Standardization of Thromboelastography: Values and Challenges

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ABSTRACT

Laboratory evaluation of hemostasis has been performed using plasma for several decades. The cell-based model of coagulation has now led to renewed interest in the global assays of coagulation such as thrombin generation and thromboelastography. These tests have remained as research tools, however, because of the lack of studies to demonstrate their reliability. The number of publications in the field of thromboelastography is growing daily, and many areas of clinical medicine are targeting the ability of this assay to evaluate in real time the process of coagulation and fibrin polymerization. It is clear that the methods employed by different investigators differ significantly, and therefore the results are not comparable. It is therefore critical to standardize the assay to achieve clinical relevance. This article summarizes the TEG-ROTEM Working Group's efforts to try and standardize thromboelastography and the challenges faced in this process. Although this has been the first effort to standardize this test, it is extremely important to continue this work, so that we may investigate the usefulness and possible applications of thromboelastography in evaluating the process of hemostasis.

KEYWORDS: Thromboelastography, standardization, hemostasis

Thromboelastography was developed by Dr. Hellmut Hartert in Germany >60 years ago. However, it was not used in clinical practice until 25 years later, by Kang in Pittsburgh, Pennsylvania, in the setting of liver transplantation for monitoring changes in coagulation and fibrinolysis commonly seen with the procedure.[1] Since its inception, advances in technology have resulted in significant improvements in the instrument, thereby making it an easy to use point-of-care device. Most of the literature refers to the use of thromboelastography in surgical and trauma units, where its main purpose has been to decrease the use of blood and blood products. However, with our current knowledge of the process of coagulation and the understanding of the role of both cellular as well as enzymatic factors in clot formation, it has become imperative that the tests we use for the assessment of the process of coagulation include all cellular elements to obtain an accurate assessment. By using whole blood, thromboelastography makes it possible to study the contribution of the various cellular and enzymatic elements in the process of coagulation. Thromboelastography also allows us to study the clot well beyond the initiation of clot formation, and therefore provides information on clot strength as well as the amount of fibrin generated. This continues until clot lysis occurs via fibrinolysis. Hartert compared the coagulation of blood to building a house and said, "TEG does not end when the foundation stone is laid, as one-stage clotting tests do."

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Global Hemostasis: New Approaches to Patient Diagnosis and Treatment Monitoring; Guest Editor, Maha Othman, M.D., Ph.D.

Semin Thromb Hemost 2010;36:707–711.
DOI: http://dx.doi.org/10.1055/s-0030-1265287.
ISSN 0094-6176.

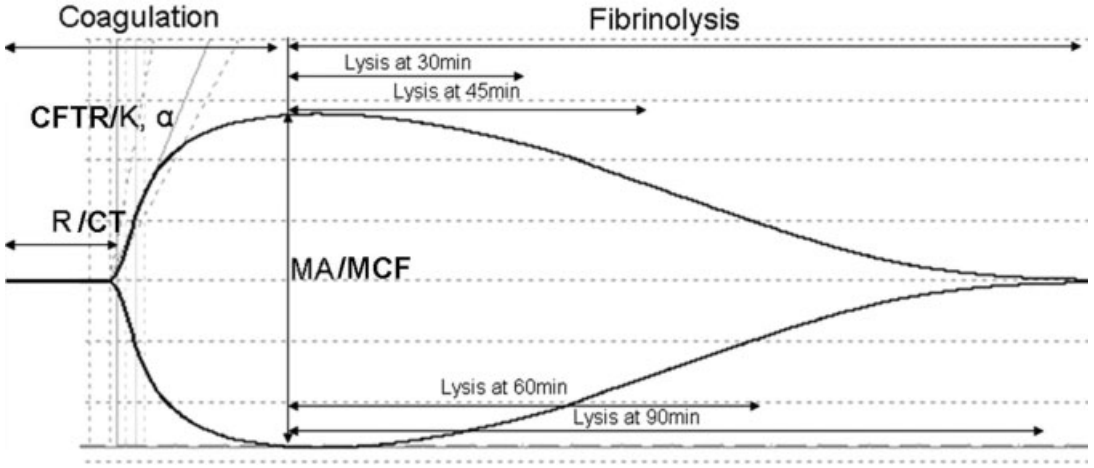

Figure 1 A thromboelastograph showing various test parameters.

