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VOLUME 21

SEMINARS IN THROMBOSIS AND HEMOSTASIS

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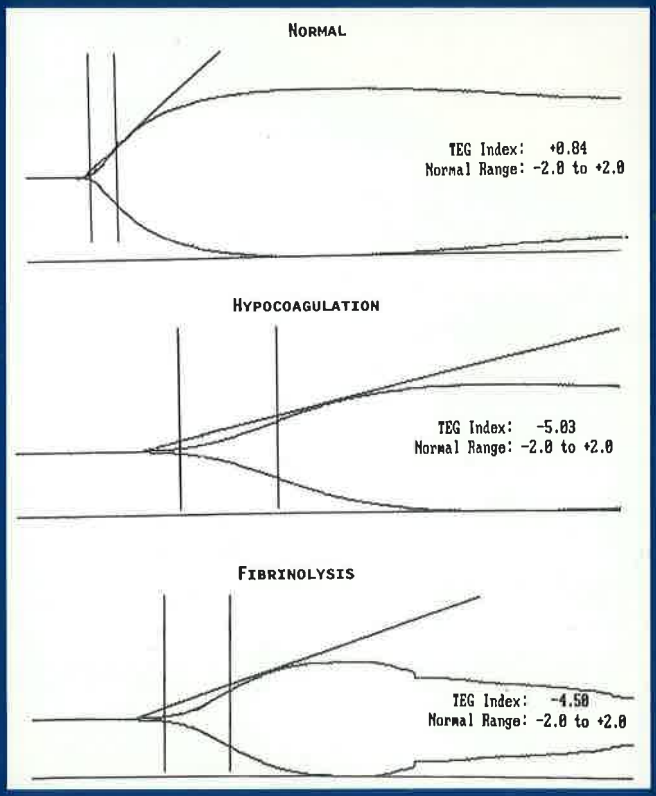

THROMBOELASTOGRAPHY

JOSEPH A. CAPRINI, M.D., CLARA I. TRAVERSO, M.D., PH.D.,
JEANINE M. WALENGA, PH.D., JUAN I. ARCELUS, M.D., PH.D.,
JAWED FAREED, PH.D., and BRUCE D. SPIESS, M.D.
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SEMINARS IN THROMBOSIS AND HEMOSTASIS

SEMINARS IN THROMBOSIS AND HEMOSTASIS

Recent and Future Issues

SEMINARS IN
THROMBOSIS
AND
HEMOSTASIS

Volume 21 Supplement 4, 1995

Thromboelastography

Joseph A. Caprini, M.D., Clara I. Traverso, M.D., Ph.D., Jeanine M. Walenga, Ph.D., Juan I. Arcelus, M.D., Ph.D., Jawed Fareed, Ph.D., and Bruce D. Spiess, M.D.

Continued

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COVER FIGURE: Thromboelastography.

In Memoriam

Professor Dr. Hellmut Hartert

This supplement to *Seminars in Thrombosis and Hemostasis* is devoted to the use of the thromboelastograph in various clinical settings. It is only appropriate to pay tribute to its inventor, Professor Dr. Hellmut Hartert who passed away in late 1993. He described the instrument in 1948 in the Klinische Wochenschrift "Blutgerinnungsstudien mit der Thrombelastographie, einem neuen Untersuchungsverfahren." The procedure allowed for the first time the measuring not only of coagulation globally, but information on when blood clots, how it clots, and when the clot dissolves. It thus revealed valuable information for the clinician regarding the functionality of the coagulation system. The concept for such an instrument arose from Professor Hartert's interest not only in clinical medicine, but in physics and electronic engineering. He was especially involved in hemorheology and invented in 1972 the Rheo-Simulator and in 1981 the concept of Resonance Thrombelastography.

Until his retirement in 1983, Professor Hartert was Professor and Head of the Medical Clinic in Kaiserslautern, a teaching hospital of the University of Mainz. He was a member of numerous scientific organizations, especially related to hemorheology, published more than 300 scientific articles, and served on the editorial board of several scientific journals. The receipt of the Poiseuille Gold Medal at the 5. International Congress of Biorheology in 1983 crowned his scientific accomplishments.

Those of us who had the pleasure knowing Professor Hartert remember him as a kind and warm-hearted person, as an excellent scientist, and a superb physician. The scientific community has lost one of its finest members.

I am grateful to Dr. Joseph Caprini and his associates for putting this issue on thromboelastography together, in honor of its inventor, Professor Dr. Hellmut Hartert.

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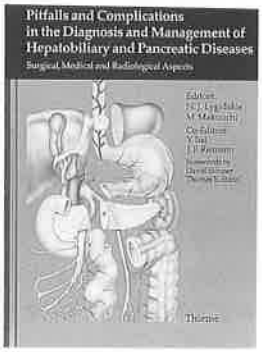

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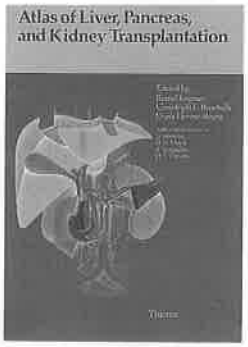

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Preface

Dr. Hellmut Hartert conceived the first thromboelastograph in Germany, in 1948. By doing so, he introduced more than a new test; his device actually changed the concept of coagulability so much that Raby coined the phrase, "The era of the thromboelastography," to reflect the importance of this new hematologic technique. Before thromboelastography (TEG) only the time until the onset of clotting could be measured. Following TEG, both quantity of clotting and stability of the clot formed could be evaluated; in other words, not only the kinetics, but also the dynamics of the entire coagulation process, from activation to clot lysis, could be measured.

Soon, TEG was tested, analyzed, and used by several European researchers, such as P. De Nicola in the 1950s, M. Audier at the beginning of the 1960s, and C. Raby at the end of the 1960s and in the 1970s. The American use of TEG began with von Kaulla and Caprini in the 1960s and minimal growth occurred until the 1980s. Its use in the United States has been growing substantially during the current decade.

TEG has probably been underestimated over the years. Why? The answer is not easy. Individual clotting defects are better characterized by specific tests other than TEG. The global nature of this test is more abstract and the same TEG result can be observed with varying levels of components, that is, low platelets and high fibrinogen may look similar to low fibrinogen and high platelets. Those who have used this test successfully have exploited the global nature of the data obtained by TEG analyses.

The goal of this issue of *Seminars* is to reach practical conclusions that can be applied to both everyday clinical practice and to research. Clinical applications, evaluation of test results, and limitations of TEG will be addressed in the course of this review. The most important aspect of this issue may be to point out the limitations of TEG, which should promote a better understanding and appropriate use of this interesting hematologic tool. A number of important investigators have shared their vast thromboelastographic experience with us and serve as a tribute to the inventor, Dr. Hellmut Hartert.

The authors wish to express their sincere gratitude to Robert Coats for his outstanding efforts editing, collating, and retyping the manuscripts. Our thanks go also to Kevin Hoffman for having the organizational skills to keep all of the participants focused in the right direction. Finally, we would like to acknowledge the editorial efforts of Stella Caprini.

Joseph A. Caprini, M.D., Clara I. Traverso, M.D., Ph.D.,
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The Thromboelastograph and the Thromboelastograph Technique

WAYNE L. CHANDLER, M.D.

The thromboelastograph was originally described in 1948 by Hartert.[1] Work by Hartert and others in the 1950s evaluated the effect of clotting factor deficiencies, anticoagulants, thrombocytopenia, and fibrinolysis on the thromboelastograph and demonstrated that it could be used as a rapid empirical screen of overall hemostatic function.[2] The thromboelastograph has been used extensively in Europe for a number of years. Its availability was limited in the United States until the 1980s, when it underwent somewhat of a rebirth with its use during liver transplantation procedures.[3,4]

The blood clot has both viscous and elastic properties. The thromboelastograph measures the elastic shear modulus or storage modulus of clotting blood. As originally described by Hartert, it is independent of sample viscosity over a wide range.[5] Figure 1 shows a diagram of the sample mechanism of the thromboelastograph and a typical thromboelastograph tracing. Whole blood is placed in the sample cup. A pin suspended by a calibrated torsion wire is lowered into the sample. To measure the elastic shear modulus of the sample, the cup is oscillated through an angle of 4° 45′ over a 10-second interval, including 1 second rest periods at the end of the rotation in each direction to prevent viscosity errors. The torque of the cup is transmitted to the pin through the sample in the cup. The width of the tracing is proportional to the magnitude of elastic shear modulus of the sample. Liquid whole blood transmits little or no torque from cup to pin, producing no deflection on the tracing even when the whole blood viscosity is high (such as in polycythemia or macroglobulinemia). As the blood clots, fibers composed of platelets and fibrin form between the cup and pin, transmitting a portion of the cup's motion to the pin. As shown later, the higher the platelet count and fibrinogen, the higher the maximum elastic shear modulus of the sample.

In early thromboelastographs, rotation of the pin was recorded using a mirror attached to the pin, a light beam, and photographic film. Analysis of the tracing was delayed by the need to develop the film. This has been simplified in current models by a mechanical/electrical transducer and amplifier, which drive a pen motor producing immediately usable tracings on heat-sensitive paper. In the latest versions of the instrument the output is converted to digital data for display on a computer terminal. All versions produce essentially equivalent tracings.

INTERPRETATION OF THROMBOELASTOGRAPH TRACING

Five standard measurements are made on each thromboelastograph tracing (Fig. 1). The reaction time (R, or r) value is measured from the start of the tracing to the point where the curve is 1 mm wide. The R value can be expressed in two equivalent ways, as a distance in millimeters or as a time in minutes. The chart speed of the thromboelastograph is 2 mm/min, so the time in minutes is equal to the distance in mm divided by 2. The R value is similar to a whole blood clotting time. Prolongation of the R value is associated with clotting factor deficiencies or inhibitors and anticoagulants such as heparin. As platelets provide the phospholipid surface for coagulation reactions in the standard thromboelastograph, thrombocytopenia may also prolong the R value.

The coagulation time (K, or k) value is the distance or time from the 1 mm wide point to the 20 mm wide point. The angle (α) is measured between the midline of the tracing and a line drawn from the 1 mm wide point tangential to the curve. The K value and angle are indications of the rate of increase of elastic shear modulus in the sample. In other words, how fast the clot structure is

From the Department of Laboratory Medicine, University of Washington, Seattle, Washington.

Reprint requests: Dr. Chandler, Department of Laboratory Medicine, University of Washington, Seattle, WA 98195.

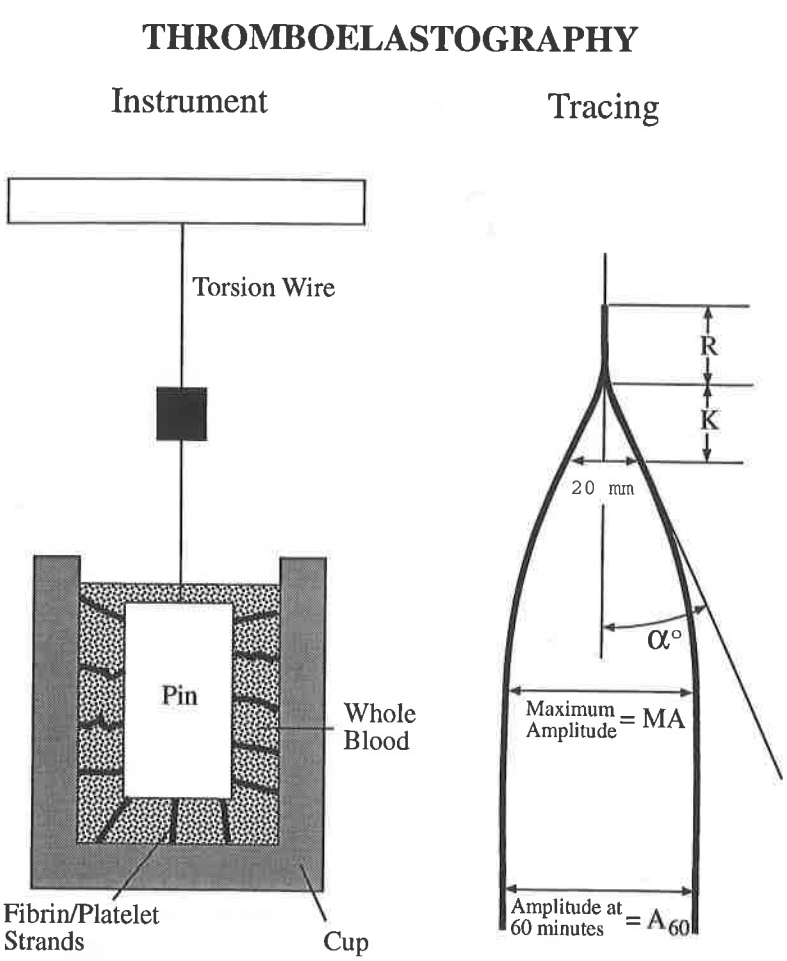

FIG. 1. Diagram of the thromboelastograph instrument and the tracing it produces. The instrument diagram shows the sample cup and measurement pin that is attached to a calibrated torsion wire. Whole blood (or recalcified/citrated blood) is placed in the space between the cup and pin and allowed to clot. Fibers composed of fibrin and platelets attach to the cup and pin, increasing the elastic shear modulus of the sample. The width of the tracing in millimeters is proportional to the elastic shear modulus. Five standard measurements are made on the tracing: R value is the distance or time from the start of the assay to the beginning of clot formation, defined as a 1 mm wide curve; K value is the distance or time from the 1 mm to 20 mm wide curve; α is the angle in degrees from the center line of the curve to a line running from 1 mm point tangenital to the curve; maximum amplitude (MA) is the maximum width of curve; A_{60} is the width of the curve at 60 minutes (or alternately width 60 minutes after the maximum amplitude point). Interpretation of various measurements is described in the text.

forming. These are the most complex results; they are abnormal in clotting factor deficiencies, platelet dysfunction, thrombocytopenia, and hypofibrinogenemia.

The fourth measurement is the maximum amplitude (MA). This is the width of the curve at its widest point and represents the maximum clot elastic shear modulus attainable. As discussed in detail later, it is primarily a function of platelet count and fibrinogen level, the structural components of the clot. In addition to thrombocytopenia and hypofibrinogenemia, very long clotting times associated with severe factor deficiencies or heparin therapy will also reduce the MA.

The elastic shear modulus of the sample increases exponentially in proportion to the amplitude of the thromboelastograph tracing (Fig. 2). Based on Hartert's original work, the absolute elastic shear modulus of the sample can be estimated from thromboelastograph amplitude as follows:

$$G = (5000A)/(100 - A) \qquad \text{Eq. 1}$$

where G is the elastic shear modulus in dynes per square centimeter and A is the width of the tracing in millimeters.

Thus, an amplitude of 100 mm is equivalent to an infinite shear modulus, whereas an increase in the amplitude from 50 to 67 mm is equivalent to a twofold increase in the elastic shear modulus. A typical clot from a healthy subject has an MA of 50 mm, equivalent to an elastic shear modulus of approximately 5000 dyn/cm^2.[5]

To simplify the clinical evaluation of shear modulus, Hartert developed a relative elasticity scale in which a normal clot with a MA of 50 mm was assigned an arbitrary elastic modulus of 100 as calculated by the following equation:

$$\epsilon = (C100A)/(100 - A) \qquad \text{Eq. 2}$$

where ϵ is the arbitrary modulus (also designated M_ϵ).[5]

After measuring the MA, the amplitude at the end of the tracing is measured. A substantial decrease in this amplitude compared to the MA indicates the presence of significant fibrinolysis. The two most common protocols

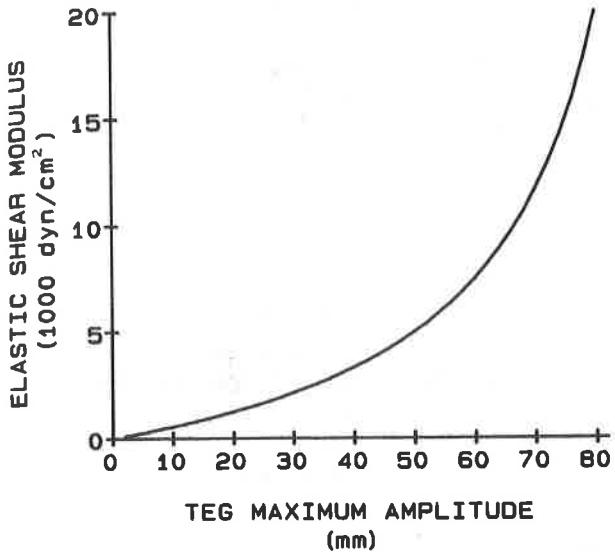

FIG. 2. Curve showing the relationship between the thromboelastograph (TEG) maximum amplitude and the associated elastic shear modulus.

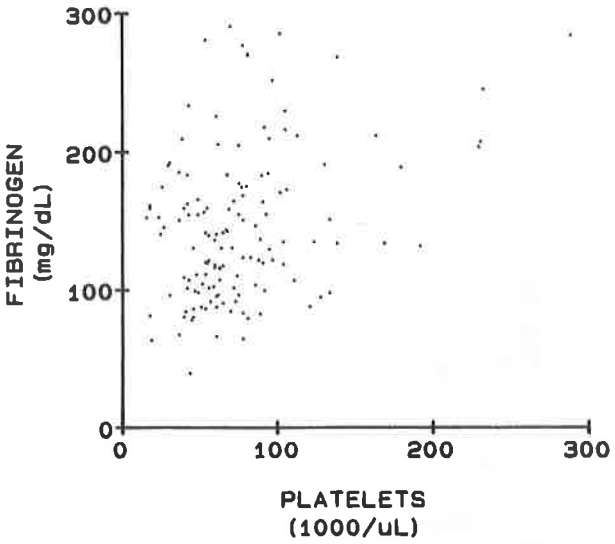

FIG. 3. Scatter plot of platelet count versus fibrinogen level during orthotopic liver transplantation.

are to measure the amplitude 60 minutes after starting the assay or 60 minutes after MA is reached. The longer you wait to measure the final amplitude, the more likely you are to detect weak fibrinolytic activity. How long you wait before measuring the final amplitude depends on how sensitive you want the assay to be for fibrinolysis. For some procedures, such as orthotopic liver transplantation, where rapid coagulation changes occur and severe fibrinolysis is common, samples may be run every 15 minutes. In these situations, allowing the thromboelastograph to run longer than 60 minutes is seldom useful.

