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Global assays in hemophilia

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ABSTRACT

The quest for the ideal method to study hemostasis in the evaluation and management of patients with bleeding disorders such as hemophilia is an ongoing effort. With the rapid evolution of biotechnology and the emergence of several new products for treatment of patients with hemophilia with and without inhibitors, there is a great need for tests that can be used to reliably evaluate and monitor our interventions. Global assays in coagulation allow the study of the interaction between the components involved in the process of hemostasis and are therefore considered by many to be more reflective of the in vivo hemostatic mechanism. Here we provide a brief review of the most widely used global assays in hemostasis (thrombin generation and thromboelastography) and their utility in the evaluation and management of hemophilia.

1. Overview of coagulation

Our understanding of the process of hemostasis has changed dramatically over the last couple of decades. We are now aware that the cascade model of coagulation does not explain the bleeding diathesis of hemophilia and other factor deficiencies or the lack of bleeding with other factor deficiencies, but is excellent for describing the action of routinely performed coagulation screening tests. The interplay between the enzymatic factors of coagulation and the cellular components of hemostasis was elegantly described by Hoffman et al [1], in the cell-based model of coagulation, and provided the necessary explanation for the bleeding tendency seen in patients with hemophilia and other bleeding disorders. While the cascade model appears to indicate that the intrinsic and extrinsic pathways are redundant, we now know that the extrinsic pathway initiates the process of coagulation and the intrinsic pathway is necessary to amplify the process of thrombin generation to form an effective clot. According to this model, a breach in vascular integrity leads to exposure of collagen and tissue factor 9TF). Circulating active factor (F)VII then binds to the exposed TF and results in the activation of small amounts of FX to FXa and resultant generation of a small amount of thrombin; this is known as the "initiation phase." This phase is followed by the "amplification phase," where the small amount of thrombin now activates platelets that adhere and accumulate activated clotting factors on their surfaces. The next phase is the "propagation phase" where the activated clotting proteins combine with their co-factors on the platelet surface to generate physiologic amounts of thrombin necessary to stop bleeding. Based on this model the defect in hemophilia is recognized to be in the propagation phase required to amplify the amount of thrombin. The initiation and amplification phases involving TF, FVII and platelets occurs normally. However, the assembly of FVIII-FIXa complexes on the platelet surface to provide a thrombin "burst" does not occur, which results in poor generation of FXa and poor fibrin clot structure, as well as the production of a weak and ineffective thrombus that is insufficient to stop bleeding. The impaired thrombin production leads to poor activation of FXIII and TAFI (thrombin activatable fibrinolysis inhibitor), and poor clot stability.

2. Overview of standard coagulation assays

Several coagulation assays in broad clinical use support physicians in both diagnosing and treating coagulation disorders. As described above, the coagulation system is like a symphony orchestra and assaying one component is akin to playing a piece of music designed for an orchestra with just the violins. As such, these assays are limited in their ability to provide a complete picture of how a patient's coagulation system is functioning. In addition, assays set up in artificial systems can lead to paradoxes such as the significantly prolonged activated partial thromboplastin time (aPTT) in a patient with FXII deficiency, a condition that is not associated with bleeding. Furthermore, sometimes results can be misleading as in the prolonged aPTT in some patients with the

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antiphospholipid antibody syndrome. Despite this, the standard coagulation assays have a role in clinical medicine. The most commonly used coagulation assays are the aPTT, prothrombin time (PT), PFA-100®, and platelet aggregation studies; while a detailed discussion of these assays is beyond the scope of this review, suffice it to say that the major difference between these assays and the global hemostasis assays lies in the fact that each of the above is used to evaluate only a part or parts of the coagulation system: the clotting factors for the aPTT, and PT and platelet function for the PFA-100 and platelet aggregation tests. The ideal coagulation assay should mimic in vivo coagulation as much as possible, combining the use of whole blood under conditions of normal blood flow over an endothelial lining. Alas, such an assay is not yet a clinical reality, although the global assays described below begin to incorporate some of these ideals.