PRINCIPLES OF THROMBOELASTOGRAPHY

The term *thromboelastograph* (TEG) was used to describe the trace that was produced from the measurement of viscoelastic changes resulting from fibrin polymerization.[2] Thromboelastography today can be performed on two instruments. These are the TEG (Haemonetics Corp., Braintree, MA) and the ROTEM (Tern International GmbH, Munich, Germany). The two instruments work on the same principle but have minor differences in mechanical operations. In both instruments blood is incubated in a heated cup. Into this blood sample, a pin is suspended connected to a computer that functions as a recording device. The change in viscosity that occurs with clot formation is detected via a torsion wire in the TEG and an optical detector in the ROTEM. In the TEG, the cup oscillates at a fixed angle, whereas in the ROTEM the pin oscillates, with the resulting clot causing a bridge between the wall of the cup and the pin. This causes impedance in the rotation of the cup in the TEG and impedance in the rotation of the pin in the case of the ROTEM, which is transmitted to the computer and a trace is generated. The resultant tracing is called a *thromboelastogram*, which provides a graphic representation of the fibrin polymerization process (Fig. 1). The mechanism of the TEG and the ROTEM is shown in Fig. 2.

WHAT NEEDS TO BE STANDARDIZED?

Standardization of a laboratory assay is extremely essential to ensure that the results are reliable and reproducible and therefore clinically useful. The ability to reproduce a test is facilitated by the use of standardized preanalytical and analytical procedures.

Preanalytical and Analytical Variables

Laboratory assessment of coagulation begins with collection of the venous sample, which is affected by factors such as size of the needle used and the venous pressure used to facilitate sample collection. Standardization therefore needs to begin with sample collection. It has been shown that inappropriate phlebotomy and sampling techniques can significantly activate the coagulation factors and platelets resulting in erroneous results. The use

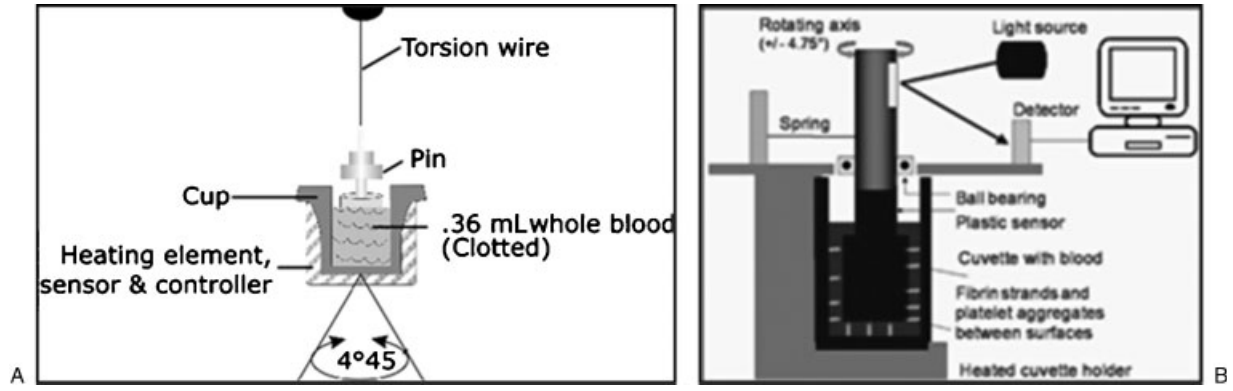

Figure 2 The mechanism of thromboelastography using (A) the TEG (Haemonetics Corp., Braintree, MA) and (B) the ROTEM (Tern International GmbH, Munich, Germany).

of butterfly needles for sample collection has been shown to result in significant contact activation.[3] The use of Vacutainer tubes (Becton Dickinson, Meylan, France) has been associated with more platelet activation compared with the use of Sarstedt S-Monovette (Sarstedt, Orsay, France) that allows slower aspiration of blood, limiting the damage to the platelets.[4] After sample collection, the addition of anticoagulants such as citrate has also been shown to influence coagulation,[5] and because it is extremely difficult to perform the testing at the bedside, it is essential to further investigate this matter and determine the ideal agent or the significance of these results. The inhibition of contact pathway by addition of corn trypsin inhibitor is also important when low concentrations of tissue factor are used as activators.[5] The time between sample collection and performing the tests is also still debated, and some investigators have recommended a resting time of 30 minutes after sample collection, whereas others have not.[6] Both instruments maintain the sample at 37°C, thereby decreasing the influence of temperature on the process of coagulation.