DETERMINANTS OF THE MAXIMUM AMPLITUDE ON THE THROMBOELASTOGRAPH

The elastic shear modulus is affected by platelet count and fibrinogen levels in whole blood.[5] Prior studies have shown that the elastic shear modulus is proportional to the fibrinogen concentration of the clot, in both pure solution and plasma.[6,7] Studies have also shown that the elastic shear modulus is proportional to the platelet count in the sample.[2] To understand these relationships in more detail, we compared the platelet count, fibrinogen level, and MA in 137 samples of whole citrated blood obtained during orthotopic liver transplantation. Studies on human subjects were carried out according to the principles of the Declaration of Helsinki. Informed consent was obtained from all participants and the study was approved by the University of Washington Human Subjects Review Committee. Blood was anticoagulated by the addition of 4.5 mL whole blood to 0.5 mL of 130 mmol/L sodium citrate. Whole citrated blood samples were recalcified in the thromboelastograph by addition of 250 μL whole citrated blood to 100 μL of 0.645 g/dL calcium chloride. Samples were rejected if they showed evidence of fibrinolysis (greater than a 5 mm drop in amplitude at 60 minutes versus MA) or if plasma obtained from the whole blood showed evidence of heparin (thrombin time greater than 40 seconds). The elastic shear modulus in dyn/cm^2 was estimated from the MA from equation 1. The fibrinogen concentration was determined using an Automated Coagulation Laboratory 100 (ACL 100) and PT/FIB reagents from Instrumentation Laboratories (Lexington, MA). Platelet counts were performed on a Coulter STKR (Miami, FL).

To evaluate the effect of platelet count and fibrinogen level separately on MA, the platelet count and fibrinogen levels in the samples must be independent. Figure 3 shows the low correlation ($r^2 = 0.11$) of platelet count versus fibrinogen levels during orthotopic liver transplantation establishing the relative independence of these parameters in this set of samples.

There was a moderate correlation (Figure 4, top panels) between the platelet count and either the MA ($r^2 = 0.59$) or the elastic shear modulus ($r^2 = 0.67$). The correlation was weaker between the fibrinogen level (Figure 4, middle panels) and either MA ($r^2 = 0.44$) or elastic shear modulus ($r^2 = 0.43$). The best correlation (Figure 4, lower panels) was between the multiple linear regression fit of platelet count and fibrinogen level versus elastic

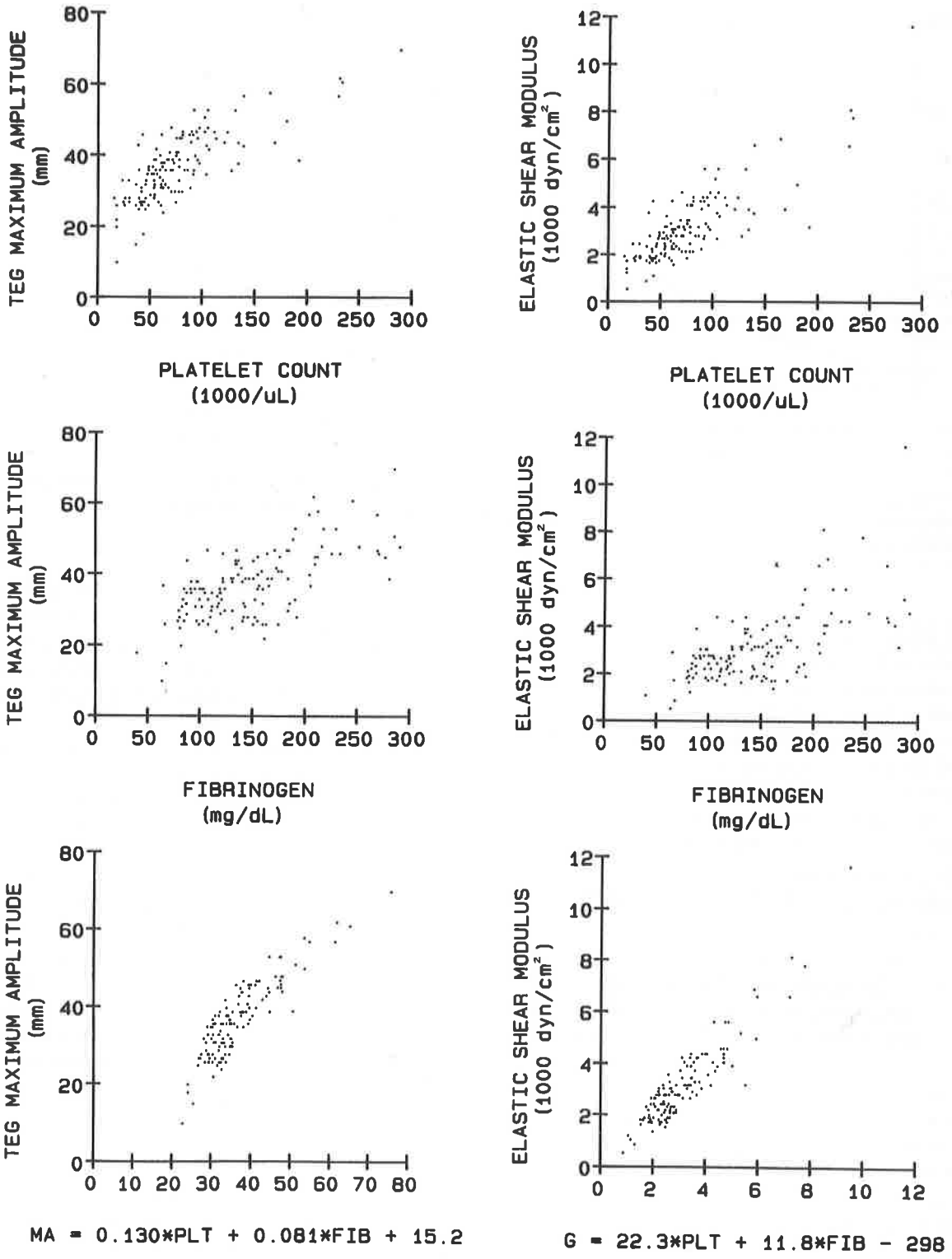

FIG. 4. Relationship between platelet count, fibrinogen level, and either thromboelastograph (TEG) maximum amplitude or elastic shear modulus. Equations on the x-axis of the lower graphs show the best multiple linear regression fit between platelet count (PLT) and fibrinogen level (FIB) versus either TEG maximum amplitude (MA) or elastic shear modulus (G).

shear modulus ($r^2 = 0.70$), with a somewhat lower correlation for MA ($r^2 = 0.60$).

As expected, this suggests that both platelets and fibrinogen are important in determining clot elasticity and that the MA is a function of both. Therefore, low MA amplitude may be due to low fibrinogen or a low platelet count or a combination of the two. It is not a specific indicator of either factor, although the data suggest platelets play a more important role in determining clot elasticity than does fibrin.

LABORATORY EVALUATION OF HEMOSTASIS USING THE THROMBOELASTOGRAPH

All thromboelastograph measurements are made at 37°C. The cup and pin are maintained at 37°C while in the instrument. It is important that cups and pins be prewarmed to 37°C prior to use. This can be done by placing them in the instrument several minutes prior to starting the assay or in a small incubator near the instrument. Cold cups or pins will slow the clotting reaction, producing falsely prolonged R values.

Two types of cup and pin sets are available, reusable stainless steel sets and disposable plastic sets. The disposable sets are potentially an advantage in low use situations or where cleaning the cups and pins is a problem. While the steel sets are initially expensive and require careful cleaning and occasional polishing, in high use situations they will pay for themselves in a few months compared to the costly disposable plastic sets.

Two options are available for running the standard thromboelastograph assay. Whole blood (350 μL) containing no anticoagulants can be placed directly in the instrument immediately after being drawn and the tracing started (native whole blood method), or citrated blood samples can be drawn and the sample recalcified to start the procedure. For citrated blood, 100 μL of prewarmed 0.645 g/dL calcium chloride is pipetted into a prewarmed cup followed by 250 μL of whole citrated blood with mixing. The tracing is started when the whole citrated blood is added to the calcium in the cup. Placing the calcium in the cup first prevents preactivation of the sample by the cup surface, which reduces the R value. After the pin is lowered into the sample, a few drops of mineral oil are placed on the sample surface to seal out air and prevent drying artifact, which appears as a rapid, ragged increase in amplitude about 15 to 30 minutes into the tracing.

Both the native blood and recalcification methods have been used successfully. The native whole blood method has longer R and K values compared with the recalcification method and requires a thromboelastograph in each location performing the test. Either an instrument must be available in each operating room, intensive care unit, and ward or the thromboelastograph must be moved from site to site in the hospital. Moving the thromboelastograph is cumbersome and time-consuming, as it requires removing the oil-filled motion dampers prior to moving followed by replacement of the dampers and careful leveling and temperature equilibration of the instrument before it can be used again.

Using the citrate/recalcification method, the thromboelastograph can be run at the sampling location or sent to a central laboratory for analysis. We use the citrate/recalcification method and run the assays in the central clinical laboratory, as it allows us to run both the thromboelastograph and standard coagulation procedures on the same sample. For example, the sample can be checked for heparin contamination using thrombin times if the sample shows evidence of unexpectedly long R and K values and a low angle. Since the citrated sample is stable for several hours, repeat thromboelastograph assays can be set up with protamine sulfate to neutralize heparin without obtaining a new sample.

Part of the value of the thromboelastograph is simple, rapid visual interpretation. We devised a novel method for transmitting results from the central laboratory to the patient care areas. Citrated blood samples are delivered to the laboratory using a dedicated pneumatic tube system (transit time, 1 minute) or by couriers. A closed circuit television (CCTV) system is used to send the image of the thromboelastograph to the ordering location in real time (Fig. 5). A signal switching system is used to direct the image from the laboratory to the appropriate monitor. We recommend the use of charge coupled device (CCD) cameras. Since the cameras view the same image constantly, non-CCD cameras are susceptible to image burn-in, and CCD cameras do not have this problem.

The CCTV system has several advantages: (1) all members of the surgical team can see the results using a wall-mounted television monitor in each operating room; (2) immediate backup is available if an instrument fails; the sample can be set up on another instrument and the image switched to the appropriate location; (3) the intensive care units can monitor the coagulation status of the patient near the end of the procedure prior to the patient being moved; and (4) the system provides real-time results to the maximum number of sites in the hospital with the minimum number of thromboelastographs and staff without moving the instrument from location to location. The cost of the CCTV system is minimal compared with the expense of even one additional thromboelastograph instrument. At present, we can cover two liver transplants and three to four cardiopulmonary bypass procedures with a single technologist running 12 channels on six instruments. When the assay is complete, the R,

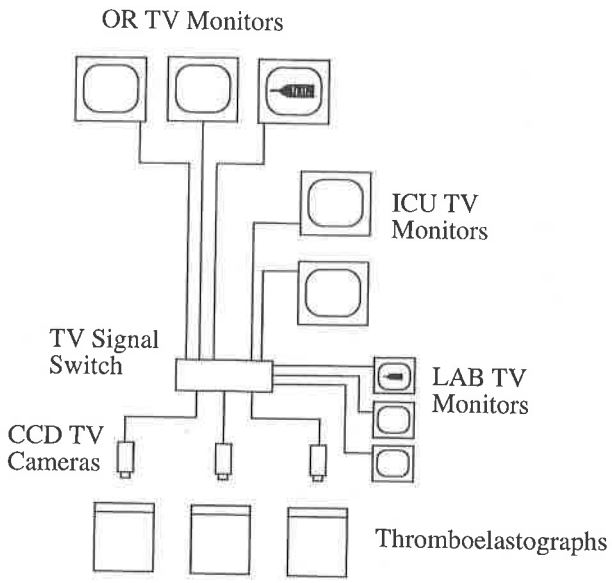

FIG. 5. Diagram of thromboelastograph closed-circuit television system for real-time transmission of tracings to operating rooms (OR) and intensive care units (ICU) from the central laboratory. Charge intensive coupled device (CCD) cameras transmit the image of thromboelastograph tracing to television signal switch, which is used to direct the signal to the appropriate ordering location.

K, angle, MA, and A_{60} values are measured and the results reported in the laboratory computer system.

QUALITY CONTROL AND QUALITY ASSURANCE ON THE THROMBOELASTOGRAPH

Quality control on the thromboelastograph is simple. The first major variable to monitor is instrument temperature. This should be done in two ways. First, a liquid crystal temperature strip should be attached to the temperature controlled plate in the instrument. These strips indicate temperature to $\pm 1°C$ and are useful in detecting major temperature variations. In addition, a digital thermometer should be used to check the temperature of the instrument daily or weekly to detect small variations in temperature. The instrument should maintain a temperature of $37 \pm 0.5°C$.

A pool of frozen plasma can be used as a daily or weekly control. The plasma should be run on each channel of each instrument as a standard recalcified sample. To determine control ranges, first check to see that the temperature of all instruments is within specifications. Next, using the calibration tool, adjust each channel so that all channels are producing the same amplitude for a given elastic shear modulus. Finally, run the control plasma 10 to 20 times on each channel over a period of days to determine the expected range for R, angle, and MA.

Once the expected control ranges have been established, the plasma control should be run at least weekly on each channel. Variations in R or the angle may indicate problems with the calcium solution, instrument calibration, or instrument temperature. Should the control show any signs of variable amplitudes between instruments or channels, the calibration tool included with the instrument should be used to adjust the output. If this fails to correct the problem, more extensive troubleshooting is needed. External quality control samples are not currently available in the United States.

Quality assurance on the thromboelastograph primarily consists of sample quality evaluation. If evacuated tubes are used for collecting the citrate sample, the tube should be at least 90% full; this assures the appropriate citrate to plasma ratio. If the tube is not full, the R values can be prolonged by excess citrate. A small amount of the whole blood can be centrifuged to check for hemolysis when blood is drawn. In vitro hemolysis occurring when blood is drawn usually indicates a difficult situation that may preactivate platelets. It is important to check samples with unexpectedly long R values or straight lines (zero MA) for the presence of heparin using a thrombin time assay on plasma from the original citrated sample. When heparin is detected, a new sample can be sent or the heparin can be neutralized with protamine sulfate. This is particularly useful during cardiopulmonary bypass when large amounts of heparin are present. Intraoperative evaluation of coagulation status can be assessed using protamine neutralized thromboelastographs.

REFERENCES

1. Hartert H: Blutgerinnungsstudien mit der Thrombelastographie, einem neuen Untersuchungsverfahren. Klin Wochenschr 26:577–583, 1948.
2. de Nicola P: Thromboelastography. Blackwell Scientific, Oxford, 1957.
3. Howland WS, O Schweizer, P Gould: Comparison of intraoperative measurements of coagulation. Anesth Analg 53:657–663, 1974.
4. Kang YG, D Martin, J Marquez, JH Lewis, FA Bontempo, BW Shaw, TE Starzl, PM Winter: Intraoperative changes in blood coagulation and thromboelastographic monitoring in liver transplantation. Anesth Analg 64:888–896, 1985.
5. Hartert H, JA Schaeder: The physical and biological constants of thromboelastography. Biorheology 1:31–39, 1962.
6. Kaibara M: Dynamic viscoelastic study of the formation of fibrin networks in fibrinogen-thrombin systems and plasma. Biorheology 10:61–73, 1973.
7. Carr ME, LL Shen, J Hermans: A physical standard of fibrinogen: measurement of the elastic modulus of dilute fibrin gels with a new elastometer. Anal Biochem 7Z:202–211, 1976.

The Normal Thromboelastogram and Its Interpretation

CLARA I. TRAVERSO, M.D., Ph.D., JOSEPH A. CAPRINI, M.D., F.A.C.S., and JUAN I. ARCELUS, M.D., Ph.D.

Thromboelastography (TEG) is an accurate test for a global study of coagulation, which it displays from the beginning of clot formation to fibrinolysis; it also evaluates platelet function. TEG provides a maximum amount of information, since its endpoint goes beyond the time of onset of clotting to assess the overall competence of the clot. To depict and better understand the worth of thromboelastographic information, Hartert[1] compared the coagulation of blood to building a house: TEG does not end when the foundation stone is laid, as the one-stage clotting tests do; TEG also reflects the speed of the building process and if it will be heavy or flimsy, strong or flabby. Therefore, TEG measures both quantity of clotting and, most importantly, quality of clotting, which is not recorded by the routine coagulation profile. In other words, TEG is a measurement of the time needed for clotting, that is, the kinetics of the forming clot, and of the tensile clot strength, that is, the mechanical properties of the clot formed, reflecting the dynamic aspect of coagulation.[2,3]

THÈ THROMBOELASTOGRAPHIC TRACING

Thromboelastographic information is obtained from an uninterrupted recorded tracing, called the thromboelastogram, which looks like a graphic representation of a burst of sound or diapason, and consists of three zones (Fig. 1): (1) An initial linear segment, or zone of precoagulation, that, corresponding to the invisible phase of coagulation, extends from the beginning of coagulation to the formation of the first fibrin strands; (2) the zone of coagulation is a sector that lies from the end of the precoagulation zone to the maximal separation of the two symmetrical branches. The progressive divergence of these two curved lines reflects the formation of the clot, and it is maximal when the clot is completely generated. It corresponds to the visible phase of coagulation; and (3) the zone of fibrinolysis starts at the end of the coagulation zone. Fibrinolysis is reflected by the slow yet progressive approach of the two curved lines. If the tracing is run long enough, the fusion of the two branches in a single straight line would indicate absolute clot lysis.

A look at these zones of the tracing provides a global appraisal of the coagulation state. Such consideration is interesting when tracings are typical; for instance, hypercoagulable states generate thromboelastograms with a typical "glass of cognac" appearance (Fig. 2). Making a first assessment by observing the tracing makes TEG an especially interesting laboratory tool to be tested in several surgical procedures that demand exact and uninterrupted monitoring in the operating room, such as cardiac surgery and the liver transplantation procedure.

The thromboelastographic tracing makes thromboelastographic information permanent, which allows readings that may be performed by several examiners. Additionally, the measurement of several well-defined parameters makes TEG an objective test and allows more accurate diagnoses.[2,4,5]

ORIGINAL THROMBOELASTOGRAPHIC PARAMETERS

Since 1948, several parameters, ratios, indexes, and even mathematical equations have been proposed to be

From the Department of Surgery and Related Specialties, University of Granada Hospital, Granada, Spain (C.I.T.); Department of Surgery, The Glenbrook Hospital and Northwestern University Medical School, Glenview, Illinois (J.A.C.); and Department of Surgery, Hospital of the Axarquía, Vélez-Málaga (Málaga), Spain (J.I.A.).

