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Can Global Assays Make Anticoagulation Safer and More Effective?

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H&O What are global assays of coagulation, and when are they preferred over specific assays?

HCH Global assays of coagulation are assays in which the whole of the coagulation mechanism cooperates to determine the result. First, however, we have to agree on what is meant by *coagulation* (or *clotting*) *mechanism*. If it refers to the entire mechanism behind normal hemostasis, which prevents thrombosis and undue bleeding, then *coagulation* is an inadequate descriptor.

For many years, scientists believed that the only purpose of the thrombin produced by the clotting mechanism was clotting. It has become increasingly clear, however, that this is not the case. Clotting occurs when just 2% or so of the prothrombin has been converted into thrombin; the remainder of the thrombin forms inside the clot. It is becoming increasingly obvious that this thrombin plays a large number of additional roles in hemostasis and thrombosis.[1] Also part of the system is the fibrinolytic mechanism, which removes clots. *Coagulation mechanism* is therefore a misnomer. Instead, it is the *thrombin generation (TG) mechanism* that ensures adequate hemostasis by providing sufficient thrombin to fulfill a number of different tasks, and the plasmin generation system that—when overactive—can cause severe bleeding despite normal TG.

Three types of global assays are available: those that use fibrin as an indicator, those that measure thrombin as it develops and disappears in clotting blood or plasma, and those that measure plasmin. Plasmin measurement is still in its infancy, so I will not be discussing it further here.

The fibrin-based tests are (1) thromboelastography, in which the mechanical resistance of the developing clot is measured; (2) turbidity measurements, in which light scattering by polymerizing fibrin monomers is measured; and (3) clotting times. Although they can all be considered global assays, some are more global than others. For example, prothrombin time (PT) is less global than partial thromboplastin time (aPTT) because the antihemophilic factors A and B contribute to aPTT but not to PT. Because all the fibrinogen is converted into fibrin long before all the thrombin has formed, each of these methods provides information only on the initial phase of TG.

Thrombin generation is triggered either in vivo, by blood coming into contact with extravascular tissue, or in vitro, by citrated blood or plasma being recalcified in the presence of tissue factor or another trigger. When this occurs, nothing appears to happen for several seconds or even minutes. Then, thrombin suddenly appears and the blood or plasma clots. During the lag time, miniscule amounts of thrombin are formed. The amounts are too small to make the blood clot, but large enough to activate elements of the clotting system—such as factors V and VIII—that are required for effective bulk TG.

The mechanism of thrombin formation thus changes over time, with the mechanism during the *lag* (or *initiation*) *phase*—which represents the clotting mechanism in the strict sense of the term—being fundamentally different from that during bulk production. Still later, after the clot has formed, physiological thrombin-limiting processes occur. These thrombin-limiting processes, which are defective in people with the factor V Leiden trait and impaired by the use of oral contraceptives, are uniquely important in preventing thrombosis but are not detected

by fibrin-based global tests. This fact is reflected in the observation that virtually no shortening of clotting times occurs in patients who have even a severe thrombotic tendency. As a result, hypercoagulability cannot be detected efficiently by fibrin-based global tests.

A much more useful definition of a global assay, therefore, is an assay that reflects that part of the phenotype of the clotting system that is relevant to the clinician, keeping in mind that the ideal test, one that represents both defective and excessive thrombin formation and fibrinolysis, does not exist.[2]

A large number of physical and chemical changes take place in clotting blood that in some way affect the blood or plasma's properties, including its turbidity, viscosity, mechanical resistance, and impedance. Although each of these properties can be analyzed with a global test, there is no escaping that the use of a test that measures TG is necessary if one wants to know about TG. Tests that use the formation of fibrin as a detector, such as thromboelastography and fibrin-related turbidity, provide information about the first phases of thrombin formation only. Still, they are well suited to detection of disorders of fibrinolysis, an area in which TG does not give direct information.

