Anticoagulation Monitoring in Cardiovascular Disease

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Introduction

Inhibition of normal hemostatic mechanisms is the cornerstone of medical therapy for many cardiovascular diseases, especially acute coronary syndromes and percutaneous coronary interventions. However, if antithrombotic therapy is to be of value, a delicate balance has to be maintained between prevention of pathologic thrombosis and and excess risk of significant hemorrhage. Thus therapeutic administration of these agents must be guided by appropriate clinical and laboratory tests to assess risk of bleeding or continued thrombosis. Multiple types of assessments are possible, including individual enzyme inhibition, quantitation of thrombosis byproducts, and overall measures of clotting efficiency. In this chapter discusses the physiological and clinical basis of the more commonly used laboratory tests for monitoring anticoagulation in patients with cardiovascular diseases. Some of the newer and promising tests will also be discussed.

Basic principles

Beneath the apparent simplicity of the routine coagulation times lies the incredible complex interplay of enzymes, coenzymes and inhibitors that combine to produce coagulation (figure 1). Blood and plasma coagulation tests are a laboratory reproduction of events resulting in fibrin polymer formation, with or without platelet assistance. However the two processes are not necessarily synonymous. Coagulation tests are initiated artificially, within the static confines of a test tube, often without the presence of erythrocytes or platelets. In-vivo thrombosis is far more complex and may not be adequately represented (or in some cases be misrepresented) by in-vitro tests. For instance, the presence of lupus anticoagulant, while prolonging in vitro coagulation, confers an increased risk of thrombosis. The two principles that govern laboratory measures of coagulation are: the need for activation of an enzyme pathway and the need for a functional cofactor to assist enzyme function. The characteristics of an
individual test are determined by the activator and whether or not there is excess of the necessary cofactors, such as phospholipids membrane. With the activator supplied in a simplified test tube reproduction, which obviates many of the regulatory and counter regulatory mechanisms, the primary limitation to thrombus initiation and growth is cofactor availability.

The endpoint of a test is the time at which sufficient fibrin has been generated to be detectable. Fibrin polymerization may be detected by a variety of methods including, the appearance of a visible fibrin clot, an increase in the optical density of the specimen, or a change in the mechanical resistance (using magnetic field manipulation or electrical conduction); [figure 213].

Specific Laboratory Tests to monitor anticoagulation

Prothrombin Time

When tissue factor and a suitable phospholipid membrane are added to recalcified, platelet-poor plasma, the extrinsic pathway is activated, producing thrombin and fibrin clot11,14,15. Devised in the 1930’s by AJ Quick16, the prothrombin time (PT) test is based on the fact that in the presence of excess tissue factor, the efficiency of fibrin formation depends on the presence and activity of factors VII, X and V, and of thrombin and fibrinogen. The test is usually performed in a glass tube with an inner coating to prevent the activation of factor XII. Briefly, citrated platelet poor plasma is added to a calcium and thromboplastin suspension and the timer is started. The end of the test is marked by detection of fibrin polymerization, usually detected by change in the optical density of the specimen. The ratio of clotting time of test plasma to control plasma gives an estimate of the function of the extrinsic pathway clotting factors. The amount of tissue factor supplied during the test makes the activity of factor VIIa, rather than the tissue factor, the primary determinant of extrinsic pathway activation17. Therefore, PT, which hinges on factor VII function can be used to estimate the severity of factor production abnormalities, such as liver dysfunction, warfarin effect or vitamin K deficiency. Of note, Heparin and the specific antithrombins, also prolong PT18,19. However the plasma activity of heparin required to prolong PT is close to 1.0 U/ml. Because most commonly used heparin regimens attempt to achieve heparin concentrations near 0.4U/ml, this is not of much clinical utility.

There may be substantial interlaboratory variation in PT of identical specimens due to the differences in the commercially available thromboplastin preparations20-22. These preparations differ in their capability to initiate and support coagulation factor activity due to many factors, including the amount and activity of tissue factor supplied and the physical properties of the accompanying phospholipid membrane23. A particularly powerful tissue factor-phospholipid preparation may overcome a mild deficiency in factor activity, producing a normal PT, whereas a weaker preparation may produce a prolonged PT and differences in the ratio of test to control PT. Although long suspected, the effect of these different thromboplastins was clearly established in a multinational study of the treatment of venous thrombosis, which reported an increased risk of bleeding when drug dosing was guided by prothrombin time ratios in North America24. A method
of controlling variations in thromboplastin sensitivity to the effect of warfarin was established in 1983\textsuperscript{23,25}. This standardization method employs the International Normalized Ratio (INR), which is a mathematical computation of the ratio of the activity of the thromboplastin to be used to a reference thromboplastin held by the World Health Organization\textsuperscript{25}. When prothrombin times using the different reagents are plotted on a logarithmic scale, there is a linear relationship between the different values. The slope of this relationship is a function of an individual thromboplastin’s sensitivity to the effects of factor depletion and is known as the International Sensitivity Index (ISI). Reference thromboplastins have an ISI value of 1.0. Less sensitive preparations have a value near 2.0. Using ISI, an INR can be calculated according to the following formula: \( \text{INR} = (\frac{\text{PT ratio}}{\text{ISI}}) \).\textsuperscript{25,26}

Whole Blood Clotting Time

LEE and White described the whole blood clotting time (WBCT) in 1913 as a method for evaluating the intrinsic capability of blood to clot\textsuperscript{27}. WBCT is based on the principle that when blood is placed in a glass tube, the intrinsic pathway to thrombin generation is activated and the initial thrombin generation is limited by the availability of cofactors that augment enzyme function, especially factors VIIa and Va, and calcium and a phospholipids membrane\textsuperscript{10,14,28,29}. The initial appearance of active thrombin, albeit in low concentrations, leads to the generation of necessary cofactors through its action on factors VIII, V and platelet activation\textsuperscript{10,14,28,30,31}. A burst of thrombin generation and fibrin polymerization ensues. This test does not utilize exogenous activators, except the glass tube.