Reprint requests: Dr. Traverso, Department of Radiation Oncology, "La Paz" Hospital, 28016 Madrid, Spain.

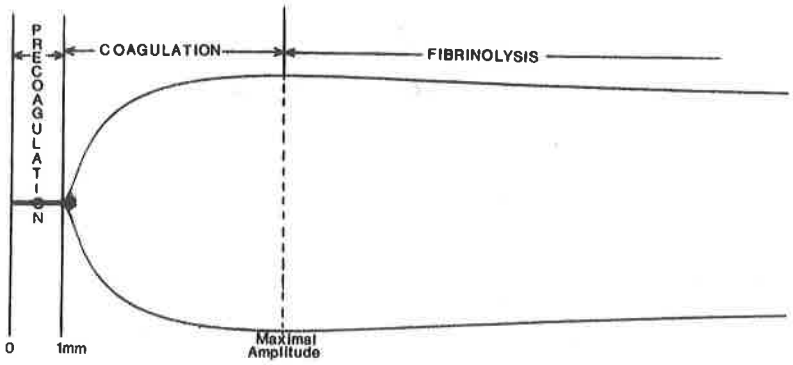

FIG. 1. Zones of the thromboelastogram. (Authors' tracing.)

added to the original constants introduced by Hartert.[4] In the course of this article we shall review all of them, although our study will be essentially focused on those that are more often utilized and the validity of which has been established.

TEG measures the time needed for the clot generation by two longitudinal parameters: R and K. R, which is called the reaction time by most authors following Hartert's nomenclature (also called the thromboelastographic parameter of thromboplastin by Marchal and his French colleagues[6]), is the distance in millimeters from the beginning of the tracing at the point where the curved lines are 1 mm apart. This is calculated by drawing a line perpendicular to the two branches at the 1 mm mark (Fig. 3). It is equivalent to the precoagulation zone described before. As R provides information on thrombin generation, it is influenced by all factors that belong to the intrinsic pathway[2] and increases linearly with increasing thrombin concentration.[7] Because coagulation begins at the time of blood withdrawal but the measurement of R is started when the blood is placed in the cuvette when TEG is performed in native whole blood, it is necessary to add 1 mm to R values measured on the thromboelastogram. When TEG is performed in decalcified whole blood, a technique introduced by Raby,[3] it is recommended that 2 mm be added because of the delay between recalcification and the start of the TEG recording.

K is called the clot formation time by most authors following Hartert's terminology, but it was also named the thromboelastographic parameter of thrombin by Marchal and associates.[6] It is the time interval in millimeters elapsed between the end of R and the point at which the distance between the two branches reaches 20 mm (Fig. 3). This point was arbitrarily defined by Hartert as corresponding to the maximal divergence obtained in normal platelet-poor plasma and is a measurement of the rapidity of clot development from the beginning of the visible phase of coagulation to a defined level of clot strength. It describes both thrombin activity and fibrin formation; therefore, the faster the minimum amount of thrombin that is able to form a visible clot, the shorter the K value. It is influenced by factor II, thrombin formation, fibrin precipitation, fibrinogen concentration, and hematocrit.[8–10] R + K is another parameter often used, which

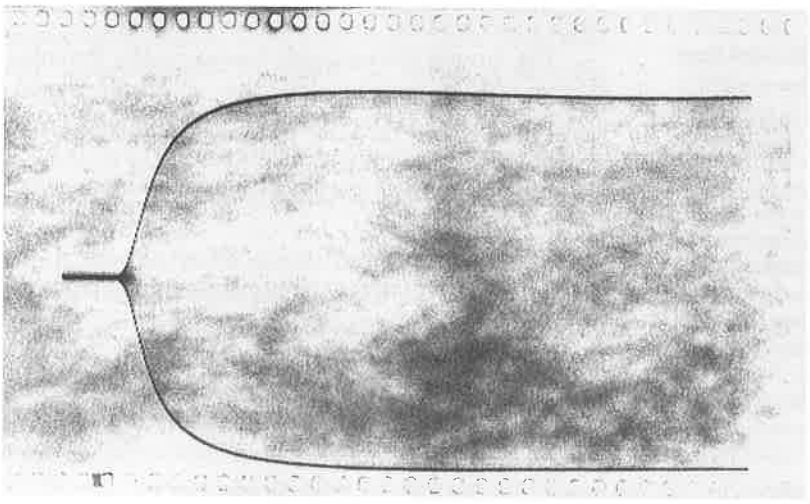

FIG. 2. Typical thromboelastogram with a "glass of cognac" appearance. A 64-year-old patient was diagnosed as having a gallbladder neoplasm. Note that hypercoagulability is very marked. (Authors' tracing.)

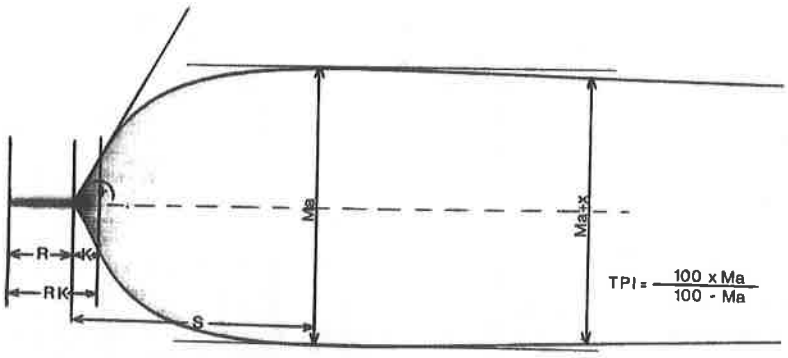

FIG. 3. Normal thromboelastogram. Parameters commonly evaluated are shown (see text).

reflects the coagulation time from its beginning to a predefined clot strength (Fig. 3).

The transverse parameter maximal amplitude (MA) or maximal elasticity, which was called maximal dynamic parameter of the thromboelastogram by Marchal and colleagues,[6] is the maximal distance in millimeters between the two diverging branches. To determine this parameter easily, two parallel lines (one per branch) to the line that results from prolonging the straight line portion of R through the tracing are drawn; both parallel lines would be united to the thromboelastogram by the peak of maximum value reached. The perpendicular line drawn from one branch to the other that intersects both parallel lines at the thromboelastographic point of maximum value corresponds to the MA (Fig. 3). At this point, the clot is entirely formed; accordingly, MA is a reflection of the final strength of the fibrin clot, that is, of the overall competence of the clot formed. Because of this valuable information on quality of the clot, MA is considered the most important thromboelastographic parameter[3,8,11–13] that makes TEG superior to other hemostatic tests.[14,15] MA is influenced by both platelet function and number,[11,16,17] which is one of its more interesting characteristics to be applied in cardiac surgery and other fields, as is stressed elsewhere in this issue of *Seminars*. MA is also influenced by fibrinogen and thrombin concentration, fibrin, factor XIII, and hematocrit.[7–11,18,19]

CLINICAL CORRELATION TO CHANGE IN THROMBOELASTOGRAPHIC PARAMETERS

R, K, and MA are the thromboelastographic parameters more widely utilized, and most parameters proposed after Hartert are based on them. As will be reviewed by the following articles in this issue of *Seminars*, these original constants can be evaluated individually or together. For instance, the slightest qualitative platelet defects disturb the MA. In contrast, thrombocytopenia has to be very marked to observe MA modifications, but it lengthens K. Afibrinogenemia generates a thromboelastogram characterized by an R infinitely prolonged, with absence of the other constants. Heparin administration induces modifications on the three parameters, which are related to dosages, but R is the parameter more often used for intravenous heparin monitoring. All three parameters will be modified if there is hypercoagulability. However, MA will be exclusively influenced if there is a prothrombotic state, since increasing fibrinogen and fibrin concentrations and platelet aggregation, which are the biologic alterations of that disorder, do not modify the other two constants. In any case, the global reading of R, K, and MA is considered to provide better information than that derived from the analysis of an isolated parameter, and it is the basis of the thromboelastographic interpretation today.

ADDITIONAL THROMBOELASTOGRAPHIC PARAMETERS

Taking these three Hartert original parameters as a reference, some authors defined other constants. Leroux[20] evaluated the interval between the end of R and MA, which he named the parameter of syneresis or the S constant (Fig. 3). This timing value could be used to evaluate the phase of fibrinogen coagulation, and it is proportional to the concentration of fibrinogen. Bosson and Dechamboux[21] termed parameter T, or global constant of coagulation, the time elapsed from the beginning of the tracing to MA. This would describe the time needed to form a stable clot. Audier and Serradimigni[2] also proposed a measurement from the end of K to MA, which they called the specific constant of coagulation or constant T. These three parameters have not been incorporated in the routine thromboelastographic analysis.

In contrast, the constant of clot retraction or constant MA + x, which was also proposed by Leroux,[20] is often used.[3,22] MA + x denotes the maximal amplitude of the tracing at several predetermined times after MA is mea-

sured, usually 30 (MA + 30), 60 (MA + 60), and 120 (MA + 120) min. It is a sector characterized by the approach of the two curved lines at the mentioned intervals (Fig. 3). This decrease in the distance between the two branches following MA is indicative of fibrinolysis. To quantify fibrinolysis, several indexes have been defined. T, F, and T + F, called the lysis time, have been parameters often utilized. T is equivalent to the constant S; thus, it describes the time needed to reach MA. F lies from MA to the point at which MA is equal to zero, that is, until fibrinolysis is completed. Both parameters have been applied to the analysis of fibrinolytic agents,[5,23,24] as well as to monitoring the fibrinolytic system in several surgical procedures,[25,26] which will be discussed by the following articles in this issue of *Seminars*. An index of fibrinolysis based on correlating MA with MA + x at several times[27] has not been incorporated in routine practice. However, the ratio MA + 60/MA is considered equivalent to F and is utilized.[26]

Derived from the relationship among Hartert's original constants, several interesting ratios and indexes have been defined. The information provided by some of them has been so useful that they have been incorporated for routine application. This is the case of the ratio MA/R + K, which was empirically obtained by Audier and Serradimigni[2] in platelet-rich plasma. This is not a mathematical equation; it is a result of plotting R + K and MA values in the same graph. The authors noted that this ratio provided useful information, which has also been reported by other authors.[28,29] It has been shown to play a special role in detecting hypercoagulable states and monitoring anticoagulant treatment, as discussed by other authors in this issue of *Seminars*.

As a result of the relationship between two important parameters, K and MA, Raby[3] developed the thrombodynamic potential index (TPI), which was defined for decalcified whole blood TEG. According to Raby, the main information provided by the thromboelastographic study is on the quality of the clot, that is, information on its hemostatic competence. Therefore the important parameters are those that are related to that kind of information. MA and S, both already defined, are those constants: MA because it reflects the overall competence of the clot and S because it defines the time needed for building the clot. Following Raby's argument, due to the fact that these two parameters usually change in an opposite way, the dynamic aspect of coagulation could be defined as the MA/S ratio. However, according to Raby, the exact measurement of S is difficult in practice because the maximal divergence of the two curved lines is the same on several centimeters of the thromboelastogram. To elude this drawback, the author used K instead of S, following some of his studies that showed that these two parameters changed in the same way. Regarding MA, he utilized the

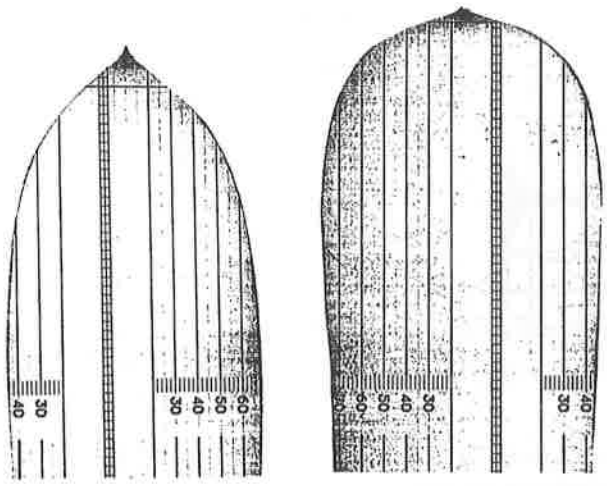

FIG. 4. Native whole blood (left) and celite-activated whole blood (right) thromboelastograms. (Authors' tracing.)

maximal coefficient of elasticity, or Emx, which is a multiple value of the MA. There are tables that provide Emx values but it can be calculated by the equation: $100 \cdot MA/100 - MA$. Substituting MA by Emx and S by K in the previously mentioned ratio, Raby[3] defined the dynamic aspect of coagulation (the TPI) as the ratio Emx/k. TPI has been shown to be well correlated to all of Hartert's original parameters,[30] and some authors have reported good results.[12,31–34] Some authors[35] have erroneously called TPI the MA/K ratio. In any case, MA/K is only used in a few centers, although good results have been reported.[36]

Shortly after Hartert, Della Santa and Duraffourd (1953) proposed the index of coagulability (cI or dI) as an attempt to merge the thromboelastographic information in this single equation: $cI = tag\ a \cdot 160$, where 160 is a constant; a is the alpha angle between the two following lines: one line is drawn by prolonging the initial straight line of R; the other is a tangent to one branch of the thromboelastogram that is drawn from the beginning of R. Most authors, however, prefer only to use the angle. For such a finality, a, which is a thromboelastographic parameter often used with good results,[7,22,32,38] is measured as already explained, but the second line is a tangent to the shoulder of the tracing instead of drawing it from the beginning of R (Fig. 3). The a is the rate of clot formation, that is it relates the strength of the clot to the speed at which it is formed; it is primarily a reflection of the function of fibrinogen, and it increases with improved platelet function. It is expressed in degrees.

Caprini and colleagues[7] introduced a modified technique in 1974. The new technique, called celite-activated TEG, consists of comparing two simultaneously performed thromboelastograms (Fig. 4): the first is a native

whole blood thromboelastogram and the second is performed in native whole blood activated with a 1% celite (diatomaceous earth).[7] The result of evaluating together, by a discriminate analysis, the data obtained from both thromboelastograms is a linear combination of the parameters, formulated as the following index:

$$I = A1 + A2R + A3RK + A4Ma + A5a + A6RC + A7RKC + A8MaC + A9aC$$

where A1 to A9 are coefficients, and the C labels the celite-activated thromboelastographic parameters. These parameters are measured as explained for native whole blood except for RC, the end of which is the point at which the two branches are 2 mm apart, instead of 1 mm. Note that this index does not involve K, but it does involve R + K (RK). The index evaluates the reserve clotting potential by what Caprini and associates called the celite-stimulating potential. In other words, by adding celite, the activated TEG compared to the native whole blood TEG exhibits accurately the coagulation state, even in those situations in which alterations of coagulation may be covered or insufficiently defined. The index was found to be well correlated with TPI when the Caprini experience was compared to that of Raby.[39] The index has been comprehensively analyzed and clinically applied by Caprini and colleagues with good results, and its role will be discussed by other articles in this issue of *Seminars*. For example, the index was shown to be a proper monitor for intravenous heparin administration,[40] as well as a guide, together with other selective tests, for identifying surgical patients who may be at risk of bleeding or developing deep vein thrombosis.[41] Several other authors have recently reported good results using this technique.[16,42]

To our knowledge, three mathematical equations have been formulated for evaluating TEG. Scott and Burnett[43] proposed two of them in 1969, both based on Hartert's original constants R, K, and MA: one described the earliest stage of clotting and the other the later stages. Recently, Wang and associates[44] have proposed a complex equation, since they considered Scott and Burnett's equation to be unable to provide information on fibrinolysis and clot softening. Because Wang and colleagues really extended the Scott and Burnett expression, their new equation is also based on Hartert's original parameters. The new equation remains to be tested in clinical use, as underscored by those authors.

ADDITIONAL IMPORTANT TECHNICAL POINTS

A critical issue regarding TEG interpretation is a careful definition of normal ranges by each laboratory. If inappropriate ranges are taken as reference, information not only may be missed, but also, and most importantly, may be erroneous. The consequences derived from this are obvious. Therefore, it is imperative for each laboratory to establish their own reference ranges or to check given normal ranges before interpreting the thromboelastogram. Consequently, we expect every author of this issue to provide their own reference ranges, related to both their specimen and technique utilized. To illustrate and emphasize the importance of this matter, we have compiled in Table 1 some normal ranges or values reported.

Another important issue regarding TEG is to adjust the duration of the recording to the information required. Obviously, the duration will depend on which parameters one wants to read. In addition to this, the duration of

TABLE 1. Some Examples of Normal Ranges According to the Thromboelastographic Technique Used*

Specimen	*R*	*K*	*MA*	*TPI*	*MA/R+K*
Native whole blood					
Hartert and Schaeder[45]	24	12	50	—	—
Caprini et al[7]	16–24	8–16	50	—†	—
Decalcified whole blood (dilution: 1/20)					
Raby and Couinaud[31]	13–14	11–12	45–47	6–10	—
Torrás-Barba et al[10]	10.5–11.1	9.9–10.3	50.7–52.3	10.2–11.0	—
Decalcified whole blood (dilution: 1/5)					
Schneider et al[47]	14.8–18.8	6.6–9.8	91.7–116.3‡	—	—
Traverso[29]	10–19	4–10	50–64	—	2–3.5
Platelet-rich plasma					
Marchal et al[6]	16–18	7	54–62	—	—
Audier and Serradimigni[2]	14–20	5–9	55–62	—	2–3
Caen et al[46]	13–20	4–8	53–64	—	—
García-Monteavaro et al[12]	17.6–19	4.5–4.9	56.7–57.9	—	3–3.2

* R: Reaction time; K: clot formation time; MA: maximal amplitude; TPI: thrombodynamic potential index.
† Normal range for Caprini's index is from −5 to 2.
‡ Emx is provided.

tracing time will depend on both the specimen and the technique used. For instance, if one uses native whole blood for monitoring intravenous heparin, the information required can be obtained in 15 to 20 minutes.[18,25,26] The celite-activated TEG reduces the recording time for all analyses.[7] Performing TEG in decalcified whole blood to assess the entire coagulation process takes 60 to 90 minutes,[2] but hypercoagulability can be analyzed in 45 to 60 minutes.[29] The study of fibrinolysis needs 3 to 4 hours of recording.[3] The computerized TEG greatly shortens these times.

