Thrombin is a multifunctional protease that, in addition to hemostasis, supports other “nonhemostatic” mechanisms, such as the regulation of vascular permeability, vascular tone, inflammation, and angiogenesis. There is also evidence that thrombin plays a role in the pathogenesis and progression of liver fibrosis (1).

Among the hemostasis functions played by thrombin, one may list the fibrinogen-to-fibrin conversion, the activation of platelets, factors V, VIII, XI, XIII, protein C (PC),2 and the thrombin-activatable fibrinolysis inhibitor. In this issue of Clinical Chemistry, Ninivaggi et al. (2) report on a new whole-blood thrombin generation test (TGT), which has been a long-awaited development in the area of coagulation testing. To understand the importance of this test to the field requires some background information on current coagulation-testing methods.

Methods to assess thrombin generation from its precursor prothrombin were developed in the early 1950s, when McFarlane and Biggs triggered coagulation in whole blood or plasma by adding calcium chloride with or without tissue factor or cephalin (3). In the original TGT, the protease generated upon the triggering of coagulation was monitored by sampling a portion of the mixture at fixed time intervals and placing the sample into a second tube containing a fibrinogen solution for testing. The resulting clotting time, which was inversely proportional to the thrombin generated as a function of the coagulation factors, was eventually converted into the thrombin concentration by interpolation from a dose–response calibration curve, which was constructed by testing known amounts of thrombin. The method was too cumbersome and time-consuming for routine use, however, and was soon abandoned in favor of simpler tests, such as the prothrombin time (PT) and the activated partial thromboplastin time (APTT). Many years later, the TGT was revisited by Hemker et al. (4), who introduced important modifications. Fibrinogen as a detection system for thrombin was replaced with a chromogenic substrate specific for thrombin, which allowed the assessment of thrombin by photometric measurement (4). Plasma was defibrinated before testing, thus minimizing the interference produced by the turbidity of fibrin formation during the measurement. Dedicated computer software was developed to calculate the parameter values for the thrombin generation curve, including the lag time for thrombin generation after triggering coagulation, the time to reach the peak, the peak thrombin value, and the area under the curve (referred to as the “endogenous thrombin potential”). The endogenous thrombin potential represents the total amount of thrombin that can be generated as a function of the balance between the procoagulant drivers (coagulation factors downstream from factor XII) and the anticoagulant drivers (namely, antithrombin and PC) operating in plasma. The TGT was further modified by the introduction of a slow-acting chromogenic substrate specific for thrombin that allowed continuous measurement of the cleaved substrate, thereby avoiding the need for the subsample technique. Modified in this way, the method can be fully automated and used on clinical chemistry analyzers (5). More recently, the chromogenic substrate was replaced by a fluorogenic one (6), thus allowing the TGT to be run without defibrination in not only platelet-free but also platelet-rich plasma, a possibility that had not been feasible until then because of the turbidity of the testing mixture. Presently, the TGT can be run on automated fluorometers with use of microplate technology, thereby allowing the simultaneous measurement of thrombin generation for many samples (7).

The most recent generation of the TGT has been used over the past decade to carry out studies aimed at investigating hypo- and hypercoagulability, including the following: (a) the elucidation of mechanisms that regulate hemostasis for situations in which such traditional tests as the PT and APTT are poorly suitable (see below); (b) the elucidation of mechanisms that regulate hemostasis for situations in which such traditional tests as the PT and APTT are poorly suitable (see below); (b) the elucidation of mechanisms that regulate hemostasis for situations in which such traditional tests as the PT and APTT are poorly suitable (see below); (c) the prediction of recurrence after a first unprovoked episode of venous thromboembolism (9); and (d) the management of patients treated with the old and new antithrombotic drugs.