The WBCT is primarily of historical interest now because the test methodology and results varied greatly. However, among its many derivatives, the \textit{activated partial thromboplastin time (aPTT)} and the \textit{activated clotting time (ACT)} are in widespread contemporary use.

Activated Partial Thromboplastin Time

In 1950s, a group of investigators developed a variation of the PT that used a lipophilic extract of mammalian (non human) brain for thromboplastin preparation\textsuperscript{32}, they used platelet poor plasma and an excess amount of a membrane substitute. They called this test PTT as they thought that their thromboplastin must be incomplete, as unlike the PT, it gave abnormal results with plasma from hemophiliacs\textsuperscript{33}. The aPTT is a modification of the above, which avoids variation in the intensity of activation by initiating activation (during a pretest incubation period) with a constant quantity of activator (kaolin, celite etc.). When citrated, platelet-poor plasma is exposed to strong contact activation, is recalcified and platelet membrane substitute is supplied, the time required for fibrin clot to form is dependent on the integrity of the intrinsic pathway. The aPTT avoids the extrinsic pathway by excluding tissue factor from its reagents and also separates coagulation from platelet function.
The normal range for aPTT for most laboratories is 20-40 seconds (sec). It is prolonged when any of the factors from contact activation to fibrin polymerization are deficient or are inhibited to less than 30% of normal activity.

When heparin is complexed with antithrombin III (AT III), the ability of the heparin-AT III to inhibit thrombin may be increased 1000 fold over that of AT III alone. As a result, the initial steps following contact activation proceed slowly through the regulatory points. The small amount of thrombin produced decays at an accelerated rate, and the activation of factor VIII is impaired. Consequently more time is required to produce thrombin in sufficient concentrations to effectively overcome the rate limiting steps in the intrinsic pathway (Figure 36.4 old chapter). This lag time is the measurable prolongation of clotting times produced by the thrombin inhibitors (such as heparin; figure 36.5 –old chapter). Because aPTT has a strong contact activation step, it is immediately sensitive to the presence of thrombin inhibitors. The aPTT is prolonged at a level of thrombin inhibition that correlates with heparin activity of 0.1-0.6 U/ml, a range known to be clinically effective for medical treatment of venous and some arterial thrombosis. The aPTT is the most widely used test for guiding heparin therapy for venous and arterial thrombosis and for medical management of acute coronary syndromes (ACS), with usual target range of 1.5-2.5 times the control value. However, when heparin is administered to achieve higher concentrations (as in the setting of percutaneous intervention [PCI] or cardiopulmonary bypass), the aPTT is nonlinear in its response to heparin and may be almost infinitely prolonged; the aPTT in these circumstances is no longer a useful test.

Direct thrombin inhibitors (DTI), in contrast to heparin are active against fibrin-bound thrombin and like heparin, not only do they inhibit thrombin production and activity, they may also inhibit thrombin induced platelet activation. Thus, because DTI have different mechanisms of action and different dose-effect relationships, the relationship between aPTT prolongation and antithrombotic effect will be different from that of heparin. A given aPTT range for DTI may correspond to an equal or greater antithrombotic effect than for heparin, especially in situations where the thrombosis is platelet dependent, as in ACS. Thus direct comparisons of drug efficacy cannot necessarily be made based on equal prolongation of aPTT. Risk/benefit ratios, with respect to measures of antithrombotic effect, must be determined separately for anticoagulants of different classes. Therefore, antithrombotic recommendations using the aPTT, must not only specify the reagents but also the type of antithrombotic therapy used.

**Point of care testing**

Conventionally, the aPTT is performed in a central laboratory. This involves a substantial delay in the reporting of results from the time of ordering the test. A survey of 79 hospitals that participated in the GUSTO 1 trial showed that the mean time from blood draw to availability of the aPTT result was 1 hour 46min. Since patients with acute coronary syndromes would benefit from a more rapid turnover time for aPTT,
several point of care anticoagulation systems are now commercially available and have clinically been tested. There are three commercially available bedside aPTT monitors in the United States designed for use in critical care settings. Each provides a rapid determination of the aPTT (typically within 3 minutes). Table 1 provides a comparison of these point of care devices.

**Activated Clotting Time**

ACT, first described by Hattersley in 1966, is essentially a modified WBCT in which an attempt has been made to standardize contact activation by using a constant amount of kaolin or celite. The ACT is similar to the aPTT in its dependence on the contact activation pathway for thrombin generation, but the ACT does not supply an excess of phospholipid membrane. In the aPTT, the phospholipid area is supplied in excess so that it will not interfere with the of coagulation enzyme function. The ACT requires that this area be developed through platelet activation during the testing process. This induces an additional variable to the function of coagulation enzymes. In the ACT following contact activation, the first thrombin to appear must activate platelets, in addition to factors V and VIII, and any alteration in endogenous platelet function may also alter ACT. Thus, rather than being a pure test of coagulation enzyme function, the ACT is also a somewhat imprecise measure of the cooperation between the intrinsic pathway and platelet function. For this reason (as will be detailed in a later section), ACT is useful in monitoring the combined use of heparin and glycoprotein IIb/IIIa. Also, the ACT is less sensitive to low levels of heparin anticoagulation than aPTT. However it maintains a good correlation with the heparin effect at higher heparin doses and thus is widely used for guiding heparin therapy in clinical situations needing high dose heparin such as cardiopulmonary bypass and PCI.