CONCLUDING REMARKS

The following articles in this issue of *Seminars* will analyze the information provided by TEG in several fields by merging the available literature and the authors' own experience. This is an attempt to establish TEG applications in various fields and to set TEG limitations. We believe that an adequate knowledge of the normal thromboelastogram and how to read it, as well as the meaning of the parameters, are essential to better understand why thromboelastography is considered to have a role in those fields.

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REFERENCES

1. Hartert HH: The proper phase of coagulation: Its physical differentiation by resonance-thrombography and thrombelastography. Clin Hemorheol 2:51–69, 1982.
2. Audier M, A Serradimigni: *Savoir interpreter un thrombo-elastogramme ou l'exploration precise de la coagulation sanguine:* Paris, Maloine, 1962, pp 135.
3. Raby C (Ed): Exploración del tiempo trombodinámico: In: *Hemorragias y Trombosis*. Toray-Masson, Barcelona, 1968, pp 190–211.
4. Hartert HH: Blutgerinnungsstudien mit der Thrombelastographie, einem neuen Untersuchungsverfahren. Klin Wochenschr 29:577–583, 1948.
5. De Nicola P: *Thromboelastography.* Springfield, Il, Charles C Thomas, 1957, pp 110.
6. Marchal G, ME Leroux, M Samama: La Thrombodynamographie (Thrombelastographie). Sa place actuelle en clinique. Presse Med 51:1919–1922, 1960.
7. Caprini JA, JB Eckenhoff, JM Ramstack, L Zuckerman, LF Mockros: Contact activation of heparinized plasma. Thromb Res 5:379–400, 1974.
8. Jaulmes A, D Querangal, J Delga (Eds): Hematologia. In: *Práctica de laboratorio*. Toray-Masson, Barcelona, 1972, pp 591–721.
9. Butler MJ: Thromboelastography during and after elective abdominal surgery. Thromb Haemostas 39:488–495, 1978.
10. Torrás-Barba J, J Duran, A Pales, J Alloza, J Monasterio, A Triginer: Valoración tromboelastográfica de la hipercoagulabilidad en pacientes traumatológicos sometidos a profilaxis con heparina cálcica. Sangre 25:35–44, 1980.
11. De Gaetano G, J Vermylen: Effect of aspirin on the thrombelastogram of human blood. Thromb Diath Haemorrh 30:494–498, 1973.
12. García-Monteavaro M, M Rodriguez, M Diez, A Navarro: Thromboelastographic assays of the clotting process in situations of obesity and caloric restriction. Rev Esp Fisiol 42:57–62, 1986.
13. Traverso CI, JI Arcelus, E Gómez, JD Luna, M López-Cantarero, JM García: Prospective assessment of the risk of deep vein thrombosis in elective abdominal surgery. Predictive role of thromboelastography. Thromb Haemorrh Disord 7:9–15, 1993.
14. Zuckerman L, E Cohen, JP Vagher, E Woodward, JA Caprini: Comparison of thrombelastography with common coagulation tests. Thromb Haemostas 46:752–756, 1981.
15. Spiess BD, KJ Tuman, RJ McCarthy, GA Delaria, R Schilo, AD Ivancovich: Thromboelastography as an indicator of post-cardiopulmonary bypass coagulopathies. J Clin Monit 3:25–30, 1987.
16. Tuman KJ, RJ McCarthy, RV Patel, RB Patel, AD Ivankovich: Comparison of thromboelastography and platelet aggregometry. Anesthesiology 75:A433, 1991.
17. Martin P, F Horkay, SM Rajah, DR Walker: Monitoring of coagulation status using thrombelastography during paediatric open heart surgery. J Clin Monit 6:183–187, 1991.
18. Howland WS, B Castro, J Fortner, P Gould: Hypercoagulability, thrombelastographic monitoring during extensive hepatic surgery. Arch Surg 108:605–608, 1974.
19. Sultan C, G Priolet, Y Benzard, R Rosa, F Josso: Exploración de la hemostasia. In: *Técnicas en Hematología.* Barcelona, Toray-Masson, 1979, pp 129–334.
20. Leroux ME: *La thrombo-elastographie. Studes theoriques, techniques, experimentales et cliniques sur la thrombo-elastographie et sur quelques problemes annexes lies a la biologie de la coagulation sanguine.* Asnieres, Les Dessins et Tirages Publibitaries M. Garcia, 1957.
21. Bosson P, R Dechamboux: *La thrombo-elastographie de Hartert, son interet dans l'etude de la coagulation.* Paris, Librairie Medicale Vigue, 1958.
22. Owen CA, SR Rettkee, EJW Bowie, TL Cole, CC Jensen, RH Wiesner, RAF Viram: Hemostatic evaluation of patients undergoing liver transplantation. Mayo Clin Proc 62:761–772, 1987.
23. Summaria L, J Sandesara, G Yang, JP Vagher, JA Caprini: In vitro comparison of fibrinolytic activity of plasminogen activators using a thromboelastographic method. In vivo evaluation of the B-chain-streptokinase complex in the dog model using pre-titered doses. Thromb Haemostas 56:71–78, 1986.
24. Kang Y, S Aggarwal, JA Freeman: Update on anesthesia for adult liver transplantation. Transplant Proc 19:7–12, 1987.
25. Kang YG, DJ Martin, J Marwuez, JH Lewis, FA Bontempo, BW Shaw, TE Starzl: Intraoperative changes in blood coagulation and thrombelastographic monitoring in liver transplantation. Anesth, Analg 64:888–896, 1985.
26. Tuman KJ, BD Spiess, RJ McCarthy, AD Ivankovich: Effects of progressive blood loss on coagulation as measured by thrombelastography. Anesth Analg 66:856–863, 1987.
27. Lee BY, FS Trainor, WR Thoden, D Kauner (Eds): Monitoring coagulation dynamics: Thromboelastography. In: *Handbook of Noninvasive Diagnostic Techniques in Vascular Surgery.* East Norwalk, CT, Appleton-Century-Crofts, 1981.
28. Fano R, H González: Valor de la tromboelastografia en la detección de la hipercoagulabilidad plasmática. Barcelona Quir 26:288–294, 1983.
29. Traverso CI: Estudio de las modificaciones tromboelastográficas inducidas por la heparina a bajas dosis y el dextrano 70 como

profilaxis de la enfermedad tromboembólica venosa postoperatoria. Granada University School of Medicine, Spain, Medical Degree Thesis, 1986.
30. Stoltz J, P Alexandre, P Voisin, A Larcan, F Streiff: Viscosite sanguine: Correlations avec les parametres du thromboelastogramme. Nouv Presse Med 9:3095–3096, 1980.
31. Raby C, C Couinaud: Les trubles de la coagulation dans les hemorragies des hypertensions portales. Chirugie 102:186–196, 1976.
32. Duraffourd P: Les conditions de la fiabilite et les avantages du thromboelastogramme. Coeur 12:447–455, 1981.
33. Blanchemaison P, P Griton, M Cloarec: Les ulceres hypercoagulables. A propos d'un cas recent. Phlebologie 40:949–954, 1987.
34. Traverso CI: Valoración del test de tolerancia a la heparina intravenosa realizado con tromboelastografia en la predicción de la enfermedad tromboembólica venosa tras cirugía general electiva. Granada University School of Medicine, Spain, Doctoral Thesis, 1989.
35. Zyryanov BN, II Tutrin, VV Udut: Risk evaluation of thromboembolic diseases according to functional test data in patients with advanced malignancies. Neoplasma 34:353–360, 1987.
36. Vara-Thorbeck R, M Lopez-Quiñones, J Guerrero, J Castro: Modificaciones tromboelastográficas inducidas por la hemodilución con dextrano en cirugía de cadera. Cir Esp 40:1061–1069, 1986.
37. Della Santa R: Une nouvelle methode de depistage des diatheses thrombophiles: la thromboelastographie selon Hartert. Praxis 43:89–93, 1953.
38. Heather BP, SA Jennings, RM Greenhalgh: The saline dilution test -a preoperative predictor of DVT. Br J Surg 67:63–65, 1980.
39. Cohen E, JA Caprini, L Zuckerman, JP Vagher, B Robinson: Evaluation of three methods used to identify accelerated coagulability. Thromb Res 10:587–607, 1977.
40. Caprini JA, JP Vagher, SJ Rabidi, JE Mitchell: Laboratory monitoring of continuous intravenous heparin therapy. Thromb Res 29:91–94, 1983.
41. Caprini JA, L Zuckerman, A Cohen, JP Vagher, V Lipp: The identification of accelerated coagulability. Thromb Res 9:167–180, 1976.
42. Confino E, A El-Roeiy, J Friberg, N Gleicher: The thromboelastogram and circulating lupus anticoagulant. J Reprod Med 4:289–291, 1989.
43. Scott GW, J Burnett: On the rates of coagulation and subsequent softening of bovine and human blood and of thrombin-fibrinogen. Biorheology 5:163–176, 1969.
44. Wang M, FY Zhuang, T Tian: Analysis of thromboelastogram on coagulation and fibrinolysis. Biorheology 25:539–944, 1988.
45. Hartert HH, JA Schaeder: The physical and biological constants of Thrombelastography. Biorheology 1:31–39, 1962.
46. Caen J, M Larrieu, M Samama (Eds): La coagulación. In: *Hemostasia: Métodos de exploración y diagnóstico práctico*. Barcelona, Toray-Masson, 1977, pp 101–119.
47. Schneider W, O-E Rodermund, H Egli: Das normale Thromboelastogramm. Thromb Diath Haemorrh 7:35–47 1962.

Thromboelastography: Measuring Statistical Probabilities

JOSEPH A. CAPRINI, M.D., F.A.C.S., JUAN I. ARCELUS, M.D., Ph.D., SOFIA GOLDSHTEYN, M.D., KEVIN N. HOFFMAN, B.A., and ROBERT W. COATS II, B.S.

The thromboelastograph, developed in 1948 by Hellmut Hartert[1] from Heidelberg, produces a global measurement of the overall dynamics of clotting, from clot formation to dissolution. Its greatest strength is the ability to give an overall picture of this process. Its greatest weakness is an inability to specify clotting abnormalities, such as factor V or IX deficiency. If one factor level is lowered and another is raised, the net effect on the thromboelastograph may be normal if the degree of abnormalities is equivalent. A number of investigators have attempted to use thromboelastography for specific diagnosis-related problems, which is difficult to achieve and accounts for a lack of enthusiasm among hematologists.

The value of this instrument lies in the global nature of the measurements, which we combine with a standard battery of tests, including platelet count, fibrinogen level, prothrombin time, partial thromboplastin time, fibrinogen split products, hematocrit, and, when indicated, template bleeding time.

This approach may be helpful in the evaluation of complex clinical problems, such as a 70-year-old patient with bowel cancer who bleeds during surgery and has the following hematologic abnormalities: defect in platelet function due to antiplatelet therapy for arthritis; vitamin K deficiency due to lack of a normal diet because of anorexia from bowel cancer (this may be further complicated by a bowel preparation including antibiotics, which further reduce vitamin K-dependent clotting factor levels); an occult congenital defect of coagulation, such as von Willebrand's disease; activation of hematologic systems due to substances entering the bloodstream producing a low-grade intravascular coagulation with accelerated fibrinolysis; extensive intra-abdominal adhesions due to previous operations, which may compound the bleeding problem. Thromboelastographic (TEG) analysis in combination with the other tests may help direct the extent and order of therapy, depending on the global pattern reflecting decreased platelet or fibrinogen levels or accelerated fibrinolysis. It is most useful when multiple hemostatic defects are present.

Another clinical situation illustrating the use of thromboelastography occurred during an aortic aneurysm repair. The graft began to ooze, as did the tissue surrounding the graft. The wound was packed, a thromboelastogram and the usual hemostatic tests were performed, and within 15 minutes, the evolving graph suggested that the physicians were faced with a marked reduction in platelet function aggravated by an accelerated fibrinolytic response. They maintained the packing for more than an hour while correcting these defects successfully with desmopressin, platelet packs, and aminocaproic acid. The packing was removed, and the patient survived without further bleeding. The evolving dynamics of hemostasis in the fully heparinized patient were quickly and efficiently tracked using thromboelastography. Subsequent tracings helped guide continued resuscitative efforts postoperatively and, in conjunction with standard hematologic testing, helped result in a successful outcome.

The use of a dynamic global hematologic measurement (TEG) during the perioperative period provides an additional tool in the management of serious bleeding problems. This is especially true in the case of accelerated fibrinolysis during an operative procedure.[2] Normally, vessels are cut during surgery and most will develop

From the Department of Surgery, The Glenbrook Hospital, Glenview, Illinois, Northwestern University Medical School, Chicago, Illinois.

Reprint requests: Dr. Caprini, Department of Surgery, The Glenbrook Hospital, 2100 Pfingsten Road, Glenview, IL 60025.

FIG. 1. Normal thromboelastographic tracing during cardiopulmonary bypass with the patient fully heparinized.

small thrombi that prevent bleeding. It usually takes 48 to 72 hours for these clots to begin dissolving as part of the body's healing process. Patients with hemostatic defects, including accelerated fibrinolysis, may experience delayed bleeding several hours postoperatively due to premature dissolution or fragmentation of these defective thrombi. The size of the vessel, pressure, blood flow, and local anatomy are all factors that may influence this process. At the time of reoperation, an oozing area or individual bleeding vessel is often found and repaired, after which the patient recovers uneventfully. It is believed that on some occasions the vessels that were missed during the original operation actually were not bleeding but contained weak hemostatic plugs that dissolved or fragmented prematurely in the early postoperative period. Additional factors affecting this risk of bleeding in the postoperative cardiac surgery patient include rewarming, relaxation of vasospasm, and higher blood pressure. All of these factors improve tissue perfusion but may unmask vessels with weak hemostatic plugs.

Figure 1 shows a normal TEG tracing during cardiopulmonary bypass with the patient fully heparinized. The dynamics of clot formation and dissolution can be measured by adding heparinase to the blood specimen to neutralize heparin effects. Figure 2 shows a grossly abnormal TEG tracing obtained during the same stage in the operation in another patient who had accelerated fibrinolysis. This situation may occur during a complex operative procedure, particularly open heart or liver transplant surgery. Utilizing standard hematologic tests and the thromboelastograph enables one to get additional insight into some very difficult situations. One may be treating a bleeding patient and produce accelerated coagulability or hypercoagulability, which may result in a postoperative thrombosis. In the example given previously, there was a great postoperative thrombosis risk for the patient, especially if the cancer was extensive and accompanied by distant metastasis.[3] This one example provides an overview of the complexities that one may experience with hematologic disorders and how the thromboelastograph may be useful in analyzing the overall response to therapy.

A reduced maximum amplitude, long reaction (R) time or negative index, when comparing celite and native blood, may indicate a hypocoagulable state. The reverse may be seen with marked hypercoagulability. Both patients may appear similar clinically. Under one circumstance, administration of blood products may be necessary to convert hemostasis to more normal levels. If the thromboelastograph gives a very hypercoagulable pattern, then, despite prolongation of some clotting tests, one may not want to pursue replacement therapy, unless the patient is overtly bleeding.

In summary, this is a very complex process and, on occasion, the thromboelastograph can be quite useful in helping to sort out these complexities.

CELITE-ACTIVATED THROMBOELASTOGRAPHY: IDENTIFICATION OF THE CLEAN SPECIMEN

The thromboelastogram has been most useful in our hands in identifying the purity of the blood specimen obtained for coagulation analysis. This involves native whole blood thromboelastography and begins with a careful two-syringe technique for drawing blood. This technique involves discarding the first 5 or 8 mL of blood, which are then used for another purpose. The blood is drawn through a thin-wall 19 g needle or its equivalent, after a clean venipuncture with little or no tourniquet application. The blood is then carefully delivered in the proper amount to a polypropylene tube containing a citrate-citric acid buffered solution, whose pH has been carefully titrated. This specimen is used for standard clotting tests, and another aliquot is deposited into an empty polypropylene tube and placed into one of the TEG cups within 4 to 6 minutes. A duplicate specimen is placed in the other TEG cup, which contains 1% (w/v) celite solution, and the test is begun. If the specimen has been cleanly drawn and is not contaminated with any tissue juice or foreign material from any of the tubings, the syringe, or the containers used to transport the blood, then activation of the blood should not occur. This will result in a significant difference between the appearance of the native and celite-activated tracings. The native specimen will start clotting later than the celite-activated sample (R), and the rate of clotting (reaction-coagulation times [RK], angle) will be slower in this specimen. The final clot strength (maximum amplitude [MA]) will be less in the native specimen compared with the celite-activated sample. Visually and statistically, these differences are obvious. In cases in which contamination has occurred anywhere in the system, this will not be true. The normally stubby or short R time of the celite-activated specimen will also be seen in the native specimen, indicat-

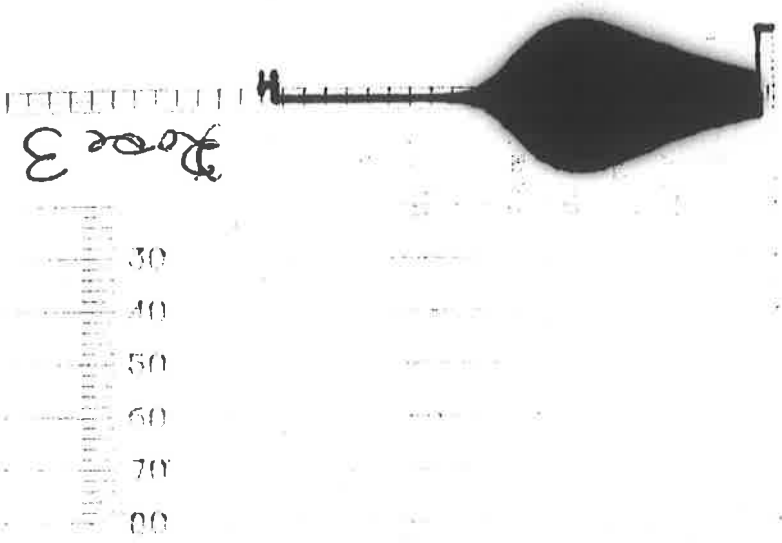

FIG. 2. Grossly abnormal thromboelastographic tracing obtained during the same stage in the operation in another patient who had accelerated fibrinolysis.

ing some contaminant that is mimicking the effect of celite in the native specimen. This problem is not rare, particularly when individuals of varying skills perform the two-syringe blood drawing technique without proper training. The presence of a severe pathologic condition, existing hemostatic defects, and poor venous access may compound the situation. A long activated partial thromboplastin time (aPTT) in the intensive care setting may be a clue that a contaminated specimen is present, but without celite-activated thromboelastography (CTEG), one cannot differentiate the long aPTT of a poor blood drawing technique from that of a deficiency state or heparin effect. The treatment of each of these two conditions is very different. In the case of heparin therapy, one may lower the heparin dose based on a long aPTT resulting from a poor blood drawing technique in a very hypercoagulable patient. Because of the accelerated clotting activity, this patient may require a higher heparin dose. The long aPTT may be appropriately seen if a deficiency state exists. One must be aware of potential clotting problems, including specimen artifacts, in these critically ill patients.