The TG test was developed in 1951 but only became popular after 2000 when a method was developed that allowed large-scale application.[5] Given that the entire coagulation mechanism exists specifically to produce thrombin, TG measurement is the global test par excellence.

An aphorism of mine—some might call it Hemker's law—is this: "The more thrombin, the less bleeding but the more thrombosis. The less thrombin, the more bleeding but the less thrombosis." The gist of this saying is that the amount of thrombin that forms is more important than the time that it takes to develop a clot, the mechanical properties of the clot, or the fibrin-related turbidity.

H&O How are global assays currently used?

HCH At this moment, thromboelastography and other similar assays are used in operating theaters and emergency departments.[6] Although TG testing has not yet been approved for clinical use, this is expected to occur in the near future. Approximately 1000 TG machines are spread among clinical and fundamental research laboratories all over the world that have been used in producing data for hundreds of published articles. The most important findings have been the observations that: (1) *all* antithrombotic therapy decreases TG; (2) there is a strong link between the amount of thrombin produced and venous thromboembolism; and (3) there is a strong link between bleeding and low TG, such as occurs in patients with hemophilia.

H&O What are some of the potential uses of global assays?

HCH As I said earlier, the fibrin-dependent global assays are ideal for surveillance of disorders of fibrinolysis and the quality of blood clot. However, as with clotting times, they are less suited to learning about excessive thrombin formation and risks of thrombosis. The potential use of TG testing directly derives from the findings cited earlier. First, because all antithrombotic therapy decreases TG, this test is perfectly suited for the control of such therapy. Second, TG directly informs about the risk that a person has to develop thrombosis, such as women or adolescents who are candidates for oral contraceptives but have a family history of thrombosis. Third, TG provides a rationale for therapeutic and prophylactic substitution therapy in people with hemophilia.[7] Finally, both the fibrin-dependent and the thrombin-dependent global tests can be used to guide management of bleeding in surgery and traumatic emergencies.

H&O Can global assays be used as hypothesis-generating tools to understand bleeding and hypercoagulable disease states?

HCH At this time, the main use of TG is as a precise tool by researchers who are investigating the mechanism behind bleeding and thrombotic disorders and who are testing new drugs. Actually, what we used in the late 1980s when we investigated the mode of action of heparins[8] was the old-fashioned TG test. The TG test is the closest thing to real life in that it is not so much a chemical test as a physiological function test on a piece of isolated organ. This test has saved the lives of thousands of laboratory animals because experimental dose-finding studies of anticoagulants can be first done via TG measurement in vitro. An additional advantage of the TG test is that it can be performed directly on human blood and plasma rather than needing to extrapolate from animal models.

H&O Do global assays have the potential to guide therapy, such as by revealing how much to anticoagulate?

HCH Yes, they have the potential to be very useful there. At this moment, low–molecular-weight heparins and novel oral anticoagulants (NOACs) are given in standard dosages. Although this is very convenient, over time it becomes clear that this is far from ideal. The reason is simple: the normal variation of TG capacity is 16%, which is as much as the variation of weight among healthy persons (with some correlation between the two). On top of that, the interindividual variation in

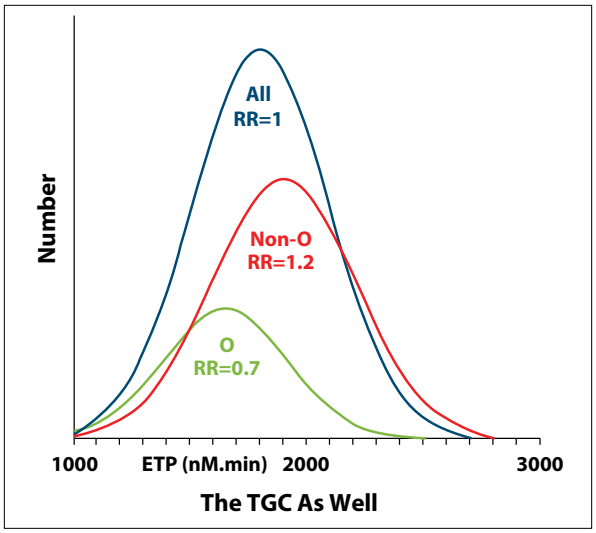

Figure. Distribution of the thrombin generation potential (endogenous thrombin potential, or ETP) in the population and in subpopulations with blood group O or non-O.