Unlike, Hattersley’s original assay, that required manual mixing of a blood sample with the contact activator and visual assessment of the tube for time to visible clot formation, commercial automated ACT monitors now in routine use. The Hemochron system (International Technidyne, Edison, NJ) uses a celite activator and detects fibrin polymerization using an oscillating magnetic field. The HemoTec (Medtronic HemoTec, Englewood, CO) uses a kaolin activator and measures the rate of plunger fall to detect fibrin polymerization. Thus, system activators and fibrin detection mechanisms differ, giving different sensitivities to factor depletion and inhibitor therapy. Although there is reasonable correlation, the values of one system cannot be extrapolated to the other systems. Thus, any recommendations for anticoagulation intensity, measured by ACT, must be qualified by the specific type of device used.

The normal ACT range in general is 90-130 seconds. Due to the range of variation in normal values, effects of low dose anticoagulants may go unnoticed in individual patients. The ACT may be shortened during ongoing thrombosis, such as in surgical procedures or unstable coronary syndromes, largely as a function of the availability of activated platelets. Contamination of blood samples with activated platelets (as with indwelling catheters) may also shorten ACT. Also, aprotinin inhibits contact activation by celite (diatomaceous earth). If this interaction is not considered, then an insufficient
dose of heparin may be administered in this setting. Thus kaolin ACT should be used in
patients receiving aprotinin.

Because the aPTT and ACT measure similar phenomenon, (except for the contribution of
activated platelets), there is a fair correlation between the tests when assessing heparin
therapy. However, DTI, in addition to impairing the feedback amplification of
coagulation enzyme function, also prevent thrombin induced platelet activation. The
resultant denial of factors VIIIa and Va and a phospholipids surface prolongs ACT. In
drug concentration ranges at which the two tests are responsive, antithrombins produce a
proportionately greater rise in ACT than aPTT. Thus, at drug concentrations producing
an equivalent rise in the aPTT, the ACT increase with specific thrombin inhibitors is
significantly greater than that with heparin. Powerful antiplatelet drugs like glycoprotein
IIb/IIIa inhibitors, may prolong ACT. Thus ACT may be a good tool to assess the
intensity of antithrombotic drugs when combined anticoagulants and antiplatelet agents
are used in a clinical syndrome that may involve endogenous platelet activation (as in
PCI setting).

More recently, another point of care ACT monitoring device has become available. The
i-STAT system (i-STAT Corp., Princeton) is modular and consists of a handheld unit into
which disposable cartridges are placed. The cartridges are self contained and they need
<1ml of blood. There are individual cartridges available to measure electrolytes, blood
gases and level of anticoagulation (ACT). Each i-STAT unit can download patient
data to a central computer for storage and subsequent retrieval. Unlike traditional ACT,
the i-STAT ACT is not based on the formation a stable thrombus. Instead a substrate
marker releases an electric signal when it is cleaved by active thrombin. The time to
generation of the electroactive marker is reported as the ACT. Therefore, the i-STAT is
less susceptible to fibrinogen levels, temperature, hematocrit, dilution and the addition of
IIb/IIIa inhibitors. Because the production of thrombin occurs earlier than the formation
of a stable clot, one would expect the value of the i-STAT to be lower than that of the
Hemochron ACT. In this regard, Schussler et al compared the i-STAT ACT with
Hemochron ACT during and after PCI. They found a high degree of correlation
between the two ACTs both at low and high level of anticoagulation. Statistically, there
was no difference between these two devices at lower levels of anticoagulation; at higher
levels of anticoagulation there was a statistically significant difference that was not
thought to be clinically significant. The use of IIb/IIIa inhibitors did not significantly
change these relationships. Due to small numbers of patients, no conclusion could be
drawn for use with DTI.

**Dry Reagent technology**

Rather than using separate solutions that must be added to a test tube, the dry reagent
technology, places the coagulation activator and cofactors on a card. A very small
amount a citrated blood is placed in a reaction chamber; the particular reagent preparation
used determines the type of clotting time that is performed. Thus, if the same detection
system is used, a single analyzer may be used for a variety of different tests. The Heparin Management Test (HMT) (Cardiovascular Diagnostics, INC., Raleigh, N.C.), based on this technology, is a measure of whole blood anticoagulation and was introduced as an alternative to conventional ACT measurements. It is similar to ACT in principal (uses celite as activator), but uses a microprocessor-controlled analyzer and disposable test cards. A reaction chamber within each test card contains paramagnetic iron oxide beads and dry chemical reagents necessary to activate the coagulation cascade in the blood sample. After a drop of blood is added, capillary action draws a small portion of this blood into the reaction chamber. An oscillating magnetic field is applied to the blood, chemical reactants and to the beads. An infrared light beam passed through the test chamber detects oscillations in the amplitude of transmitted light coincident with the bead movement in the test chamber. As clot formation occurs, the beads become enmeshed within the clot, reducing the amplitude of light oscillations to trigger the end time for the HMT measurement Figure 3. The system come as an analyzer (TAS/ Rapidpoint Coag analyzer®) and a variety of test cards that can be used to monitor different aspects of anticoagulation. The low HMT cards are used to monitor low to moderate levels of unfractionated heparin (heparin concentration from 0.25 to 3.0 U/ml) such as during PCI while the HMT test cards are intended to monitor the effects of higher doses of heparin (1.0-10.0 U/ml). The test system can use both citrated and non citrated whole blood. Table 2 compares the salient features of some of the available whole blood coagulation analyzers. The TAS HMT has been compared to the Hemochron and HemoTec ACT in prospective studies in the PCI setting. Tsimikas et al noted that the the TAS-HMT gave significantly higher readings (about 15% higher) than the HemoTec ACT but a good correlation was noted between the two methods \((r = 0.77)\), and the relation was similar in patients who received IIb/IIIa inhibitors. However, at ACT values greater than 300 seconds the correlation was less strong. When compared to the Hemochron ACT, the two systems gave fairly similar values \((HMT292 \pm 33\) sec and ACT \(284 \pm 31\) sec) and there was a reasonable correlation \((0.66)\). Also HMT was shown to have a good correlation with anti-factor Xa activity in patients undergoing cardiovascular surgery.