Studies have been done in volunteers, mimicking the poor blood drawing technique and introducing contaminants into the blood to reproduce this pattern of TEG tracings. They are immediately obvious on inspection of the tracings with the naked eye. With today's computer analysis of the graphs, this determination can be done with even greater precision (Figs 3, 4).

In addition, changes in the R time, the RK time, and the angle, which reflect the rate of the coagulation process, will be statistically similar between the celite and native tracings after a poor blood drawing technique. Similarly, the MA will have the same problem and, occasionally, when there is a contaminant in the cup of the thromboelastogram, artifacts will occur with premature retraction or uneven lines on the graph, reflecting these contaminants.

Part of the initial blood drawing protocol includes a citrated specimen for analysis. This specimen, when it is brought back to the laboratory after the native blood is put into the cups, is immediately centrifuged under refrigeration to separate the plasma from the cells as quickly as possible. It is important to spin down this sample of citrated whole blood, since studies in our laboratory indicate that allowing the cells to incubate with refrigerated plasma may alter the aPTT results in heparinized patients. Using these criteria, one is able to achieve a high level of reproducibility of the partial thromboplastin time test.

Recently, a 75-year-old woman recovering from a hip operation sustained a postoperative deep vein thrombosis. She was treated with intravenous heparin and several days later developed signs of a pulmonary embolus, suggesting that her thrombotic process had extended. On further review of the scans and on clinical symptoms, it was reasoned that the pulmonary embolus had been there all the time and, as she improved clinically on the heparin, the clot in the lungs began to dissolve and go more peripherally toward the pleural surface, producing transient symptoms. The issue here was making sure that she did not represent a failure of adequate anticoagulation, which may have necessitated the placement of a vena cava filter. On the night before her recurrent chest pain, she had a partial thromboplastin time of 90 seconds and her dose of heparin was lowered from 800 to 500 U/hr. When asked to see this patient, the referred physician did a coagulation profile including the CTEG analysis. An aPTT of 42 seconds with a normal TEG pattern was recorded and her heparin was increased to 800 U/hr. Four hours later, the tests were repeated. (They were

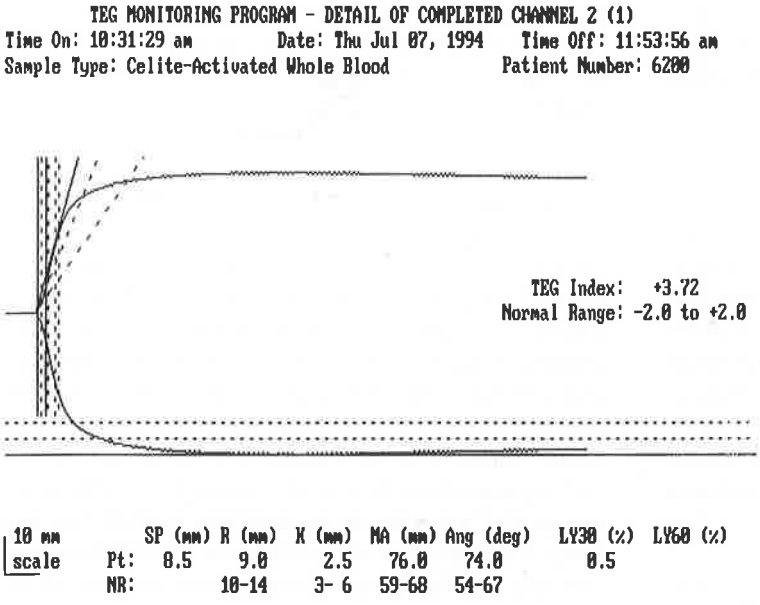

FIG. 3. Computerized thromboelastography.

also inadvertently reordered from the regular laboratory, which reported an aPTT of 90 seconds.) The result from the repeated tests was 60 seconds, with a normal CTEG pattern. Since the repeated tests had a normal CTEG graph, it was concluded that there was no contamination of the specimen and that 60 seconds was a valid test result. The patient eventually made a full recovery.

The reagents and the test methods for the partial thromboplastin time were the same in both laboratories. The result of 90 seconds from the regular laboratory suggested that specimen contamination with tissue juice occurred during the blood drawing, causing a very slight clotting of the plasma and producing a longer aPTT. The patient with thrombosis may receive an incorrect heparin dose unless this phenomenon is detected.

When investigating sick surgical patients with thromboelastography and the coagulation profile in 1969, we encountered approximately 1000 instances, over a period of time, in which differences in test results were seen between the two laboratories. A protocol was devel-

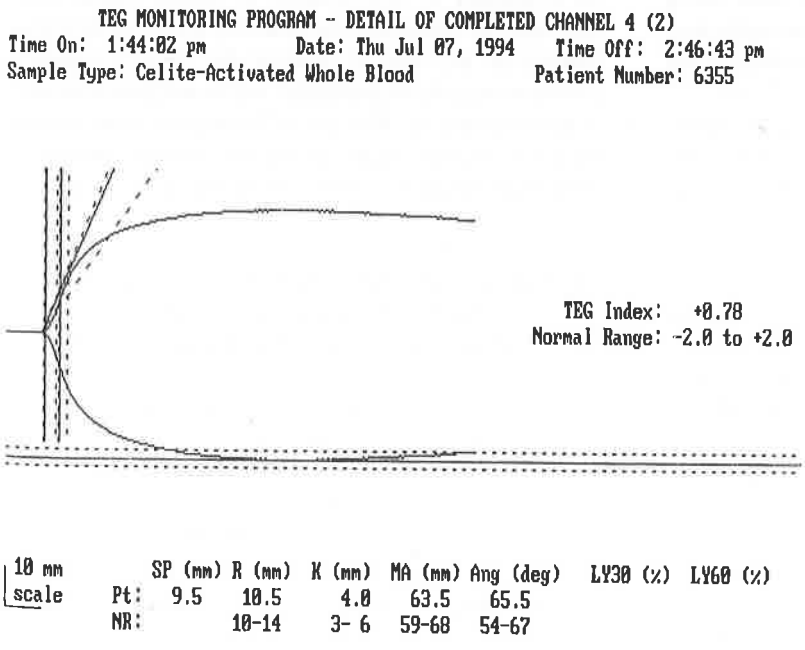

FIG. 4. Computerized thromboelastography.

oped to control and monitor all aspects of venipuncture and specimen handling. The number of errors was reduced, but the problem would not have been identified without CTEG.

Problems usually arise in very sick patients in which a prolonged aPTT may represent a poor drawing technique, excessive fibrinolysis, factor deficiency, disseminated intravascular coagulation (DIC), or other conditions. The TEG tracing is an additional tool in the evaluation of very critical hematologic situations, providing the clinician with more data for managing these complex problems. The thromboelastograph not only aids in the identification of specimen collection and handling problems, but the graphs may yield clues regarding the etiology of the hemostatic defects. Further double-blind studies should be done to characterize this process and minimize the possibility of venipuncture errors.

THROMBOELASTOGRAPHY AS AN ADJUNCT TO THE MANAGEMENT OF CARDIAC AND TRANSPLANT PATIENTS

The thromboelastograph provides a graph that represents a global pattern of the overall hemostasis and fibrinolysis processes. The overall effects of factor deficiencies, anticoagulants, and fibrinolytic drugs can be observed. Recently, this process has been expanded to operations in which the patient is fully heparinized, such as cardiopulmonary bypass, as described elsewhere in this issue of *Seminars*. Heparinase is added to the sample, and the global clotting characteristics in a given patient can be assessed during the pump run. Although in its infancy, this technology may predict those who are at risk for postoperative bleeding, as well as allow individual dose changes in heparin or protamine, depending on the patient's clotting characteristics.[4]

Limited experiments involving platelet-rich plasma infusions and the monitoring of hematologic patterns during and after bypass have been conducted. The factors that may predict bleeding during the operation or in the immediate postoperative period are being studied. In these circumstances, postoperatively the patients have shown a statistically significant increase in chest tube drainage in the first 24 to 48 hours or have been returned to the operating room for bleeding. The data are very preliminary, but it is believed that accelerated fibrinolysis, either alone or in conjunction with congestive heart failure, may result in excessive postoperative bleeding. If one can identify bleeding tendencies during cardiopulmonary bypass, then therapy can be instituted to minimize the risk of postoperative hemorrhage by neutralizing these factors so that reoperation is not necessary.[4] The TEG tracing may predict the likelihood of increased postoperative bleeding and possible reoperation for bleeding. See Spiess elsewhere in this issue for a more detailed analysis of TEG use in monitoring patients undergoing cardiac surgery.

When compared with normal MAs, narrow MAs on the TEG tracing correlate with statistically increased chest tube drainage. These effects have been linked to accelerated fibrinolysis and suppressed platelet function. These changes may be potentiated by existing conditions, including compensated DIC, or by factor defects seen in patients with advanced valvular disease associated with congestive heart failure. This area of investigation is exciting because the TEG can measure global, dynamic, ongoing characteristics of the hemostatic process both during cardiopulmonary bypass and in the presence of full heparinization. As a result, it may be possible to use therapeutic measures during and after surgery that may result in a reduced incidence of bleeding problems postoperatively, providing an increased understanding of the hemostatic responses during and immediately following extracorporeal cardiopulmonary bypass.[5]

Further research should include hemostatic studies in patients who are reoperated on for bleeding when a mechanical bleeder is found. Is it possible that altered hemostatic systems contributed to the premature dissolution of a hemostatic plug, which dissolved in several hours rather than gradually disappearing as part of the healing process. What is the role of factor XIII in maintaining the integrity of these hemostatic plugs? Finally, during liver transplantation, similar dynamic hemostatic events occur that are compounded by the anhepatic phase. The importance of global TEG measurements during this complex, dynamic operative procedure has been shown by a number of investigators to reduce overall blood loss and are described in detail by Kang in this issue. Physicians are able to identify and treat a variety of problems ranging from platelet and factor defects to accelerated fibrinolysis. The use of heparinase TEG methods may be of great value and provide further insight into hemostatic responses during liver transplant surgery.

POSTOPERATIVE HYPERCOAGULABILITY AND ITS RELATIONSHIP TO VENOUS THROMBOEMBOLISM

Considerable work has been done evaluating hemostatic systems in the postoperative patient, indicating that some patients develop defects in hemostasis, most notably in the direction of accelerated coagulability, which may be due to reduced or defective fibrinolysis. Authorities disagree on the definition of the hypercoagulable state. Hypercoagulability may represent an increased statistical probability of developing thrombosis that may or may

not be detectable, depending on the available screening methods of diagnosis. (Most postoperative thrombi are asymptomatic, and 70% of pulmonary emboli are never diagnosed or treated.) It would be reasonable to assume that a person with a defective fibrinolytic system postoperatively may be more likely to develop a clot than those without, but a clot does not occur in every single case. In many patients with cancer, thrombosis occurs following surgery and is a common cause of death in those with metastatic disease.[3] These cancer patients frequently have defects in their fibrinolytic system components. Thromboelastography has shown tumor-induced hypercoagulability in an experimental model of rat carcinoma that subsided after complete tumor excision.[6]

Over 25 years ago, Caprini et al. performed an experiment based on the theory that every person's blood has far more potential clotting activity than actually is needed for hemostasis. Patients with clotting factor deficiencies achieve competent hemostasis under most normal circumstances, despite factor levels far below the normal range. It was noted that native blood in thromboelastography exibited a much faster onset of coagulation, a faster rate of coagulation, and a greater clot strength when celite was added. Statistically significant differences between the two tracings were observed and used to develop an equation based on a discriminate analysis between normal and known cancer patients, the TEG index. Based on these studies, a normal range was between +2 and −2. Patients with +10 or above were frequently found to have cancer, thrombosis, or severe infection, and may have been more likely to develop thromboembolic problems. Prospective randomized trials with appropriate endpoints are necessary to test these postulates in the future.

During the past 8 years, we have assumed that individuals with a TEG index over +5 were more likely to develop a clot than those with the index in the normal range. On this basis, these patients were placed on subcutaneous heparin, twice a day, for 1 month postoperatively to normalize the TEG studies. Utilizing this protocol, we dramatically reduced the rate of leg thrombosis, without clinical or fatal pulmonary emboli, and less than 0.5% incidence of minor bleeding in approximately 500 patients.[7]

The TEG index is also useful for monitoring heparin. An index of -8 to -15 corresponds to an aPTT of twice control when the platelet count, platelet function, fibrinogen levels, and fibrinolytic activity are normal. When these conditions exist, the incidence of bleeding from heparin is less than 0.5%. The combination of a straight-line TEG result (absence of clotting) and an aPTT over 80 seconds is associated with an increased incidence of bleeding. Depending on the level of activity of the various hemostatic parameters, one may see different TEG patterns in patients with similar aPTT results. One example would be a 1.5× control aPTT along with a straight-line graph indicating no clotting activity in the thromboelastogram over 30 minutes. Usually, one could document another defect, such as a prolonged bleeding time or low platelet count to explain these findings. Under these circumstances, one may not increase heparin levels to obtain a twice control aPTT value. Another example would be finding a positive index in the presence of an aPTT twice control. This may be seen when the platelet count is high, fibrinogen levels are high, or hyperaggregable platelets are present. One may consider increasing heparin levels to produce a negative TEG index. These postulates must be tested in a prospective fashion. It would be attractive if one test could evaluate the quality of the blood drawing and simultaneously depict a global pattern of clotting that can be used to titer heparin therapy and avoid bleeding. It may also represent a cost-effective approach if complications can be avoided. This is our clinical impression based on more than 20 years' experience with celite-activated thromboelastography. Spending more "up front" for an improved measurement may produce a better long-term outcome and less cost than treating complications of therapy. Finally, using the TEG with other tests will not only help to improve its credibility, but will also provide the extra measure of laboratory data that helps to improve the clinical result.

THROMBOELASTOGRAPHY: A GUIDE TO THE CLINICAL AND RESEARCH USES OF FIBRINOLYTIC DRUGS

The use of modern fibrinolytic drugs has improved the treatment of certain thromboembolic situations; however, these drugs may be associated with serious side effects, including bleeding, or immunologic problems in the case of streptokinase. The dose of this drug may vary widely, depending on any previous exposure to streptococci, resulting in antibodies that will require a large drug dose to overcome. Utilizing a combination of selected drug doses with the patient's blood in the thromboelastogram, and, depending on the degree of lysis, one can calculate a dose that would provide a degree of systemic lytic activity without total afibrinogenemia. These results can be obtained in 15 to 30 minutes using the aforementioned global assay. Other drugs have also been used in this assay to predict the best approach in a given patient. The global clotting effects in patients receiving both heparin and lytic drugs can readily be evaluated using the thromboelastogram. This method improves clinical management, reduces the likelihood of bleeding and has been used to measure the effects of various fibrinolytic activators in an experimental clot model in the dog.[8] The applications of thromboelastography in experimental and clini-

cal situations involving fibrinolytic drugs are beginning to be explored. Summaria in another article in this issue provides an excellent summary of some previous experiments using thromboelastography. The global and dynamic properties of thromboelastography are attractive in quantitating the effects of fibrinolytic studies. Future research will be necessary to refine these capabilities for improved safety and effectiveness of these compounds. Outcome studies need to be done to demonstrate that the costs of this method are worth the investment in personnel and equipment over the long term.

CONCLUSION

Global evaluation of the dynamics of clot formation and dissolution using a modern computerized thromboelastograph may provide a new tool for improving clinical care in a cost-effective manner. An important clinical application of the TEG is the identification of a contaminated blood specimen due to venipuncture technique or processing of the sample. The differential diagnosis of a prolonged aPTT includes these artifacts, which are readily uncovered by comparing celite and native TEG tracings. This may require redrawing blood from the patient rather than reducing the heparin dose or giving replacement therapy when a prolonged aPTT is present. The cost-effectiveness of discovering a laboratory error by having a built-in quality control monitor is unique to celite-activated thromboelastography. Diagnosis of coagulation defects and a decrease in postoperative bleeding may be provided by using heparinase neutralized TEG tracings during cardiopulmonary bypass, in the presence of systemic heparinization. The thromboelastographic equation and index may identify subgroups of patients who are at increased risk for developing postoperative thromboembolic complications. Limited data have indicated that this technology may be of benefit in monitoring patients receiving fibrinolytic drugs. Finally, TEG monitoring during liver transplantation has minimized bleeding and transfusion requirements during these difficult and challenging operative procedures. It is hoped that future research will further exploit this dynamic global technology.

REFERENCES

1. Hartert H: Blutgerinnungsstudien mit der Thrombelastographie, einem neuen Untersuchungsverfahren. Klin Wochenschr 26:577–583, 1948.
2. Kang YG, DJ Martin, J Marquez, JH Lewis, FA Bontempo, BW Shaw, TE Starzl: Intraoperative changes in blood coagulation and thromboelastographic monitoring in liver transplantation. Anesth Analg 64:888–896, 1985.
3. Caprini JA, L Zuckerman, E Cohen, JP Vagher, V Lipp: The identification of accelerated coagulability. Thromb Res 9:167–179, 1976.
4. Tuman KJ, RJ McCarthy, M Djuric, V Rizzo, AD Ivankovich: Evaluation of coagulation during cardiopulmonary bypass with a heparinase-modified thromboelastographic assay. J Cardiothorac Vasc Anesth 8:144–149, 1994.
5. Mongan PD, MP Hosking: The role of desmopressin acetate in patients undergoing coronary artery bypass surgery. Anesthesiology 77:38–46, 1992.
6. Raina S, CR Spillert, SM Greenstein, EJ Lazaro: Effect of surgery on tumor-induced accelerated coagulation in a rat carcinoma. J Surg Res 38:138–142, 1985.
7. Caprini JA, JI Arcelus, M Laubach, G Size, KN Hoffman, RW Coats, S Blattner: Postoperative hypercoagulability and deep vein thrombosis after laparoscopic cholecystectomy. Surg Endosc 9: 304–309, 1995.
8. Summaria L, J Sandesara, G Yang, JP Vagher, JA Caprini: In vitro comparison of fibrinolytic activity of plasminogen activators using a thrombelastographic method. Thromb Haemostas 56:71–79, 1986.