RR, relative risk of venous thromboembolism.

responsiveness to a standard dose of heparin or NOAC is approximately 20%.[9] In other words, someone with low TG and high responsiveness may develop a dangerous bleeding tendency on the same dose of anticoagulant on which someone with high TG and low responsiveness might as well eat candy (or swallow a placebo).

Proof that the variation in a normal population matters as to bleeding and thrombosis tendency comes, among others, from a study on TG in different blood groups. Based on epidemiologic studies, we have known for a long time that group O blood bleeds more easily than non-O blood, and has a thrombosis risk that is almost half of that of the non-O groups. In a study published in *PLoS One* in 2015, we showed that the non-O blood groups produce approximately 25% more thrombin than O-group blood (Figure).

H&O Have global assays been validated sufficiently to be ready for use in individual clinical assessment?

HCH The potential usefulness of TG testing for use in evaluation and treatment of venous thrombosis and hemophilia has been demonstrated in sufficiently large patient populations. Unfortunately, there is still no standard procedure that can be used in any laboratory; problems of standardization remain. The availability of good standard plasma appears to be an absolute must. Owing to the large inter-individual variation, comparison with a baseline person is of no use. Instead, pooled plasma is required.

H&O Are global assays likely to be more useful in some disease states than in others?

HCH I think that these tests will be equally useful in the treatment and prevention of bleeding in the hemophilias as in that of venous thrombosis, but laboratories that see patients with hemophilia are usually more specialised and less cautious about applying sophisticated techniques, so I expect to see global assays used earlier in hemophilia.

H&O What is the cost of the equipment required to perform these tests?

HCH The cost of a TG machine is approximately $30,000 at this time. We expect the test to be reimbursed at the same rate as any other useful laboratory test; it does not need to be more expensive than any other clotting factor determination.

References

1. Mann KG, Brummel K, Butenas S. What is all that thrombin for? *J Thromb Haemost*. 2003;1(7):1504-1514.

2. Hemker HC, Béguin S. Phenotyping the clotting system. *Thromb Haemost*. 2000;84(5):747-751.

3. Lancé MD. A general review of major global coagulation assays: thrombelastography, thrombin generation test and clot waveform analysis. *Thromb J*. 2015;13:1.

4. Brummel-Ziedins KE, Wolberg AS. Global assays of hemostasis. *Curr Opin Hematol*. 2014;21(5):395-403.

5. Hemker HC, Giesen P, Al Dieri R, et al. Calibrated automated thrombin generation measurement in clotting plasma. *Pathophysiol Haemost Thromb*. 2003;33(1):4-15.

6. Lier H, Böttiger BW, Hinkelbein J, Krep H, Bernhard M. Coagulation management in multiple trauma: a systematic review. *Intensive Care Med*. 2011;37(4):572-582.

7. Dargaud Y, Negrier C. Thrombin generation testing in haemophilia comprehensive care centres. *Haemophilia*. 2010;16(2):223-230.

8. Béguin S, Lindhout T, Hemker HC. The effect of trace amounts of tissue factor on thrombin generation in platelet rich plasma, its inhibition by heparin. *Thromb Haemost*. 1989;61(1):25-29.

9. Bloemen S, Hemker HC, Al Dieri R. Large inter-individual variation of the pharmacodynamic effect of anticoagulant drugs on thrombin generation. *Haematologica*. 2013;98(4):549-554.

10. Kremers RM, Mohamed AB, Pelkmans L, et al. Thrombin generating capacity and phenotypic association in ABO blood groups. *PLoS One*. 2015;10(10):e0141491.