**Ecarin Clotting Time**

In most large clinical trials aPTT has been used to monitor heparins; it is also the most commonly used assay to monitor heparin in clinical practice. Also, the manufacturers of most direct thrombin inhibitors (DTIs) recommend aPTT to these agents. However, unlike heparins that indirectly inhibit factors IIa, Xa, IXa, XIa and XIIa, DTIs only inhibit thrombin. On the other hand, aPTT reflects inhibition of factors IIa, IXa and Xa. Thus intuitively, aPTT would not be the optimal method of monitoring DTIs because its values are affected by clotting factors not directly influenced by DTIs. Data in human studies has showed only a moderate correlation of the concentration of these agents to aPTT values. The ecarin clotting time (ECT), on the other hand is used specifically to monitor the effect of DTIs. Ecarin, used as a thrombin generating agent in the ECT, is derived from the venom of a snake (Echis carinatus). Ecarin cleaves the
arginine 320-isoleucine 321 protein bond of prothrombin, thereby generating meizothrombin. Meizothrombin possesses thrombin-like proteolytic activity. Like thrombin, its active site is inhibited by direct thrombin inhibitors (DTI). The principle behind the ECT is that after the addition of a specific quantity of ecarin to blood containing a DTI, meizothrombin is generated. Meizothrombin then reacts with the DTI to neutralize it. Once the inhibitor is neutralized, the remaining free Meizothrombin can activate the clotting process by stimulating the conversion of fibrinogen to fibrin. Since, heparins are poor inhibitors of meizothrombin, the ECT is relatively specific for DTIs.

Clinical use of ECT has been reported. One study examined the precision of ECT compare to aPTT for monitoring lepirudin in 10 patients with history of heparin induced thrombocytopenia, undergoing open heart surgery. These investigators found a strong linear relationship between the ECT values and lepirudin concentrations that ranged from 0.35-5.88µg/ml (r=0.94), whereas the aPTT demonstrated only a weak correlation (r=0.61). Other studies also have shown that ECT correlates better with lepirudin levels and demonstrates less interpatient variability compared with the aPTT or the ACT.

The thrombin inhibitor management test (TIM) (Pharmanetics, Morrisville, NC), a point of care test, based on the ECT has been developed using the dry reagent technology similar to that in the HMT test cards. Cho et al compared the TIM ECT test and ACT [using the Hemochron ACT (International Technidyne, Edison, NJ) and Coagucheck Pro/DM (Roche Diagnostics, Indianapolis, Indiana) with central laboratory anti-factor IIa assay for monitoring bivalirudin-mediated anticoagulation in the setting of non-emergency PCI. All 64 patients received bivalirudin bolus of 0.75mg/kg followed by a 1.75mg/kg/hr infusion. 55 patients also received concomitant IIb/IIIa inhibitors. Samples were drawn at baseline, after administration of bivalirudin, after IIb/IIIa inhibitor bolus and during the procedure and at sheath pull. The results showed that TIM ACT provided more accurate assessment of bivalirudin during PCI than ACT Figure 4. After IIb/IIIa inhibitor administration, no significant change in bivalirudin concentration or ECT values was noted, though the Hemochron ACT values increased. Thus, serum DTI concentrations correlate with the degree of clotting time prolongation and the ECT may be an alternative to monitoring patients’ DTI levels.

Viscoelastic Measures of Coagulation

Initially developed in the 1940s, viscoelastic measures of coagulation have undergone a resurgence in popularity. These tests are unique as they can measure the entire spectrum of clot formation from early fibrin strand generation, through clot retraction and eventual fibrinolysis. Currently two such devices are in clinical use and will be described.
**Thromboelastography**

Thromboelastography was first described in 1948 by Hartert. With the advent of computerization, this has evolved from a research tool into a compact, commercially available point of care instrument: The Thrombolestatagraph (TEG) (Hemoscope; Morton Grove, Ill).

It consists of a heated (37°C) cuvette that holds the blood (0.36ml) as it oscillates through an angle of 4°45’. Each rotation lasts 10 seconds, which includes a 1 second rest period at the end of excursion. A pin, which is suspended freely in the blood by a torsion wire, is monitored for motion (Figure 5). The torque of the rotating cup is transmitted to the pin once the clot starts to form. Therefore, the strength and rate of these fibrin-platelet bonds affect the magnitude of pin motion. When the clot lyases, the bonds are broken and the transfer of cup motion is diminished. The rotation of the pin is converted by a mechanical-electrical transducer to an electrical signal that can be monitored and recorded by a computer. Thus, TEG documents initial fibrin formation, clot rate strengthening, and fibrin platelet bonding via GP IIb/IIIA to eventual clot lysis (Figure 6).

The strength of a clot is graphically represented over time as a characteristic cigar shaped figure. There are 5 parameters of the TEG tracings which measure different stages of clot development (Figure 6):

- **R**: The R value or the reaction time is a period of time from initiation of the test to the initial fibrin/clot formation (normal: 7.5-15min). It is considered comparable to the whole blood clotting time and may be accelerated by adding celite to the sample cuvette. The R value is prolonged by a deficiency of one or more plasma coagulation factors and shortened in hypercoaguable states.

- **K**: This is measured from R time (i.e. from the beginning of clot formation) until the level of clot firmness reaches 20mm (divergence of the lines from 2-20mm). Therefore, K is a measure of clot strengthening. It is shortened by an increased fibrinogen level and, to a lesser extent, by increased platelet function and is prolonged by anticoagulants.

