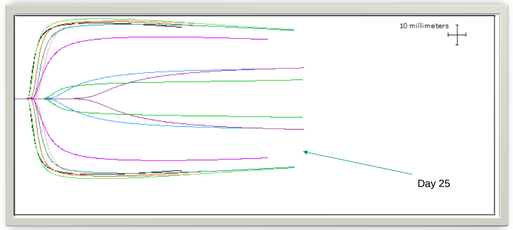

Figure 5: Example of disease state:
- Long R time consistent with deficiency in clotting factors
- Long K time consistent with deficiency in fibrinogen
- Low α angle and MA consistent with thrombocytopenia

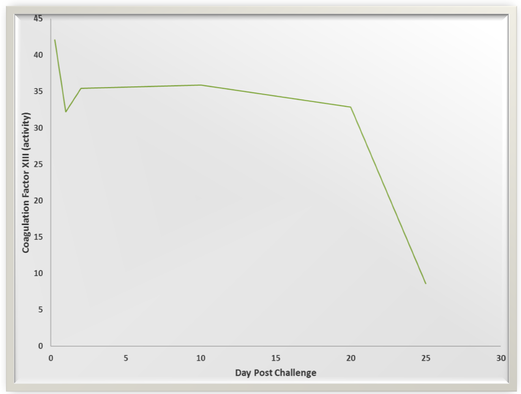

Figure 6: Blood levels of Coagulation Factor FXIII correlate with TEG tracing, illustrating decrease in at final time point (Day 25)

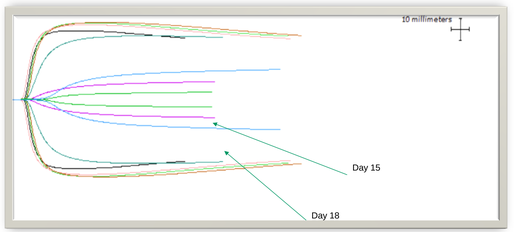

Figure 6: Benefit of measurements over time: TEG tracing tracks the development (day 15) and recovery (day 45) of thrombocytopenia (Low MA)

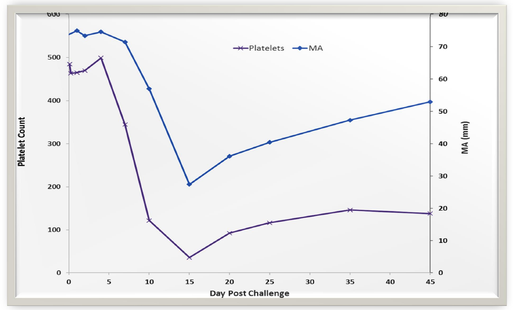

Figure 7: Hematology measurements corresponding to timepoints illustrated in Figure 6 correlate platelet count decrease to nadir (Day 15) and improvement observed at Day 45

Conclusion

- Data indicated that coagulation factors VIII, XI, XII, and XIII were markedly altered during the critical point of thrombocytopenia.
- These data support the continued use of multiple approaches to evaluate the coagulation cascade to provide the most meaningful interpretation.