Both instruments share the basic principle, and the basic test methodology has already been defined by the manufacturer. However this may require fine tuning in the form of defining the order in which the reagents are introduced and how they are mixed in the cup because this has been shown to determine the distribution of the reagents in the sample and therefore to alter the results.[7] In the TEG, the reagents have to be mixed manually in the cup by re-aspiration using the pipette or by stirring, whereas in the ROTEM this can be done with the automatic pipette or manually. These minor changes may result in differences in the results and need to be investigated further. Currently, investigators use different activators to initiate coagulation. Reagents provided by the manufacturer include Kaolin for the TEG and INTEM for the ROTEM, as well as tissue factor. Because these activators vary in the strength of activation, the results from each instrument vary and are not comparable. These variables may have a significant impact on the results and need further investigation for standardization. For detailed factors that affect the TEG trace, refer to the article by MacDonald and Luddington in this issue of *Seminars in Thrombosis and Hemostasis*.[8]

THE TEG-ROTEM WORKING GROUP

The TEG-ROTEM Working Group was established in 2006 by one of the authors (Meera Chitlur) and consists of an international group of investigators with an interest in thromboelastography. Table 1 lists the 12 centers from around the globe. The primary objective of this group is to standardize the test methodology to achieve results that are reliable, reproducible, and clinically relevant. The initial studies from this group were presented at the International Society on Thrombosis and Hemostasis (ISTH), Scientific and Standardization Committee (SSC), which met in Vienna, Austria in 2008 (manuscript in progress). Two tasks have been undertaken by this group so far. Because whole blood cannot be transported without significant detriment to the process of coagulation, it was determined that plasma would be the sample of choice. Standard pooled normal plasma as well as factor VIII (FVIII)-deficient plasma were used for the experiments. The parameters measured were clotting time (R/CT), time to clot formation (K/CFTR), maximum clot firmness (MA/MCF), clot strength (G), and rate of clot polymerization (Angle) because these are the primary measures of clot formation on the thromboelastograph. Six assays split into three sessions were performed for each parameter.

Table 1 The TEG-ROTEM Working Group

Country	Centers
United States	Wayne State University, Detroit, MI
	Gulf States Hemophilia and Thrombophilia Center, Houston, TX
	Children's Hospital of Orange County, CA
	Children's Hospital, Los Angeles, CA
	University of Vermont, VT
	Comprehensive Bleeding Disorders Center, Peoria, IL
Israel	National Hemophilia Center and Thrombosis Unit, Sheba Medical Center, Tel Hashomer
Europe	University Hospital Skejby, Aarhus, Denmark
	University of Oslo, Oslo, Norway
	Hemophilia Center and Hemostasis unit, Royal Free Hospital, London, United Kingdom
Canada	Hôpital Ste-Justine, Montréal, Canada
	Queen's University, Kingston, Canada

The initial task (task 1) was to determine if reproducibility could be demonstrated across centers by using the same methodology and reagent on pooled normal plasma. An intrinsic activator was chosen as the first choice because standard reagents are provided by manufacturers of both instruments. The activators used were kaolin for TEG and INTEM for ROTEM. The samples for task 1 were run on five ROTEMs and six TEGs from five different laboratories. Provided each channel is considered as one run, we had 33 runs on the ROTEM and 28 on the TEG. This study was mainly limited by the availability of pooled normal plasma.

After having gone through this exercise and once our efforts were better organized, we included 9 laboratories from around the globe with 11 ROTEMs and 6 TEGs for task 2. In this exercise, we used both pooled normal plasma as well as FVIII-deficient plasma, and had 264 runs on the ROTEM and 72 on the TEG.

Analysis of the data showed that the coefficients of variation (CVs) varied from 6% to 60% and varied significantly for the different parameters, with the lowest CVs seen with the R/CT and MA/MCF. The intra-laboratory variance was also significant with CVs >10%. Even though these results were not satisfactory, this has been the first effort to standardize this methodology. Significant work remains to be done to improve the reliability and reproducibility.