- **α**: Alpha angle is formed by the slope of the TEG tracing at R from the horizontal line. It represents the acceleration kinetics of fibrin build up and cross linking. Like K it is also increased by increased fibrinogen levels and, to a lesser extent, by increased platelet function and is decreased by anticoagulants. In hypercoaguable states, in which the clot amplitude never reaches 20mm (i.e. K is undefined), the angle is more comprehensive than K time.

- **MA**: Maximum amplitude (MA) reflects strength of a clot which is dependent on the number and function of platelets and its interaction with fibrin. It may be
decreased by either qualitative or quantitative platelet dysfunction or decreased fibrinogen concentration. Normal MA is 50-60mm.

**CI:** The coagulation index describes the patients overall coagulation status. It is derived from the R, K, MA and α of native or celite activated whole blood tracings (CI for celite activated blood = 0.3258R – 0.1886K + 0.1224 MA + 0.0759 α – 7.7922). Normal values range from -3.0 to +3.0, which is equivalent to 3 standard deviations about the mean of zero. Positive values outside the range (CI>3) indicate that the sample is hypercoaguable, whereas negative values outside this range (CI<3) indicate that the sample is hypocoaguable.

**Ly30/LY60:** These values measure percentage lysis at 30 and 60 minutes, respectively, after the MA is reached. Measurements are based on the reading of the area under the TEG tracing from the time MA is measured until 30 and 60 minutes after the MA. Therefore, when these values are high, the fibrinolytic activity is high.

TEG tracings can be qualitatively and quantitatively analyzed. Various patterns can be easily recognized as hypocoagulation, normal coagulation, hypercoagulation and fibrinolysis. However by using measurements and established normal ranges and indices, the patterns can be quantified as to the degree of abnormality, which allows better monitoring of therapies. Measurements derived from these diagrams have been related to more traditional measures of coagulation, such as ACT. TEG monitoring has been used in liver transplant surgery to rapidly analyze and treat the changing coagulation profile of the patients. Studies have evaluated the utility of TEG in cardiac surgery after cardiopulmonary bypass (CPB) and to assess blood product transfusion requirements. Shore-Lesserson et al. compared transfusion requirements in a randomized, prospective trial of high risk cardiac surgical patients. Patients were randomly assigned to TEG guided transfusion therapy or standard laboratory based transfusion therapy. Patients in both group received antifibrinolytic therapy with EACA. They noted that patients in the TEG group received fewer total transfusions and a significantly less volume of fresh frozen plasma. The authors concluded that TEG based transfusion algorithm reduced transfusion requirements. The TEG may also be used to differentiate surgical bleeding from coagulopathy following cardiac surgery. The TEG has also been used successfully to monitor hirudin therapy during CPB in the setting of heparin induced thrombocytopenia. Some of the other clinical applications of TEG have been to assess the coagulation status in obstetrical patients receiving low molecular weight heparins in the peripartum period and in disseminated intravascular coagulation.

**Sonoclot Analyzer**

The Sonoclot® (Sienco, Inc; Morrison, Colo) provides an alternative viscoelastic measure of coagulation. Compared with TEG, the sonoclot immerses a rapidly vibrating probe into a 0.4ml sample of blood. As the clot formation occurs, impedance to probe
movement through the blood increases and generates an electrical and a characteristic ‘clot signature’. The sonoclot may be used to derive the ACT as well as provide information regarding clot strength and clot lysis. The Sonoclot generates both a qualitative graph, known as the Sonoclot signature and quantitative results on the clot formation time (ACT-onset) and the rate of fibrin polymerization (Clot rate) for identifying numerous coagulopathies including platelet dysfunction, factor deficiencies, anticoagulation effect, hypercoaguable tendencies and fibrinolysis. As the blood sample clots, numerous mechanical changes related to the performance of the patient’s hemostasis system occur that alter the clot signal value. The record of the clot evolution is saved as a graph of the clot signal versus time. Both celite and kaolin activated Sonoclot ACT tests are available.

In a Sonoclot signature, the coagulation cascade reactions develop from the beginning and continue throughout the liquid phase (represented by the initial horizontal portion of the graph in figure). This phase ends when the viscosity of the sample increases with thrombin generation and resulting initial fibrin formation and represents the ACT (above figure). This followed by the continued conversion of fibrinogen to fibrin and its polymerization into a gel. This is affected by both the rate of conversion to fibrin and the availability of fibrinogen. This phase is represented by the slope of the graph (clot rate) and by the height of the signature when the gel formation is completed. This information is important in clinical applications including monitoring anticoagulants (figure from monograph showing heparin effect), hypercoaguable states and fibrin hemodilution.

Sonoclot also responds to clot retraction occurring within the test sample. As the clot retracts it tightens causing the signature to rise. Eventually, the clot often pulls away from some of the surfaces of the cuvette or probe. This results in a fall in the graph. The sonoclot has been used to monitor the coagulation status in a multitude of conditions including cardiac surgery, hemodialysis patients and malignancy associated hypercoaguable states.