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2 Nonstandard abbreviations: PC, protein C; TGT, thrombin generation test; PT, prothrombin time; APTT, activated partial thromboplastin time.
One may wonder what makes the TGT superior to conventional tests such as the PT and APTT. The end point for the PT and APTT is fibrin formation, which is assessed by recording the time (in seconds) elapsed from the time of coagulation initiation (after the addition of appropriate triggers) to clot formation. In these tests, plasma has been estimated to start to clot as soon as 5% of the total amount of thrombin has been generated (10), thus leaving the remaining 95% undetected. Furthermore, owing to the relatively short interval (10–30 s) from the initiation of coagulation to clot formation, naturally occurring anticoagulants operating in plasma (i.e., antithrombin and PC) cannot be fully activated, especially if one considers that they require heparin-like substances (antithrombin) and thrombomodulin (PC) for their activation. These substances are located on endothelial cells, and neither plasma nor the reagents needed to run the PT or APTT test contain heparin-like substances or thrombomodulin. Therefore, the 2 tests are unlikely to account for the total amount of thrombin generated as a function of the procoagulant drivers and for its inhibition as a function of the anticoagulant drivers. The PT and APTT have been surmised from their design to be sensitive to the procoagulant factors but to be much less sensitive to the anticoagulant factors (11). The PT and APTT are well known to be suitable for detecting congenital deficiencies of the procoagulant factors, conditions that are characterized by hypocoagulability (i.e., reduced thrombin generation), but they are not suitable for detecting congenital deficiencies of the anticoagulant factors, conditions that are characterized by hypercoagulability (i.e., heightened thrombin generation). The times for these tests are, in fact, abnormally prolonged in patients with hemophilia (APTT) or congenital factor VII deficiency (PT), but they are normal in patients with congenital deficiencies of antithrombin or PC. In such cases, the 2 tests should instead yield times for fibrin formation that are shorter than normal. Compared with the PT and APTT, the TGT is sensitive to both opposing drivers of thrombin generation (i.e., pro- and anticoagulants) for optimizing the activation of PC, especially if the TGT is modified to include soluble thrombomodulin (11). The lesson to be learned from these observations is that although the PT and APTT are suitable tests for investigating patients suspected of having a congenital deficiency of the procoagulant factors, they are probably unsuitable for the investigation of patients with a congenital deficiency of the anticoagulant factors, or of patients with an acquired deficiency of coagulation in which both pro- and anticoagulant drivers are decreased. Typical examples of acquired deficiencies of coagulation are those associated with liver cirrhosis (12) and the neonatal period (13) in which both pro- and anticoagulants are decreased to the same degree because of impairment in the liver’s synthetic ability. The logical consequence is that the balance between the pro- and anticoagulant drivers in these 2 conditions is restored by their concomitant deficiency (12, 13). In support of this concept, investigators have provided evidence for normal thrombin generation in cirrhosis and in the neonatal period if it is measured in the presence of thrombomodulin (12, 13), despite prolongation of the PT and APTT.

The above observations suggest that the TGT provides a better representation of the in vivo hemostatic process than the traditional coagulation tests when the test is performed with platelet-free or platelet-rich plasma in the presence of thrombomodulin. Nevertheless, the TGT is still performed under in vivo conditions that are far from real, even when performed in platelet-free or platelet-rich plasma, because crucial components of whole blood, such as red and white blood cells, are still missing. Although red blood cells have long been thought to play no important roles, they are now considered one of the possible determinants of hemostasis. They may expose negatively charged procoagulant phospholipids on their surfaces (14) that are able to support the assembly and activation of plasma coagulation factors needed to generate thrombin. White blood cells, especially monocytes, are able to express tissue factor, which is the main trigger of coagulation. Hence, a TGT that would be truly representative of the situation that occurs in vivo would require all crucial blood components, including plasma, platelets, and red and white cells. Until now such a test has been missing from the array of coagulation tests because of technical limitations arising from the variable quenching signals generated by red blood cells and hemoglobin. Thromboelastometry (15) has been used for many years as a whole-blood hemostasis test, but it does not measure thrombin generation specifically.

The report of Ninivaggi et al. (2) in this issue of Clinical Chemistry describes a new whole-blood TGT and provides evidence that its performance with whole blood is feasible with the technology already being used for the plasma TGT. Preliminary evaluations with blood from hemophilia patients demonstrate that the whole-blood TGT is correlated with factor VIII (2), which is one of the most important determinants of thrombin generation. Further evaluation in other clinical conditions characterized by hypo- or hypercoagulability will tell us whether the TGT has new potentials for investigating hemostasis and thrombosis in a system that more closely mimics what occurs in vivo, compared with conventional tests. Efforts in the future should be devoted to standardizing the test (i.e., reagent concentrations, calibration, and data analysis) to make it applicable in clinical practice.
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