Thromboelastography for the Assessment of Hypercoagulability During General Surgery

JUAN I. ARCELUS, M.D., Ph.D., CLARA I. TRAVERSO, M.D., Ph.D., and JOSEPH A. CAPRINI, M.D., F.A.C.S.

Thromboelastography (TEG) provides comprehensive and permanent graphic documentation of the overall clotting process, from the formation of the first fibrin strands to clot dissolution. Furthermore, TEG measures the viscoelastic properties of the blood clot and has the advantage of being objective and reproducible.[1–3]

Since its introduction in Europe in 1948,[4] TEG has proven to be a valuable tool for the study of bleeding disorders[5–7] and in the assessment of anticoagulant drugs.[8–10] Recently, North American anesthesiologists have been utilizing TEG for patients undergoing procedures with significant hemorrhagic potential, such as liver transplantation[11–14] and cardiopulmonary bypass surgery.[15–18] On the other hand, TEG has been shown to be very sensitive in the identification and measurement of hypercoagulability.[19–23]

This article reviews the use of TEG to assess hypercoagulability in general surgical patients, and as an aid to monitor heparin administered to prevent postoperative venous thromboembolism (VTE).

DETECTION OF POSTOPERATIVE HYPERCOAGULABILITY

During the postoperative period there is a transient hypercoagulable state that can be detected by different laboratory tests.[24–27] This activation of the hemostatic system is part of the normal response of the body to the surgical aggression, and it is aimed at preventing excessive bleeding. On the other hand, hypercoagulability represents a key factor in the pathogenesis of postoperative VTE.[28]

TEG has been used to document postoperative hypercoagulability in patients undergoing surgery.[21,27,29,30] Other clinical settings associated with hypercoagulability detected by TEG include cancer,[31,32] insertion of cut-down intravenous catheters,[33] and the administration of nonionic contrast media.[34]

Modifications of TEG parameters, described in detail in other articles of this issue of *Seminars*, indicative of hypercoagulability are: shortening of the reaction time (R) and the coagulation time (K) and an increase of the maximum amplitude (MA) and the angle of divergence. Other TEG parameters that increase during hypercoagulable states are the computerized index obtained from native and celite-activated whole blood[19,20] and the index of thrombodynamic potential (ITP).[6]

THROMBOELASTOGRAPHY RESPONSE AFTER OPEN ABDOMINAL SURGERY

Hypercoagulability, as detected by TEG, has been reported after hepatic surgery[21] and general abdominal surgery.[16,29,30] In 1988 we conducted a prospective study in a group of 30 patients undergoing general abdominal surgery who did not receive any anticoagulant drug as prophylaxis against VTE.[35] Using a two-syringe technique, blood was drawn from each subject and whole-blood TEG tracings were obtained using a three-channel thromboelastograph before surgery and on the third and seventh postoperative days. TEG tracings revealed a statistically significant shortening of the K compared with baseline values (Fig. 1). The reaction time did not reveal significant changes (Fig. 2). On the other hand, MA was

From the Department of Surgery, Hospital General de la Axarquía, Vélez-Málaga, Málaga, Spain (J.I.A.), Department of Surgery, Hospital Universitario, Granada, Spain (C.I.T.), and Department of Surgery, The Glenbrook Hospital, Glenview, Illinois, and Northwestern University Medical School, Chicago, Illinois.

Reprint requests: Dr. Arcelus, Departamento de Cirugía, Hospital General Básico, Finca el Tomillar s.n., 29700 Velez-Málaga, Spain.

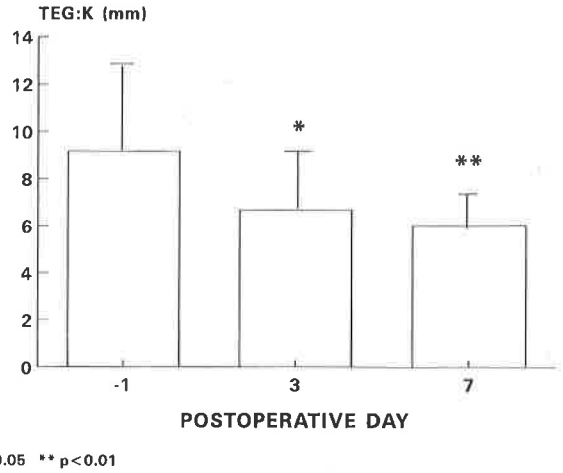

FIG. 1. Postoperative modifications of coagulation time (K) after general abdominal surgery. (Paired *t* test.)

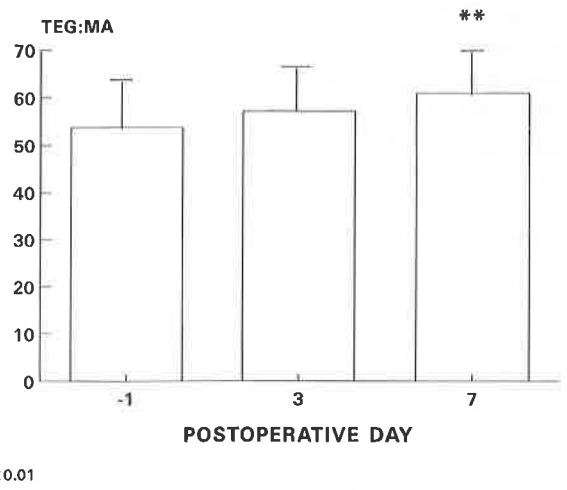

FIG. 3. Postoperative modifications of maximum amplitude (MA) (mm) after general abdominal surgery. (Paired *t* test.)

significantly increased on the seventh postoperative day (Fig. 3). These results revealed significant and progressive hypercoagulability, as detected by TEG performed on native whole blood, throughout the first postoperative week in patients undergoing general abdominal surgical procedures. Our results are similar to that of Butler[29] who also found a reduction of R and K, and an increase of MA. This author attributed the TEG changes to increasing fibrinogen concentrations.

Other investigators, analyzing plasma samples instead of whole blood, have reported postoperative shortenings of activated partial thromboplastin time (aPTT)[25] and recalcification time,[26] and an increase in fibrinogen.[25,29] Regarding platelets, it has been shown that there is an increase in the number of platelets and their aggregability during the postoperative period.[36]

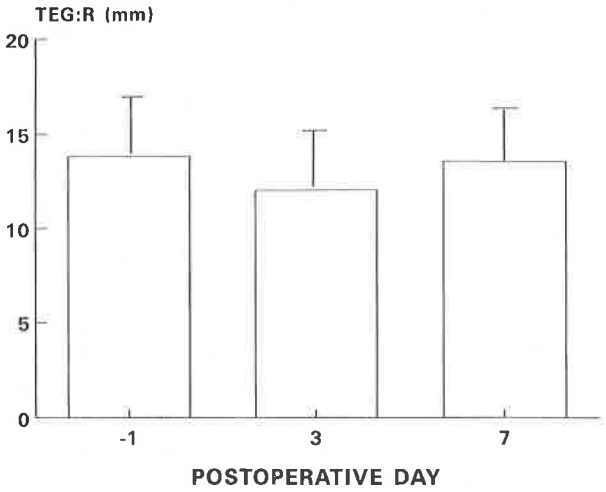

FIG. 2. Postoperative modifications of the thromboelastographic parameter of the reaction time (R) after general abdominal surgery. (Paired *t* test.)

THROMBOELASTOGRAPHY RESPONSE AFTER LAPAROSCOPIC SURGERY

Traditional cholecystectomy has been associated with an activation of blood coagulation.[26,37,38] Laparoscopic cholecystectomy does not require large surgical incisions and patients resume their normal activities soon after a short hospital stay.[39] Because of this, laparoscopic cholecystectomy is considered a minimally invasive surgical procedure. Nevertheless, there are several factors intrinsic to the procedure, such as the tilting of the patient in a reverse Trendelenburg position during the procedure and the creation of pneumoperitoneum. Both have been shown to induce venous dilation and stasis.[40–42]

We conducted a study to assess the hemostatic response to this operation and to conventional open cholecystectomy.[43] Our results indicate that laparoscopic cholecystectomy is associated with significant hypercoagulability on the first postoperative day, as detected by whole blood TEG and aPTT (Figs. 4 and 5). The results were similar in the group of patients undergoing open or converted cholecystectomy. This activation of blood coagulability in both groups probably reflects the organism's reaction to general anesthesia plus the aforementioned factors inducing venous stasis.

In this study we found statistically significant correlations between age and preoperative TEG index ($r = 0.29$; $p < 0.01$), MA ($r = 0.25$; $p < 0.01$), and aPTT ($r = 0.39$; $p < 0.001$). In addition, there were significant correlations between R and aPTT preoperatively ($r = 0.25$; $p < 0.01$) and postoperatively ($r = 0.35$;

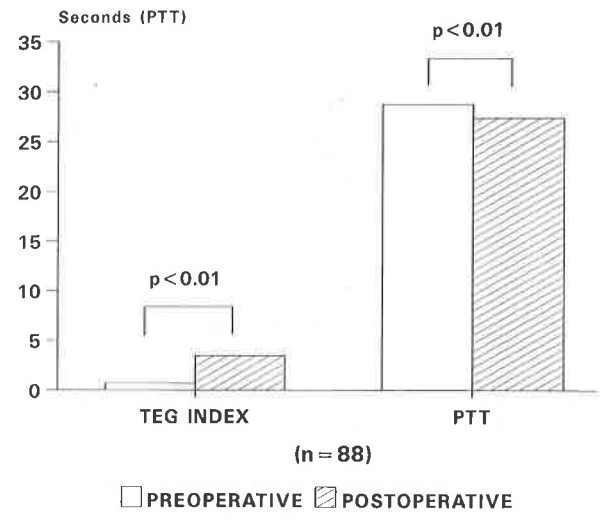

FIG. 4. Postoperative modifications of the thromboelastographic index (normal range, −2 to +2) and partial thromboplastin time in patients undergoing laparoscopic cholecystectomy. (Paired *t* test.)

$p < 0.001$). The parameter K was not correlated with aPTT or age either before or after surgery. There was also significant correlation between MA and the platelet number, both preoperatively ($r = 0.33$; $p < 0.01$) and postoperatively ($r = 0.32$; $p < 0.01$).

HYPERCOAGULABILITY AND PREDICTION OF VENOUS THROMBOEMBOLISM

The identification of hypercoagulability in surgical patients is important, since it represents a major risk factor for developing VTE. Chayen and coworkers[27] found that the TEG was able to predict postoperative VTE accurately in high-risk patients. Besides, Heather and coworkers[22] developed a thromboelastographic technique consisting of in vitro mixing of whole blood and normal saline solution to reduce the hematocrit to 75 to 85% of its initial value. This saline dilution test increases TEG sensitivity to hypercoagulable states and helps in the prediction of postoperative VTE. Another modification proposed by Rodzynek and associates[44] consists of mixing plasma of the patient under study with other control plasma whose coagulation parameters are known. This transfer test would be a valuable screening tool to detect hypercoagulable states and thrombosis.

In a recent study, we evaluated the role of preoperative decalcified whole blood TEG in the identification of patients at risk for developing VTE after elective abdominal surgery.[45] Our results reveal a significant higher value of preoperative MA in patients developing postoperative deep vein thrombosis compared with those who did not have thrombosis. We have developed a predictive index based on the preoperative values of MA after a discriminant analysis of the data from that study: thrombosis: $-56.09 + 1.79$ MA; no thrombosis: $-48.29 + 1.66$ MA.

By replacing the MA value of a given patient, the equation with the highest result will predict the odds of thrombosis. When MA = 60 mm, the odds are the same. Accordingly, when MA is more than 60, there is an increased risk of developing postoperative deep vein thrombosis. The specificity of this index is 72.2% and the sensitivity is 69%.

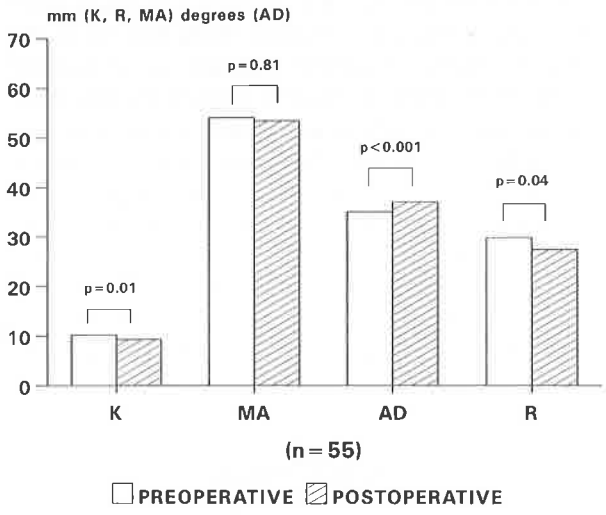

FIG. 5. Postoperative modifications of the different thromboelastographic parameters in patients undergoing laparoscopic cholecystectomy. (Paired *t* test.)

THROMBOELASTOGRAPHY AND PHARMACOLOGIC PREVENTION OF POSTOPERATIVE VENOUS THROMBOEMBOLISM

Heparin has become the current gold standard for pharmacologic prevention of postoperative VTE in general surgery. Recent meta-analyses of the literature have shown that heparin, given at low doses (10,000 to 15,000 U/24 h), is effective and safe.[46,47] Nevertheless, there is a potential risk for the development of hemorrhagic complications as a result of the anticoagulant action of heparin.[48–50] On the other hand, there is great interindividual variation in the response or tolerance of patients to the same dose of heparin.[51,52] This is especially true when this drug is given subcutaneously.[53,54] Gurewich and associates[52] have reported that 15% of the patients receiving subcutaneous low-dose heparin were hyperresponders, experiencing significant prolongations of the aPTT and a higher rate of bleeding complications. However, most investigators have reported minimal or no modifications of aPTT after a subcutaneous injection of 5000 U of

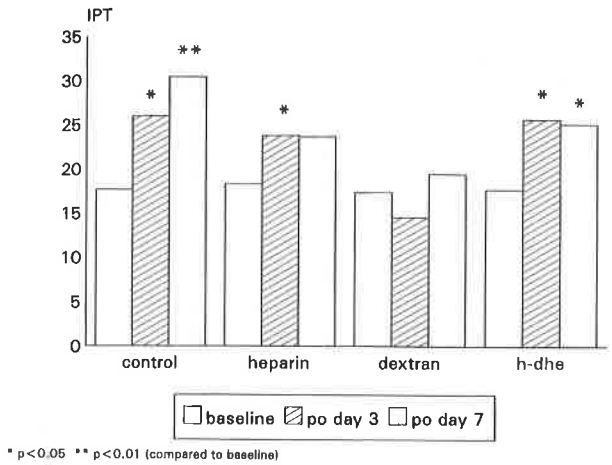

FIG. 6. Postoperative modifications of the index of thrombodynamic potential in patients undergoing general surgery and receiving different prophylaxis against venous thromboembolism. (Paired *t* test.)

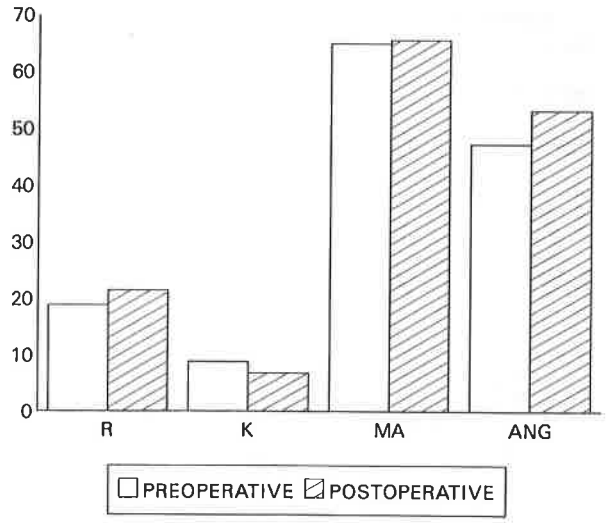

FIG. 7. Thromboelastographic parameters in patients operated on for gastrointestinal cancer and receiving, during the postoperative period, 7500 U of Fraxiparin as prophylaxis for venous thromboembolism.

heparin.[25,55] Similarly, low-dose heparin does not prolong bleeding time[52] or prothrombin time.[25]

We conducted a study to assess the postoperative thromboelastographic response in general surgical patients receiving low-dose heparin, dextran 70, and heparin-dihydroergotamine.[56] This study also included a group of patients who did not receive any pharmacologic agent as prophylaxis against VTE. The results of this study, regarding the modifications of the ITP, are summarized in Figure 6. Low-dose heparin at fixed doses (5000 U/12 h) did not neutralize early postoperative hypercoagulability, since IPT increased significantly on the third postoperative day. However, heparin did control the progressive hypercoagulable trend observed in the control group throughout the first postoperative week. Similar results were found with heparin-dihydroergotamine. On the other hand, dextran achieved a slight reduction of ITP on the third postoperative day. Similar results have been reported by other investigators using low-dose heparin[57,58] and dextran.[59]

Our current practice in high-risk general surgical patients consists of adjusting or "tailoring" the doses of subcutaneous heparin, based on preoperative and serial postoperative TEG performed, on alternate days, 4 hours after the heparin injection. We target the TEG index, described by Caprini and associates,[19] to a normocoagulable range (−2 to +2). With this approach, the rates of VTE in our surgical patients has been negligible. Furthermore, we have not seen significant bleeding complications as serial TEG monitoring avoids inducing significant hypocoagulability.[60]

In recent years, low molecular weight heparin fractions have become the new standard for VTE prophylaxis in many European countries. These heparin fractions have the advantage over conventional heparin of having a better bioavailability with at least the same prophylactic efficacy and a theoretical lower risk of bleeding complications.[61] However, there is a problem when monitoring these agents, since conventional coagulation tests, such as aPTT, are not reliable.[62] We are conducting some studies to assess the sensitivity of TEG to two of these heparins (Fraxiparin and Fragmin). Our preliminary results reveal that TEG is able to monitor reliably the effect of different doses of these agents on blood coagulation. Figure 7 shows the average TEG parameters in a group of 12 surgical patients with gastrointestinal malignancies receiving 7500 anti-Xa units (Institute Choay) of Fraxiparin. The preoperative baseline TEG parameters evidence the hypercoagulability typically associated with cancer. On the fourth postoperative day, blood was drawn 4 hours after the subcutaneous injection of Fraxiparin as described before. Our TEG results indicate that low molecular weight heparin is able to control the trend to develop further postoperative hypercoagulability, since the differences between the baseline and postoperative TEG parameters are less dramatic than those shown in Figures 1 to 3 when no prophylaxis is given. Nevertheless, some patients required higher doses of Fraxiparin to prevent significant hypercoagulability.