**ENOX Test**

The ENOX test (Pharmanetics Inc., Morrisville, NC) measures the combined anti-Xa and anti-IIa activity of the low molecular weight heparin (LMWH) enoxaparin. Unlike heparin that has an anti-Xa/anti-IIa activity ratio of one, the LMWHs have a much higher relative anti-Xa inhibition. This is an important distinction as the ability to prolong the aPTT and ACT is proportional to the anti-IIa activity. The ENOX test was developed for exclusive use with the LMWH enoxaparin. In the ENOX test, factor X is rapidly converted to factor Xa by a specific activator of factor X, initiating the clotting process. Enoxaparin, from the patients blood, complexes with antithrombin to inhibit factor Xa and proportionately lengthen the clotting time. Conventional chromogenic anti-Xa assays provide drug concentrations only in dilute, supplement plasma and are not suitable for point of care use. The ENOX test is commercially available for point of care use and is based on dry reagent technology. The ENOX test cards are manufactured to be used with the Rapidpoint® Coag analyzer (Bayer Corp). As with all similar tests, all the components needed to perform the test, with the exception of the patient sample, are included in the
reaction chamber of the test card\textsuperscript{101}. The test uses one drop (35\mu l) of citrated venous or arterial blood sample. The ENOX test was designed to measure citrated whole blood clotting times corresponding to enoxaparin concentration of 0.0-3.0 anti-Xa IU/ml in derived plasma\textsuperscript{102}. In a clinical trial that included patients undergoing PCI using enoxaparin, the ENOX clotting times correlated well (r=0.80) with chromogenic anti-Xa assay derived plasma enoxaparin concentrations (range <0.1 – 1.8IU/ml)\textsuperscript{103}. Based on prior studies, a proposed targeted window for PCI anticoagulation with enoxaparin is 0.8-2.0 IU/ml\textsuperscript{104-106}. This corresponds to an ENOX clotting time of 250-450 seconds\textsuperscript{104-106}. The recently completed ELECT trial (Evaluation of ENOX clotting times) will shed further light on the applicability of ENOX test in PCI.

**Monitoring of Platelet function**

The pivotal role of the platelet in arterial thrombosis in general, and in acute coronary syndromes in particular, is well established. It is also clear that inhibition of platelets with an antiplatelet agent (such as aspirin, the thienopyridines, or the platelet glycoprotein IIb/IIIa inhibitors) can reduce progression to myocardial infarction in unstable coronary syndromes and reduce the incidence of ischemic complications during PCI. However despite the effectiveness of these agents, they fail to prevent thrombotic events in all patients. One of the explanations for this, is a potential heterogenous response among individuals to standard dosing regimens. Current clinical practice (and practice guidelines)\textsuperscript{107} do not include any measurement of the effectiveness of platelet inhibitor therapy. Significant variation in the response to aspirin has been demonstrated by various studies\textsuperscript{108,109}.

However, only recently, after the introduction of glycoprotein IIb/IIIa inhibitors has there been an expansion in our capabilities to assess platelet inhibition\textsuperscript{110}. Platelet function tests measure the capacity of platelets to adhere, activate, aggregate and secrete. The goal of platelet function testing is to provide information about the platelet contribution to the risk of thrombosis or hemostasis. Important clinical questions in acute coronary syndromes are whether the antiplatelet agent is having the desired effect on platelet inhibition (inhibition) whether the patient has sufficient platelet function to avoid significant bleeding (safety).

Platelet thrombus formation involves a number of processes, including activation of the platelet, secretion of vasoactive and prothrombotic chemicals, promotion of the clotting cascade, and platelet aggregation, each of these individual platelet functions may be affected by platelet therapy and could form the basis for a functional assay\textsuperscript{110}. It is not clear which function is most important to influence therapy or monitor with testing. A variety of platelet function tests are available for use in the central laboratory, of which the **Photo-Optical Tubidometric Aggregometric Assay** is considered the gold standard. This test involves, addition of a platelet aggregation agonist (eg. ADP, collagen, epinephrine, or thrombin receptor activating peptide [TRAP]), to platelet rich plasma. As platelets aggregate, light transmission increases. Maximum transmittance is calibrated
using platelet poor plasma while minimum transmittance is determined with platelet rich plasma prior to addition of the agonist.

For rapid clinical use in the critical care/ cardiac catheterization laboratory setting, various point-of-care tests have been described, of which the following are more commonly used clinically in cardiovascular settings.

**Rapid Platelet Function Assay**

The Ultegra-RPFA (Accumetrics, San Diego, CA) is an automated, whole blood, cartridge-based optical aggregometer that utilizes fibrinogen-coated polystyrene beads. After addition of an agonist (iso-TRAP), these beads agglutinate in whole blood in proportion to the number of unblocked glycoprotein IIb/IIIa inhibitors receptors\(^{111}\). The light transmittance increases with progressive binding of the platelets to the beads, leading to their agglutination. Current use involves, a baseline measurement and repeat tests after drug administration. The results can be reported as a percentage of baseline aggregation or as an absolute rate of aggregation. One of the drawbacks of the test, as is the case with most currently available tests, is that it differs from in vivo conditions in that it is not conducted under flow conditions. The RPFA has been been validated versus turbidometric aggregometry\(^{112}\). At increasing concentrations of abciximab, the percentage inhibition of the RPFA correlated well with the turbidometric assay (\(r^2=0.95\)). This study also showed an excellent correlation with the degree of glycoprotein IIb/IIIa inhibitors receptor occupancy (measured using radiolabelled abciximab) \(r^2=0.96\).