CHALLENEGES FACED DURING THE PROCESS OF STANDARDIZATION

The monumental efforts of the members of this group were instrumental in the successful completion of the tasks that the group set out to accomplish. The first major hurdle was related to the sample that would be used for the purpose of standardization. The objective of thromboelastography is to be able to use "whole blood" to obtain a "global" picture of coagulation. However, because plasma is more stable than whole blood for storage and transportation, plasma was used for the interlaboratory assays. A pool of normal as well as FVIII-deficient plasma was provided by Ingerslev and Sorensen at the Center for Haemophilia and Thrombosis, Aarhus University Hospital, Skejby, Denmark. A major problem encountered was the large volume of plasma required for the task, and in the future, for studies of the same nature, a commercial source of plasma should be considered. Because these studies were conducted by a group of individual investigators with a personal interest in the field, these studies were performed with financial assistance from individual institutions, and support from the manufacturers for the disposables and activators. Obtaining a reliable source of funding for studies of this nature would be highly recommended. Several protocols were established for each step in the process to ensure accurate results. All plasma samples were transported frozen with the utmost precautions to prevent any freeze-thaw effects. A protocol was followed to ensure all samples were thawed in the same manner to prevent any activation of coagulation factors and eliminate any discrepancies that might arise. It was determined that all instruments had undergone a maintenance check within 6 months of conducting this task to ensure they were all in good working order. The procedure to conduct each test was based on the protocol per the manufacturer's recommendation. These procedures, although time consuming, are an essential aspect of standardization of any laboratory assay. In spite of these rigorous efforts the results were not as expected and may be related to several issues such as the use of plasma instead of whole blood or minor procedural variations that will need to be addressed. In addition, the preanalytical variables mentioned previously have not been addressed as yet.

CLINICAL FOCUS OF THE TEG WORKING GROUP

In spite of the fact that the assay remains investigational, several researchers have attempted to study the usefulness of thromboelastography in various clinical situations. As previously mentioned, thromboelastography was initially used to evaluate the coagulopathy associated with liver transplantation and cardiac surgery. Today, the uses have expanded beyond this realm into the field of bleeding disorders as well as thrombosis. The TEG-ROTEM Working Group was established to address the need for standardization of this assay, which we believe has a significant potential in the assessment of coagulation. The group will continue its efforts to standardize the methodology of thromboelastography and facilitate its use in the diagnosis and management of patients with bleeding disorders especially those with hemophilia with or without inhibitors. We strongly believe this technology may enable us to characterize the phenotypic variation in bleeding patterns seen in patients with bleeding disorders and also be useful in the monitoring treatment with bypassing agents.

CURRENT STATUS OF THE WORKING GROUP AND FUTURE PLANS

Even thought the results have not been totally satisfactory, we now understand the issues that need to be tackled and can move forward with a better focus. At the last meeting convened by the group, clinical scenarios where this technology may be used or has been used were discussed, to help us better understand the potential of this assay and assist in consideration of methods for standardization. The Working Group will continue its efforts in this area.

It must be noted that the FVIII/IX subcommittee of the ISTH SSC has now established a Working Party for the Standardization of Thromboelastography. The objective of this group is to standardize the methodology to facilitate the evaluation and management of patients with hemophilia. The Working Party is chaired by Benny Sorensen (United Kingdom), with Meera Chitlur (United States) as co-chair, and members include several members from the TEG-ROTEM Working Group and other established investigators.

The interest generated by this technology has shown that researchers would like a significant paradigm shift in the assessment of hemostasis from conventional plasma-based assays to assays that can evaluate coagulation in a more physiological manner.

CONCLUSION

Thromboelastography is an old test that is newly modified, giving us the opportunity to evaluate bleeding and clotting in a new light. The ability to use whole blood

samples gives us the opportunity to assess the contribution of the cellular and enzymatic factors in the process of coagulation. In the management of acute bleeding associated with trauma and surgery, the thromboelastograph has proved its mettle. Now it remains to be established if the same can be said for its ability to diagnose and monitor treatment of patients with bleeding and thrombotic disorders. To establish this, standardization is an absolutely essential step, which the TEG-ROTEM Working Group hopes to achieve. Standardization will establish that the test is reproducible and reliable, which will then make it a clinically relevant assay. However, standardization for this assay may need to be individualized based on the specific pathway in coagulation of interest. Therefore, the test for detection of coagulation deficiency may be different from that used to diagnose a thrombotic disorder because the sensitivity of the activator to the process of coagulation in question may differ significantly. These issues remain to be established and will assist in defining the future of this assay. Thromboelastography appears to hold great promise, and by modifying the assay to make it more specific and sensitive we may be able to provide an assay that may be of great clinical utility for multiple bleeding as well as thrombotic conditions.

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