3. Overview of global hemostasis assays

The concept for a global hemostasis assay is not new. Hartert described the first thromboelastography (TEG) in 1948 as one example [2]. There are several so-called global hemostasis assays that have been developed over the past two decades and some (TEG) are licensed for clinical use in particular as point of service devices in the operating room to assist in hemostasis management for patients undergoing major surgery. With respect to hemophilia, a modest but growing literature is available for TEG and thrombin generation assays (TGAs) and this review will focus on these devices. The two assays differ in a number of important ways. First, although both assays can accommodate whole blood, platelet-rich and platelet-poor plasma, the vast majority of studies done thus far with each device have used whole blood for TEG and platelet-poor plasma (hereafter referred to as just plasma) for TGA. Second, the TEG demonstrates the dynamics of clot formation over time while the TGA (as its name implies) demonstrates thrombin generation over time. The advantages of the TEG are that it uses whole blood and measures clot formation, which comes closer to the idealized coagulation assay, while the TGA measures a surrogate marker for clot formation. The main advantage of the TGA is that by using plasma, samples can be stored and shipped, which allows for testing to be done at any time and in a central or reference laboratory whereas the TEG assay must be done within 2 hours of sampling, requiring it to be done locally. Furthermore, the utilization of plasma allows sharing of samples between labs facilitating collaborative studies and external quality-assurance schemes. Regardless, both assays have been demonstrated to be useful for both demonstrating different hemophilia phenotypes and for assessing the response to hemostatic therapy. In fact, the assays can be considered complementary with each one demonstrating different biomarkers of hemostasis. The sections below will review some of the key studies that have been conducted to improve our understanding of hemophilia and its treatment.

4. Thromboelastography

4.1. General principles

The thrombelastographic principle introduced by Hartert [1] has been modernized in the computerized version of the TEG 5000 device manufactured by Haemonetics (Braintree, MA, USA) and the ROTEM manufactured by Tem International (Munich, Germany). Although the qualitative and quantitative results produced by the two devices are identical, the nomenclature is different, mostly arising due to proprietary concerns (Table 1). Thus, although the term thromboelastography is a general term, it mostly refers to the TEG 5000, while for the ROTEM the term thromboelastometry is more often used. Hereafter, “TEG” will be used generically to refer to both devices and where one device is referred to specifically, either TEG 5000 or ROTEM will be used.

The devices consist of a heated cylindrical sample cup into which a pin is suspended. In the TEG 5000, the cup oscillates at $\pm 4^{\circ}45'$ every 5 seconds and the pin is suspended freely into the cup by a torsion wire. In the ROTEM, the cup is stationary while the pin transduction system oscillates at $\pm 4^{\circ}45'$ every 6 seconds. Once the pin is inside the cup and the oscillation begins, the forming clot results in a physical connection (by strands of fibrin) between the cup and the pin, transferring the torque of the cup to the pin. The elastic strength of this connection increases gradually as the properties of the forming clot change and a mechanical-electrical transducer is used to convert the rotation of the pin to an electrical signal, which is then recorded by a computer. The computer software produces both quantitative parameters (Table 1) and a graphical representation (Fig. 1), which allows one to evaluate the phases of clot formation and judge the adequacy of a patient’s coagulation system. Importantly, both assays can also demonstrate fibrinolysis thereby providing unique information on clot stability.

4.2. Methods

Given that both the TEG 5000 and ROTEM use whole blood (at least preferentially) the adequacy of sample procurement is paramount. A difficult or poorly performed venipuncture can leave artifacts, which can result in either enhanced or reduced clot formation. Further details on proper sample procurement can be found in Chitlur et al [3]. Once the sample is procured, several options exist with respect to activation of the samples. Coagulation may be initiated purely by contact activation with the cup (called “native”); however, in general, specific activators that can target the contact pathway or the extrinsic pathway are used as they both reduce the time to clot formation and improved the reliability of the assays. Activators for both the intrinsic and extrinsic system are available from the manufacturers. In addition, some investigators have used “homemade” reagents as activators. Further details regarding the activators and the controversies surrounding this issue can be found in the literature [3,4].

Table 1
Terms and units for the TEG 5000 and ROTEM.

TEG 5000 term	ROTEM term	Units	Clot formation property
Reaction time (R)	Clotting time (CT)	Seconds/minutes	Clot initiation
Kinetic time (K)	Clot formation time (CFT)	Seconds/minutes	Clot propagation
Alpha angle	Alpha angle	Degrees	Clot propagation
Maximal amplitude (MA)	Maximum clot firmness (MA)	Millimeters	Clot rigidity
Clot elastic modulus (G)	Shear elastic modulus (G)	Dynes/cm^2	Clot elasticity