Initial animal studies with monoclonal antibody 7E3 demonstrated that blockade of \(\geq 80\%\) of glycoprotein IIb/IIIa inhibitors receptors with suppression of platelet aggregation to \(\leq 20\%\) of baseline was necessary to prevent in vivo thrombosis\(^{113}\). This level of platelet inhibition was used as the basis for the early dosing studies for abciximab (c7E3 Fab, ReoPro, Centocor, Inc., Malvern, PA, USA)\(^{114}\) and the importance of maintaining this level of platelet inhibition in the clinical setting was not tested directly. Numerous small studies have used RPFA to measure platelet inhibition after administration of glycoprotein IIb/IIIa inhibitors\(^{115,116}\). The multicenter GOLD trial correlated the degree of platelet inhibition (using the RPFA device) to the clinical outcomes, in five hundred patients undergoing PCI with adjunctive glycoprotein IIb/IIIa inhibitors use\(^{117}\). Major adverse cardiac events (MACEs) were prospectively monitored./ One quarter of all patients did not achieve \(\geq 95\%\) inhibition 10 minutes after the bolus and experienced a significantly higher incidence of MACEs (14.4\% versus 6.4\%, \(p=0.006\)). Patients whose platelet function was \(< 70\%\) inhibited at 8 hours after the start of therapy had a MACE rate of 25\% versus 8.1\% for those \(\geq 70\%\) inhibited (\(p=0.009\)). By multivariate analysis, platelet function inhibition \(\geq 95\%\) at 10 minutes after start of therapy was associated with a significant decrease in the incidence of a MACE ( OR 0.46, 95% CI 0.22 to 0.96, \(P=0.04\)). The authors concluded that there was substantial variability in the level of platelet function inhibition achieved with glycoprotein IIb/IIIa inhibitors among patients undergoing PCI. Also that the level of platelet function inhibition as measured by RPFA was an independent predictor for the risk for MACEs after PCI.
**Platelet Function Analyzer (PFA-100)**

The PFA-100 test (Dade Behring, Miami, FL) evaluates primary hemostasis through platelet-platelet interaction as whole blood flows under shear stress conditions through an aperture\(^{18}\). The instrument uses citrated whole blood which is drawn by means of a vacuum through a capillary tube producing high shear forces and then through a precisely defined aperture in a membrane that has been coated with either collagen and epinephrine or collagen and ADP. The platelets adhere and aggregate at the aperture until it is occluded, and the results are reported as the closure time (CT). The testing process takes about 10 minutes. The use of whole blood allows for the interaction of platelets with red blood cells and other whole blood components that may play a role in thrombus formation. Also the addition of shear stress into platelet activation may make this test theoretically more physiologically sound\(^{119}\). The test is prolonged by platelet counts below 50,000 and hematocrit levels below 25%. The normal range for CT for healthy subjects is 59-120 seconds\(^{120}\). The maximal CT has been set at 300 seconds, which represents nonclosure of the aperture after 300 seconds. In a study of healthy volunteers treated with different concentrations of Tirofiban, there was found to be a good linear correlation of turbidometric aggregation with PFA with collagen-epinephrine membrane percent maximal CT prolongation \(r^2=0.97\), and a strong quadratic correlation of turbidometric aggregation and PFA with collagen-ADP membrane percent maximal CT prolongation \(r^2=0.098\).

**Conclusions**

A variety of laboratory and point of care methods are available to help guide antithrombotic therapy. Depending on the clinical situation at hand, the appropriate test varies. For example, there is substantial evidence and clinical experience supporting the use of the standard aPTT in guiding heparin therapy for the prevention of pulmonary thromboembolism in patients with deep venous thrombosis. In contrast ACT is used to assess heparenization for angioplasty or bypass surgery. For direct thrombin inhibitors the conventional methods like aPTT or ACT may not be optimal and newer tests such as the ECT are being investigated. Viscoclastic measures of coagulation are also being investigated in various clinical settings including during cardiopulmonary bypass. Point of care tests are becoming increasingly popular especially in the acute care setting. In this regard it is important to have stringent rules and guidelines to ensure proper quality measures and calibration to ensure reliability. This is especially true as these devices typically have higher coefficients of variability than standard laboratory tests\(^{121}\). Finally, it should be remembered that though two test may measure the same parameter (for example ACT), there numeric values may not be (and usually are not) identical. Thus
each coagulation test value should be preferably qualified by the type of device used to measure it.

References:


98. Sonoclot coagulation & platelet function analyzer: An overview (Product monograph). In: *Sienco, Inc., CO, USA.*


Figure Legends:

1. The Coagulation cascade. The central precipitating event is considered to involve tissue factor (TF), which under physiological conditions is not exposed to blood. With vascular or endothelial cell injury, TF acts in concert with activated factor VIIa and phospholipids (PL) to convert factor IX to IXa and X to Xa. The intrinsic pathway includes ‘contact’ activation of factor XI by factor XIIa/activated high-molecular weight-kininogen (HKA) complex. It should be noted that the contact system contributes to fibrinolysis and bradykinin formation in vivo. Factor XIa also converts factor IX to IXa and factor IXa in turn converts factor X to Xa, in concert with factor VIIIa and PL (the tenase complex). Factor Xa is the catalytic ingredient of the prothrombinase complex and converts prothrombin to thrombin. Thrombin cleaves the fibrinopeptides (FPA, FPB) from fibrinogen, allowing the resultant fibrin monomers to polymerize, and converts factor XIII to XIIIa, which crosslinks (XL) the fibrin clot. Thrombin accelerates the process (interrupted lines) by its potential to activate factors V and VIII, but continued proteolytic process also dampens the process by activating protein C, which degrades factors Va and VIIIa. Natural plasma inhibitors retard clotting: C1-inhibitor (C1 INH) neutralizes factor XIIa, tissue factor pathway inhibitor (TFPI) blocks factor V/via/TF, and antithrombin (ATIII) blocks factors IXa and Xa and thrombin. (Arrows, active enzymes; filled rectangles, sites of inhibitor action; dashed lines., feedback reactions.) From reference 122

2. Fibrinopolymer detection methods. A: Light transmission of a plasma sample increases as fibrinogen leaves the solution in fibrin polymers. A threshold value or percentage of light transmission may be set to stop the timer. B: As fibrin polymers form, specimen viscosity rises. When a plunger is lifted and then allowed to fall, the rate of fall is a measure of specimen viscosity. C: Two electrodes may be placed in a specimen and a current established. One of the electrodes may be withdrawn from the specimen at preset
intervals. When viscosity rises, contact between the moving electrode and the specimen will not be broken and the timer will stop. D: If a ferromagnetic specimen is placed in a specimen, motion may be established by an oscillating magnetic field. When motion is restricted to a preset value, timing will stop. E: A probe may be placed in the specimen and vibrated at a preset frequency. A sensor positioned outside the specimen, regulates the output of the probe to maintain a constant value. Fibrin polymerization and increased viscosity decrease sound transmission, increasing the output required of the probe.