CONCLUSION

TEG provides a rapid and objective measure of the dynamic changes in viscoelastic properties of blood from

the formation of the first fibrin strands to the dissolution of the clot. This test also identifies hypercoagulable states associated with the postoperative period. Moreover, TEG is also very sensitive to the effect of heparin and other anticoagulants on hemostasis. This allows for a reliable monitoring and dose adjustment of these drugs to improve their prophylactic effect without increasing the risk of bleeding.

REFERENCES

1. De Nicola P, GM Mazzetti: Evaluation of thrombelastography. Am J Clin Pathol 25:447–452, 1955.
2. Elliot RL, K von Kaulla, SG Blount: Thrombelastographic studies with a twenty-four hour schedule for subcutaneous heparin. Circulation 24:1206–1214, 1976.
3. Lee BY, FS Trainor, WR Thoden, D Kavner: Monitoring coagulation dynamics: thrombelastography. *In:* Lee BY, FS Trainor, WR Thoden, D Kavner (Eds): *Handbook of Noninvasive Diagnostic Techniques in Vascular Surgery.* Appleton-Century-Crofts, New York: 1981:pp 266–286.
4. Hartert H: Blutgerinnungsstudien mit der Thrombelastographie, einem neuen Untersuchungsverfahren. Klin Wochenschr 26:577–583, 1948.
5. Marchal G, ME Leroux, M Samama: Thrombodynamographie (Thrombelastographie). Sa place actuelle en clinique. Presse Med 68:1919–1922, 1960.
6. Raby C: *Biologie des hemorragies et des thromboses.* Masson, Paris, 1966.
7. Franz RC, WJ Coetzee: The thrombelastographic diagnosis of hemostatic defects. Surg Annual 13:75–107, 1981.
8. Bloom WL, S Brewer: The independent, yet synergistic effect of heparin and dextran. Acta Chir Scand 387:53–57, 1968.
9. Lee BY, FS Trainor, D Kaynar, WJ McCann: Monitoring heparin therapy. Surg Gynecol Obstet 149:843–847, 1979.
10. De Gaetano G, J Vermylen: Effect of aspirin on the thromboelastogram of human blood. Thromb Diath Haemorrh 30:494–498, 1973.
11. Howland WS, GM Ryan, RE Bettigole, JG Fortner: Coagulation abnormalities associated with liver transplantation. Surgery 68:591–596, 1970.
12. Kang YG, DJ Martin, J Marquez, JH Lewis, FA Bontempo, BW Shaw, PM Winter: Intraoperative changes in blood coagulation and thrombelastographic monitoring in liver transplantation. Anesth Anal 64:888–896, 1985.
13. Owen C, SR Rettke, EJW Bowie, TL Cole, CC Jensen, RH Wiesner, RAF Krom: Hemostatic evaluation of patients undergoing liver transplantation. Mayo Clin Proc 62:761–772, 1987.
14. Chapin JW, GL Becker, BJ Hurlbert, MC Newland, DJ Cuka, RP Wood, BW Shaw: Comparison of thromboelastogram and sonoclot coagulation analyzer for assessing coagulation status during orthotopic liver transplantation. Transplant Proc 21:3539, 1989.
15. Spiess BD, KJ Tuman, RJ McCarthy, GA DeLaria, R Schillo, AD Ivankovich: Thrombelastography as an indicator of post-cardiopulmonary bypass coagulopathies. J Clin Monit 3:25–30, 1987.
16. Tuman KJ, BD Spiess, RJ McCarthy, AD Ivankovich: Comparison of viscoelastic measures of coagulation after cardiopulmonary bypass. Anesth Analg 69:69–75, 1989.
17. Martin P, F Horkay, SM Rajah: Monitoring of coagulation status using thrombelastography during pediatric open heart surgery. Int J Clin Monit Comput 8:183–187, 1991.
18. Mongan PD, MP Hosking: The role of desmopressin acetate in patients undergoing coronary artery bypass surgery. Anesthesiology 77:38–46, 1992.
19. Caprini JA, L Zuckerman, E Cohen, JP Vagher, V Lipp: The identification of accelerated coagulability. Thromb Res 9:167–180, 1976.
20. Cohen E, JP Caprini, L Zuckerman, P Vagher, B Robinson: Evaluation of three methods used to identify accelerated coagulability. Thromb Res 10:587–604, 1977.
21. Howland WS, EB Castro, JB Fortner, RN Gould: Hypercoagulability, thrombelastographic monitoring during extensive hepatic surgery. Arch Surg 108:605–608, 1974.
22. Heather BP, SA Jennings, RM Greenhalgh: The saline dilution test—a preoperative predictor of DVT. Br J Surg 67:63–65, 1980.
23. Moriwaki K, N Sato, M Kubota, T Maekawa, M Nomura, H Sasaki, K Nakatani, O Yuge: Thromboelastography as a bedside monitor of coagulation and fibrinolysis during surgery—a report of three cases. Masui 41:1145–1150, 1992.
24. Sharnoff JG, HH Kass, BA Mistica: A plan of heparinization of the surgical patients to prevent postoperative thromboembolism. Surg Gynecol Obstet 115:75–80, 1962.
25. Hedlund PO, M Blombäck: The effect of prophylaxis with low dose heparin on blood coagulation parameters. A double blind study in connection with transvesical prostatectomy. Thromb Haemostas 41:337–344, 1979.
26. Lundqvist PB, J Swedenborg: Postoperative hypercoagulability, detection and measurement using a modified recalcification time system, effects of low dose heparin and types of surgery. (Abst.) Thromb Hemostas 46:16, 1981.
27. Chayen D, SD Blair, CN McCollum, RM Greenhalgh: Prediction of postoperative DVT by saline dilution. (Abst.) Thromb Hemostas 58:384, 1987.
28. Wu KK: Thrombogenesis, atherogenesis, and hypercoagulability. *In:* Wu KK (Ed.): *Pathophysiology and Management of Thromboembolic Disorders.* APG, Littleton, MA 1984: pp 5–18.
29. Butler MJ: Thromboelastography during and after elective abdominal surgery. Thromb Hemostas 39:488–495, 1978.
30. Blair SD, S Menashi, D Samson, RM Greenhalgh: Can the hypercoagulability of surgery be measured? Br J Surg 73:500, 1986.
31. Zuckerman L, E Cohen, JP Vagher, E Woodward, JA Caprini: Comparison of thrombelastography with common coagulation tests. Thromb Haemostas 46:752–756, 1981.
32. Raina S, CR Spillert, SM Greenstein, EJ Lazaro: Effect of surgery on tumor-induced accelerated coagulation in a rat carcinoma. J Surg Res 38:138–142, 1985.
33. Spillert CR, PJ McGovern, RM Vidaver, EJ Lazaro: Thrombelastographic evaluation of cut-down induced accelerated coagulation. (Letter.) Thromb Hemostas 46:567, 1981.
34. Parvez Z, R Moncada, J Fareed, HL Messmore: Effect of non-ionic contrast media on the components of coagulation and complement systems. Invest Radiol 18:279–284, 1983.
35. Arcelus JI, CI Traverso, M Lopez-Cantarero, JM García, F Navarro, E Gómez, F Bravo: Estudio de la modificaciones tromboelastograficas inducidas por la heparinizacion profilactica en cirugia general. Cir Esp 43:518–526, 1988.
36. Negus D, DJ Pinto, WW Slack: Effects of small doses of heparin on platelet adhesiveness and lipoprotein-lipase activity before and after surgery. Lancet 1:1202–1204, 1971.
37. Sandset PM, HE Hogevold, T Lyberg, TR Andersson, U Abildgaard: Extrinsic pathway inhibitor in elective surgery: A comparison with other coagulation inhibitors. Thromb Haemostas 62:865–860, 1989.
38. Kemen M, H Sperling, A Mumme, V Zumtobel: The thrombin-antithrombin III complex ELISA test in diagnosis of surgery-induced blood coagulation activation. Infusionstherapie 18:199–201, 1991.

39. National Institutes of Health Consensus Conference. Gallstones and laparoscopic cholecystectomy. JAMA 269:1018–1024, 1993.
40. Arcelus JI, JA Caprini, CI Traverso, G Size, JH Hasty: The role of elastic compression stockings in prevention of venous dilatation induced by reverse Trendelenburg position. Phlebology 8:11–115, 1993.
41. Millard JA, BB Hill, P Cook, ME Fenoglio: Intermittent sequential pneumatic compression of the legs in the prevention of venous stasis associated with pneumoperitoneum. 100th Scientific Session of the Western Surgical Association 1992; San Antonio, Texas. 32.
42. Beebe DS, MP McNevin, KJ Belani, JG Letourneau, MR Crain, RL Goodale: Evidence of venous stasis after abdominal insufflation for laparoscopic cholecystectomy. Anesthesiology 77 (3A):148, 1992.
43. Caprini JA, JI Arcelus, CI Traverso, D Vittum: Hypercoagulability after laparoscopic cholecystectomy. Thromb Haemostas 65:1347, 1991.
44. Rodzynek JJ, P Leautaud, T Martin, P Schoenfeld, P Wettendorf, A Delcourt: The transfer test: A new screening procedure for thrombotic diseases. J Surg Res 35:227–233, 1983.
45. Traverso CI, JI Arcelus, E Gomez, JD Luna, M Lopez-Cantarero, JM Garcia: Prospective assessment of the risk of deep vein thrombosis in elective abdominal surgery. Predictive role of thrombelastography. Thromb Haemorrh Disord 7:9–15, 1993.
46. Collins R, A Srimgeur, S Yusuf, R Peto: Reduction in fatal pulmonary embolism and venous thrombosis by perioperative administration of subcutaneous heparin. N Engl J Med 318:1162–1173, 1988.
47. Clagett GP, JS Reisch: Prevention of venous thromboembolism in general surgical patients. Ann Surg 208:227–240, 1988.
48. Gallus AS, J Hirsh, SE O'Brien, JA McBride, RJ Tuttle, M Gent: Prevention of venous thrombosis with small, subcutaneous doses of heparin. JAMA 235:1980–1982, 1976.
49. Pachter L, T Riles: Low dose heparin: Bleeding and wound complications in the surgical patients. Ann Surg 186:660–674, 1977.
50. van Ooijen B: Subcutaneous heparin and postoperative wound hematomas. Arch Surg 121:937–940, 1986.
51. De Takats G: Heparin tolerance revisited. Surgery 70:318–322, 1971.
52. Gurewich V, T Nunn, TT Kuriakose, M Hume: Hemostatic effects of uniform, low-dose heparin in surgical patients. Arch Intern Med 138:41–44, 1978.
53. Cooke ED, MJ Lloyd, S Bowcock: Monitoring during low dose heparin. N Engl J Med 294:1066–1069, 1976.
54. Nicolaides AN, T Harbourne, RCG Russell: Subcutaneous heparin, plasma heparin levels and postoperative wound haematoma. Br J Surg 71:62–64, 1984.
55. Lahnborg G, K Bergstrom: Clinical and hemostatic parameters related to thromboembolism and low-dose heparin prophylaxis in major surgery. Acta Chir Scand 141:590–595, 1975.
56. Arcelus JI, CI Traverso: Repercusiones tromboelastograficas inducidas por la heparina-dihidroergotamina utilizada como profilaxis de la enfermedad tromboembolica venosa. Actual Med 75:141–148, 1989.
57. Sagar S, J Massey, JM Sanderson: Low-dose heparin prophylaxis against fatal pulmonary embolism. Br Med J 4:257–263, 1975.
58. Vernese NA, CR Spillert, EJ Lazaro: Thromboelastographic evaluation of mini-dose heparin in minor surgical procedures. Am Surg 52:112–113, 1986.
59. Vara-Thorbeck R, M Lopez, JA Guerrero: Modificaciones tromboelastograficas inducidas por la hemodilucion con dextrano en cirugia de la cadera. Cir Esp 40:1061–1069, 1986.
60. Caprini JA, RA Natonson: Postoperative deep vein thrombosis: Current clinical considerations. Semin Thromb Hemost 15:8–13, 1989.
61. Nurmohamed MT, FR Rosendaal, HR Büller, E Dekker, DW Hommes, JP Vandenhroucke, E Briët: Low-molecular-weight heparin versus standard heparin in general and orthopedic surgery: a meta-analysis. Lancet 340:152–156, 1992.
62. Doutremepuich C, MC Lalanne, M Guyot, F Doutremepuich, O De Seze, F Toulemonde: Laboratory control of heparin and heparin fragment treatment: an experimental study. Semin Thromb Hemost 15:373–377, 1989.

Thromboelastography and Cardiopulmonary Bypass

BRUCE D. SPIESS, M.D.

A surgeon may from time to time place a clot between his or her thumb and forefingers and rub it back and forth. By doing so, the surgeon is testing the tensile strength of the clot or the shear elastic modulus of that clot. For many years, such qualitative assessments have provided "clinical" data to the practitioners regarding clot strength. Knowledge of clot elasticity has some very unique advantages in the operating room. Four fundamental questions can be asked about clot viscoelastic strength: (1) How fast does the clot begin forming? (2) how rapidly does clot growth occur? (3) what is the maximum strength of the clot? and (4) is that strength maintained or does the clot undergo early lysis? If all four questions can be answered that the clot in question behaves normally, then there is no chance that the patient will hemorrhage due to a coagulopathy.

The thromboelastograph (TEG) provides a quantitative measurement of the four questions just asked and provides a scientific measurement for what the surgeon is attempting to feel in the clot. The application of the TEG to cardiopulmonary bypass (CPB) patients has evolved from experience with liver transplantation. Arguably, one can say that the cases undergoing the largest shifts in coagulation function during routine surgery occur in liver transplants and CPB. The coagulopathies seen in CPB have been extensively investigated and as yet are still not fully characterized.[1,2] It is known that platelet activation and consumption, in conjunction with near universal fibrinolysis, are major contributors to the CPB coagulopathy.[3,4] The routine use of large dose heparin and protamine sulfate for anticoagulation and reversal create an imperfect ("un-normal") system for patients on bypass. Length of bypass run, type of oxygenator, temperature, volume of prime, as well as multiple other factors, all add to a complex picture of coagulation dysfunction. The presently available coagulation monitoring technologies remain inadequate. The activated clotting time (ACT) has become the standard for monitoring heparin and reversal, but it has proven ineffective in predicting postoperative hemorrhage. Tests of the routine coagulation profile have not proven to be uniquely useful after bypass, as many of the activators used in the test systems are interfered with by the heparin-protamine complex. As a result, transfusion therapy varies widely among institutions. There has not been a universally accepted method of following coagulation therapy and transfusion of coagulation product efficacy. A coagulation monitoring gap exists in the CPB operating rooms.

PERIOPERATIVE HEMORRHAGE CLINICAL STUDIES

Much of the work to date with the TEG and CPB has focused on this technology's ability to predict perioperative hemorrhage. Two independent studies, at the same institution, examined predicted accuracy of the TEG for abnormal chest tube bleeding postoperatively.[5,6]

First Study

The first study of 37 patients compared routine coagulation screening tests (prothrombin time [PT], activated partial thromboplastin time [aPTT], fibrinogen, platelet count, and fibrin(ogen) split products [FSP]), ACT and the TEG (reaction and coagulation times, [R and K] angle, of divergence, maximum amplitude [MA], and A60) at baseline before bypass, and 10 to 20 minutes after protamine was administered.

Correlations showed statistically significant, although poor correlations, between the TEG R value and

From the Division of Cardiothoracic Anesthesia, University of Washington, Seattle, Washington.

Reprint requests: Dr. Spiess, Associate Professor, Chief of the Division of Cardiothoracic Anesthesia, University of Washington, Seattle, WA 98195.

the ACT and the aPTT before bypass. Also, the TEG MA and the platelet count before bypass had weak but significant correlations. It appears reasonable that the correlated measures of the onset of coagulation would have some relationship, as they all measure in some way the activity of the intrinsic cascade. However, the TEG as performed for this study was not activated. Furthermore, coagulation did not use the ACT and the aPTT. The MA of the TEG also may be expected to have some correlation with the platelet count as total clot strength is dependent on the platelet fibrin interaction.

The correlations noted before bypass did not exist after termination of CPB and administration of protamine. In retrospect, that appears logical as the TEG measures the interdependent parameters assessing interaction of the platelets and procoagulant proteins. The coagulation screening tests examine independent parameters. Platelet function is greatly changed in CPB, so there is no reason why platelet count after CPB should correlate with a functional test (TEG). The PT and aPTT have a number of problems with their activators and high levels of circulating heparin-protamine complex can inhibit these activators. Indeed, it would be quite surprising if the same weak correlations that existed before bypass followed through after bypass.

The data for coagulation testing were blindly and independently divided into two categories (either normal or abnormal) as were the first 8 hours of chest tube output. Percentages of agreement were examined and reported as reflecting the predictive accuracy of an individual test for abnormal hemorrhage. Chest tube outputs were classified as excessive if they exceeded 7 cc/kg for the first 8 hours. The TEG as an entire test with its multiple interdependent parameters had the best agreement with normal or excessive chest tube drainage (87%). The routine coagulation tests were approximately 50% accurate in prediction (Table 1). Of more importance, the TEG had no false-positive results, whereas the coagulation screens had a large number of false-positive results. That would be significant if clinicians were guiding their transfusion replacement therapy based on the coagulation screening tests. In today's climate of blood product conservation due to viral transmission, the use of tests with a high degree of false-positive results could have implications for public health.

Second Study

The results of this first study were quite encouraging. Another whole blood viscosity test, the Sonoclot (Sienco, Morrison, CO), was also available, and therefore a second protocol was undertaken in 42 patients at high risk for postoperative hemorrhage.[5] Patients included were those undergoing reoperation, valve replacement, ventricular or aortic arch aneurysm resection, or complex cardiac procedures. Blood samples were drawn at similar times as in the first study. Abnormal chest tube drainage for this study was defined as greater than 150 cc/h for 2 hours or 300 cc/h for any 1 hour in the first 8 hours.