3. **The internal mechanism of the Rapidpoint Coag® heparin management test (dry reagent technology).** Above the platform for the test card is a photo-detector and a photo-diode. Below the platform are the bias and electro magnets which, during the test procedure, influence the PIOP to move. As a clot begins to form, fibrin strands attach themselves to the particles, impeding their movement. Movement slows until the particles are entrapped in the clot and stop moving. The particle movement is monitored optically as changes in reflected light from the surface of the test card. When the movement stops no light changes are seen and the test is complete. For fibrinolysis tests the process is reversed. When a sample is added to the test card a clot is formed immediately and the particles are trapped. The analyzer monitors time for the clot to dissolve and free the particles to begin moving. (From Cardiovascular Diagnostics, INC., Raleigh, N.C.)

4. **Correlation between ECT, ACT and bivalirudin levels.** Considering samples from all time points, the correlations between clotting time and bivalirudin concentration were r=0.90 for ECT and r=0.71 for Hemochron ACT. The ellipse shown on each graph represents the 90% density contour (confidence curve for the bivariate distribution). The ellipse becomes more circular in shape as the correlation decreases. From reference 82.

5. **The Thromboelastograph®** (Hemoscope, Morton Grove, Ill). See text for details.

6. **Thromboelastography tracing parameters.** See text for details. (From Hemoscope, Skokie, IL)

7. **Characteristic thromboelastograph tracings.** Modified from reference 88.

8. **Representative description of the the Sonoclot Signature and variables.** From reference 99. See text for details.
<table>
<thead>
<tr>
<th>Instrument</th>
<th>Coaguchek Plus&lt;sup&gt;a,b&lt;/sup&gt;</th>
<th>Hemochron Jr.</th>
<th>Thrombolytic Assessment system (TAS)&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manufacturer</td>
<td>Roche &amp; Boehringer- Mannheim Diagnostics</td>
<td>International Technidyne Corp.</td>
<td>Cardiovascular Diagnostics Inc.</td>
</tr>
<tr>
<td>Blood sample</td>
<td>Fresh whole blood</td>
<td>Whole blood or citrated plasma</td>
<td>Citrated whole blood or plasma</td>
</tr>
<tr>
<td>Sample volume</td>
<td>1 drop</td>
<td>1 drop</td>
<td>1 drop</td>
</tr>
<tr>
<td>Time to result</td>
<td>Minutes</td>
<td>Minutes</td>
<td>Minutes</td>
</tr>
<tr>
<td>Setting of clot formation</td>
<td>Capillary flow of unclotted blood</td>
<td>Forced movement of unclotted blood through a narrow channel</td>
<td>Chamber with magnetic particles with oscillating electric field</td>
</tr>
<tr>
<td>Clot detection method</td>
<td>Laser photometry</td>
<td>Optical light transmission</td>
<td>Optical light transmission</td>
</tr>
<tr>
<td>Correlation with standard laboratory</td>
<td>r =0.78-0.89&lt;sup&gt;125,126&lt;/sup&gt;</td>
<td>R = 0.87</td>
<td>R = 0.84&lt;sup&gt;121&lt;/sup&gt;</td>
</tr>
<tr>
<td>Therapeutic aPTT&lt;sup&gt;a&lt;/sup&gt;</td>
<td>60-85 sec (GUSTO III)</td>
<td>50-70 sec (PARAGON)</td>
<td>Customized to each hospitals standard aPTT range</td>
</tr>
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</table>

Modified from reference 52

<sup>a</sup> Corresponding to heparin level of 0.2-0.4U/ml
Table 2. Salient features of point-of-care whole blood coagulation analyzers

<table>
<thead>
<tr>
<th>Feature</th>
<th>Hemochron</th>
<th>HemoTec</th>
<th>TAS/HMT</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Manufacturer</strong></td>
<td>International Technidyne Corp, Edison, NJ</td>
<td>Medtronic, Inc, Englewood, CO</td>
<td>Cardiovascular Diagnostics, Inc, Raleigh, NC</td>
</tr>
<tr>
<td><strong>Amount of blood</strong></td>
<td>2ml</td>
<td>0.2-0.4ml</td>
<td>1 drop (35µl)</td>
</tr>
<tr>
<td><strong>Contact activator</strong></td>
<td>Celite</td>
<td>Kaolin</td>
<td>celite</td>
</tr>
<tr>
<td><strong>Testing vehicle</strong></td>
<td>Test tube</td>
<td>Test cartridge</td>
<td>Test card</td>
</tr>
<tr>
<td><strong>Sensor method</strong></td>
<td>Electro-mechanical Magnet in test tube</td>
<td>Electro-mechanical Plastic flag</td>
<td>Electro-optical Iron oxide particles on test card</td>
</tr>
<tr>
<td><strong>Detection system</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Data management/storage</strong></td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Modified from reference 65
Figure 1.

Figure 2.
FIGURE 5

FIGURE 6
Characteristic Thromboelastograph Tracings

Normal
Thrombocytopenia
Severe Platelet Dysfunction
Coagulation Factor Deficiency
Fibrinolysis
Hypercoagulable State

Figure 7

Clot signal

Time to peak (TP, [min])
Peak amplitude (PA, [units])
Clot rate (CR, [units/min])
Activated clotting time (ACT, [sec])

Time

Figure 8