The predictive accuracy showed TEG to be 88% in agreement with the abnormal bleeders and the Sonoclot was not statistically different (74% accurate). Routine coagulation screen tests, again either in individual instances or grouped together, were unsatisfactory (Table 1). It is interesting that in both studies 2 years apart TEG accuracy was the same (87 and 88%).

Both whole blood viscoelastic tests did show individual parameters that changed in the group of patients with abnormal bleeding compared with those patients with normal blood loss. The MA of the TEG was below 40 mm in the abnormal hemorrhage group, whereas in the nonbleeders it was 49.9. That variance represents a decremental doubling of clot strength in the group that did not bleed. It should be pointed out that patients in both studies (n = 79) had normal coagulation data preoperatively and had not received platelet-inhibiting compounds within the 7 days prior to surgery. None of the 79 patients included in data analysis had to return to the

TABLE 1. Accuracy of Predicting Hemorrhage from Abnormal Results After Cardiopulmonary Bypass

Coagulation Monitor	*Overall Accuracy (%)*	*False Negative (%)*	*False Positive (%)*
Study 1*			
Thromboelastography (TEG)	87		
Activated clotting time	30		
Coagulation screen	51		
Study 2†			
TEG	88	0	15
Sonoclot	74	0	33
Coagulation screen	33	44	73

* Data from Spiess et al[6] in 37 patients undergoing coronary artery bypass grafting.
† Data from Tuman et al[5] in 42 high-risk patients undergoing cardiopulmonary bypass.

operating rooms for mediastinal reexploration. In the first study one patient who was returned to the operating room for bleeding had a normal TEG and a bleeding site was found at the anastomosis of the vein graft to the aorta.

Pediatric Study

Data from pediatric cardiac surgery report 100% agreement between abnormal TEG and excessive bleeding.[7] This study examined 22 patients undergoing a number of congenital repairs. It also noted that the TEG had a 73% specificity for abnormal hemorrhage with some false-positive results. These authors note that the MA after CPB was the best indicator of abnormal bleeding, although some change in the coagulation time value was also seen.

Other Studies

A recent publication in the surgical literature represents the only negative study to date.[8] One hundred and one patients undergoing all types of operation with CPB underwent both coagulation screen and TEG analysis. Seventeen patients developed abnormal chest tube hemorrhage and five had to be returned to the operating room. This study attempted to make correlations between the chest tube outputs in real numbers with the values for the individual TEG parameters as well as the coagulation screen numbers. That is in contrast to the binary analysis technique utilized in our two reported studies.[5,6] There may be little correlation between actual chest tube output and each of the TEG parameters rather than the TEG test as a whole.

The best correlation for all coagulation testing and overall chest tube output was between the platelet count and the MA. However, the chest tube drainage data may have some inaccuracies. Patients with surgical bleeding may not have been excluded from this analysis; they would have very high blood loss outputs with presumably normal coagulation. The study does not analyze the data of subgroups (surgical versus generalized groups) who had abnormal hemorrhage in the same manner (that is, correlations of output and TEG parameters). They do note that the patients with surgical bleeding all had normal TEGs (accuracy, 100%). Those whose bleeding cause was not explained had an almost equal number of normal and abnormal TEGs. If these surgical bleeders were not explored and eventually tamponaded, there would be no way to know without reexploring every patient. To assume that a patient with bleeding and a normal TEG does not have reason to be reexplored introduces a bias that skews all the later analysis.

The authors also note, in contrast to all other published work, that there were no correlations before bypass between the TEG data and the coagulation screen data. On closer examination, the ACT mean values of this patient population were quite prolonged at 156 seconds and there was a significant, almost 50%, incidence of fibrinolysis.[8] These two facts suggest that the patients studied may have had a large amount of preoperative heparinization as well as fibrinolytic therapy. Unfortunately, the article does not give any demographics to explain this discrepancy. Such drug therapy, specifically avoided in all other studies, would have a major effect on platelet function and thereby alter the relationship between the whole blood clot testing of TEG and the routine coagulation screening tests.

After bypass, a significant percentage of fibrinolysis and hypercoagulability was found. The incidence of hypercoagulability had no relationship to a zero incidence preoperatively, suggesting two points: patients before bypass were receiving heparin or fibrinolytic agents or both; or after CPB, large amounts of coagulation products were transfused. This article does not list criteria for transfusion, demographics, or timing of coagulation products used; thus, no relationship between such transfusions and the coagulation data can be drawn. Certainly, use of fresh-frozen plasma, platelets, and cryoprecipitate would influence chest tube output but not a TEG drawn earlier. The entire study therefore represents a patient series with few controls and a number of problems. Although it stands alone as a negative study, it should be looked at with the understanding that it was not a controlled study.

CLINICAL ADVANTAGES

Recent work as yet unpublished examining 43 patients undergoing coronary artery bypass grafting who had no prior coagulation abnormalities utilized TEG as well as other tests of coagulation.[9] Unlike prior research, these patients donated platelet-rich plasma (PRP) prior to CPB and randomly received their autologous PRP either prior to CPB, just after protamine, or on arrival in the intensive care unit (ICU). Although the findings were not supportive of PRP usage in saving chest tube bleeding or blood product utilization, there were significant coagulation testing changes in platelet count and more significantly in the MA value. A continuous multiple regression analysis of TEG parameters found that TEG parameters plus age were an excellent predictor of chest tube output (correlation coefficient, 0.82). Furthermore, a similar regression analysis showed correlation with blood product administration (coefficient, 0.74). These authors concluded that "TEG is the most powerful predictor of bleeding and blood product utilization following CPB." Other researchers published results comparing TEG changes with PRP salvage but have had mixed results.[10–12]

TABLE 2. Impact of Thromboelastographic Management on Blood Utilization, Donor Exposure, and Reoperation Rates

	Before TEG (n = 488)*	*With TEG (n = 591)*	*Significance (p Value)*
Patients receiving any transfusion (%)	86.3	78.5	0.001
Patients receiving no transfusion in operating room (%)	33.8	42.1	0.005
Red cells (%)	83.2	73.9	0.0001
Platelets (%)	59.2	48.2	0.0001
Plasma (%)	36.1	26.4	0.001
Total donor exposure (mean ± SD)	8 (15)	6 (11)	0.0001
Red cells (mean ± SD)	4 (4)	2 (5)	0.0001
Platelets (mean ± SD)	0.4 (8)	0 (6)	0.0001

* TEG: thromboelastography.

Unpublished retrospective data from our own institution have shown an impact of TEG monitoring on blood utilization. In a retrospective analysis of all CPB patients for 6 months prior to the TEG system installation and after its commencement, the use of red cells, fresh-frozen plasma, and platelet concentrates was all reduced (Table 2). However, chest tube output did not change. Demographically, the groups were not different; other major factors changed in the period of time, but the attending anesthesia and surgical staffs were unchanged.

Most impressive were changes in the rate of mediastinal reexploration (Table 3). Surgeons and house staff began utilizing the TEG to assess coagulopathy rapidly prior to bringing patients back to the operating room. If a normal TEG tracing existed and abnormal chest tube output existed, that patient would be reexplored immediately. However, if the TEG was hypocoagulable, attempts would be made to normalize it unless a patient became unstable. The reduction in mediastinal exploration rates was significant and can be further translated into reductions in morbidity, perhaps mortality, and certainly cost.

Recent work utilizing a mathematical formula to create a TEG index has shown that the TEG is predictive of abnormal hemorrhage.[13] The TEG index utilizes multipliers of the values for each TEG parameter from whole blood and blood stimulated with 1% celite. The formula utilized has the multipliers derived from prior discriminate analysis evaluating the greatest impact on TEG function. The end result of using such a formula is that the five interdependent parameters are shrunk to a single digital value:

$$\begin{aligned}\text{TEG index} = {} & +3.0903 + \text{R}\,(0.2662) + \text{K}\,(-0.9469) \\ & + \text{MA}\,(-.0499) + \alpha(-0.0933) \\ & + \text{RC}\,(-.367) + \text{KC}\,(0.865) \\ & + \text{MAC}\,(0.0199) + \alpha\text{c}\,(0.1483)\end{aligned}$$

where R is the reaction time, K is the coagulation time, and C is the TEG parameters from TEG with 1% celite activation.

Note that this equation for the TEG index is for samples citrated and recalcified. That single digital value, presented as either a positive or negative number, represents the tendency to be more or less hypercoagulable or hypocoagulable. Normal values exist on a continuum between −2 to +2.

TABLE 3. Reoperation Rate of Patients at the University of Washington Before and After Thromboelastography Installation

	Before TEG (n = 488)*	*With TEG (n = 591)*	*Significance (p Value)*
Overall (%)	5.7	1.5	0.0001
Coronary artery bypass grafting (%)	4.5	1.4	0.007
Valves and complex cases (%)	9.0	2.0	0.009

* TEG: thromboelastography.

CARDIOPULMONARY BYPASS

The mean TEG index before bypass was hypercoagulable (3.22) which became more hypocoagulable after bypass (−5.13). That would be expected with the coagulation changes, particularly platelet dysfunction previously discussed. The TEG index mean slowly improved over the next 24 hours. Correlations between TEG index and chest tube output were highly significant at 4 hours after entrance to the ICU (correlation coefficient, 0.85; $p = 0.003$). Also, the subgroups of patients with the most and least chest tube drainage (greatest and least, 25%) had a strong trend toward different TEG indexes ($p = 0.090$). The TEG index has not been used clinically yet, but perhaps it can be a prediction of those patients (roughly, 15 to 20%) with a truly abnormal hemorrhagic risk. No patient after CPB was profoundly hypercoagulable by this monitoring technique; however, further study

TABLE 4. Significant Relationships with Thromboelastography Analysis

1. Preoperative activated partial thromboplastin time prolonged and before bypass thromboelastography (TEG) hypocoagulable due to anticoagulation/factor deficiency, $p \leq 0.02$
2. Preoperative and postoperative activated clotting time and TEG hypocoagulable due to anticoagulation/factor deficiency, $p \leq 0.0001$
3. Preoperative heparin therapy and TEG hypocoagulable due to anticoagulation/factor deficiency, $p \leq 0.01$
4. Calcium channel blocker utilization and TEG postbypass platelet–fibrin interaction abnormality, $p \leq 0.04$
5. Postoperative renal insufficiency and postoperative hypercoagulability by TEG, $p \leq 0.02$
6. Pump time and postbypass TEG hypocoagulability platelet-fibrin interaction, $p \leq 0.01$

is targeted to examine any relationship between TEG index and risks associated with hypercoagulability.

A large series of 500 patients undergoing CPB with routine TEG analysis was analyzed for a descriptive assessment.[14] Abnormalities in coagulation function before and after bypass were significant; however, characterization of those abnormalities shifted from an anticoagulation protein dysfunction prior to CPB to a platelet–fibrin interaction deficit afterward. Indeed, after bypass only 2% of patients exhibited TEG evidence of hypocoagulability due to residual anticoagulation or protein deficiencies. The TEG found 48% of patients with platelet–fibrin interaction problems and platelet function abnormalities would fit well in this category. A significant number of patients exhibited fibrinolysis and hypercoagulability as well. Hypercoagulability had a 50% agreement before and after CPB.

A discriminate analysis showed a number of unique associations between drug therapy, outcome, and TEG (Table 4). This discriminate analysis further validates TEG whole blood coagulation testing as an indicator of changes affecting coagulation in the entire coagulation system.

TECHNICAL ASPECTS

The University of Washington model for TEG utilization is unique and has been described in this issue of *Seminars* by Chandler. The key to this system is a closed-circuit television system with displays in the cardiac operating rooms as well as the postoperative ICUs. Although outwardly appearing expensive, the system is quite inexpensive, and with data showing decreased rates of reoperation, the system can be justified as a real cost savings in the days of future reimbursement limitations.

The usefulness of a technology is dependent not only on the correlation of its data with a clinical situation (hemorrhage), but also availability, cost, and tie to data analysis. TEG data requires up to 1 hour or longer for complete (to the A60) analysis. However, the most important data (R, K, α angle, and MA) may be gathered within 30 to 40 minutes. The usefulness of this assay system could be enhanced if changes occurring during the CPB run could be characterized prior to separation from bypass.

Heparinase, an enzyme-targeting heparin, can be utilized to assay blood samples during fully heparinized bypass.[15] Heparinase is quite expensive (1000 times the cost of protamine) and recent evidence has compared small aliquots of protamine added to whole blood before placing samples in the TEG.[16] Protamine-reversed TEG-collected samples during heparinized CPB had a better correlation to the postprotamine TEG than did heparinase-reversed TEG samples. Routine use of protamine-reversed blood samples during heparinized CPB may in the future allow for advanced diagnosis of developing coagulopathies. Further work is required to decide how useful this system will become.

ADJUVANT DRUG TREATMENTS

A number of pharmacologic adjuvants have been utilized with some success in reducing CPB bleeding: antifibrinolytics, aprotinin, and desmopressin. Each has proven to be of some use in certain patient populations. TEG analysis has been applied to some of these therapies.

Fibrinolysis

Fibrinolysis is a nearly universal problem with CPB; the formation of FSP can be found in more than 80% of patients. Several studies have shown that FSP values do not correlate with TEG A60 or lysis index measurements. Although one study noted the TEG A60 did not correspond with the FSP, TEG overall significantly predicted blood loss.[17,18] The TEG provided an excellent monitor for plasmin activation in controlled animal models[19] and it makes one wonder if the etiology of FSP generation on CPB is due to subclinical plasmin activation by thrombin or tissue plasminogen activator, or mechanical destruction of fibrinogen. Antifibrinolytics can be used as a prophylactic therapy or as a treatment. The TEG can be used not only to diagnose early clot lysis, but to provide an in vitro test of antifibrinolytic efficacy for treatment.[20]

Desmopressin

Desmopressin is thought to enhance the release of von Willebrand factor. It is of use in uremic patients, but its use in CPB patients remains controversial.[21–24] A recent

study utilized TEG to characterize post-CPB coagulation states as normal and abnormal prior to administering desmopressin.[25] MA values less than 50 were considered abnormal and presumptively attributed to platelet–fibrin dysfunction (most probably platelet function abnormalities). Inside each group, a randomization process produced groups to receive desmopressin or placebo. The group with low MA values who received desmopressin benefited from such therapy. This report represents a unique application of TEG monitoring and one that should be studied more in depth. Perhaps TEG can be used to characterize subpopulations of patients at risk who may benefit from certain interventions.

Within this study's data but not discussed in the article were some outcome data. If all patients with normal TEGs were compared with those with abnormal values, there appeared to be differences in myocardial infarction and death rates. The interaction of platelets with vascular surfaces, perhaps damaged, (cardioplegia, surgery, hypothermia) may lead to adhesion. It makes sense that those patients with normal or hypercoagulable conditions would have a greater chance of developing perioperative ischemia. No study has yet examined this exact question, but perhaps TEG whole blood testing in the future may be able to detect a subgroup of patients undergoing coronary revascularization who are at an increased risk for early graft thrombosis.

Aprotinin

Data with aprotinin, a nonspecific serine protease inhibitor, and TEG have not been widely published. In redo or complex cardiac cases, chest tube output and blood product utilization can be decreased by prophylactic aprotinin administration. Personal communication with those experienced in its use indicate some mild tendencies toward hypocoagulability with aprotinin.

Patients receiving aspirin in the few days prior to CPB are at risk for more hemorrhage than those not taking such anti-inflammatory agents.[26] One TEG study in volunteers has shown that the TEG is less sensitive to early changes of small dose aspirin ingestion than is the bleeding time.[27] The TEG MA value is a parameter dependent on both fibrin and platelet interaction. The platelets may become activated through many mechanisms and the TEG does not necessarily reflect the best way to assess the activation partially blocked by aspirin. The bleeding time has recently been widely criticized as having no predictive value for surgical hemostasis or hemorrhage.[28] Its use has been abandoned by our medical center. The TEG, however, may be insensitive to the subgroup of patients with new aspirin ingestion. In contrast, however, discriminate analysis of our series of patients did find significant correlations between TEG MA and prior aspirin usage, so perhaps the question remains unsettled.

Platelet function studies have been tested and correlated with TEG MA values.[29] In laboratory settings the changes seen with adenosine disphosphate, epinephrine, and collagen-activated platelet aggregometry correlated with changes in TEG MA values.

The TEG is being applied to many other areas of basic and applied research that may have implications for CPB patients. Several studies investigating new anticoagulants have used TEG analysis in their techniques.[30,31] The ACT test may be ineffective for such pharmacologies, so TEG testing as well as other monitoring technologies need to be evaluated.

SUMMARY

The TEG tracks postoperative hemorrhage after CPB and is useful in guiding therapy. Its ability to characterize the overall interaction of all procoagulant participants in a final outcome (clot strength) is unique. Much work in the future is needed to establish the particular applications for TEG monitoring in CPB patients.

REFERENCES

1. Ellison N, D Jobes: *Effective Hemostasis in Cardiac Surgery.* WB Saunders, Philadelphia, 1988.
2. Schwartz AJ, FA Hensley (Eds): *Case Conference.* J Cardiothorac Anesth 4:499–517, 1990.
3. Tanaka K, M Takao, I Yada, H Yuasa, M Kusagawa, K Deguchi: Alterations in coagulation and fibrinolysis associated with cardiopulmonary bypass during open heart surgery. J Cardiothorac Anesth 3:181–188, 1989.
4. Horrow JC, J Hlavacek, MD Strong, W. Collier, I Brodsky, SM Goldman, IP Gobel: Prophylactic tranexamic acid decreases bleeding after cardiac operations. J Thorac Cardiovasc Surg 99:70–74, 1990.
5. Tuman KJ, BD Spiess, RJ McCarthy, AD Ivankovich: A comparison of viscoelastic measures of coagulation after cardiopulmonary bypass. Anesth Analg 69:69–75, 1989.
6. Spiess BD, KJ Tuman, RJ McCarthy, AD Ivankovich: Thromboelastography as an indicator of post-cardiopulmonary bypass coagulopathies. J Clin Monit 3:25–30, 1987.
7. Martin P, F Horkay, SM Rajah, DR Walker: Monitoring of coagulation status using thromboelastography during paediatric open heart surgery. Int J Clin Monit Comput 8:183–187, 1991.
8. Wang JS, CY Lin, WT Hung, MF O'Connor, RA Thisted, BK Lee, RB Karp, MW Yang: Thromboelastogram fails to predict postoperative hemorrhage in cardiac patients. Ann Thorac Surg 53:435–439, 1992.
9. Hofer B: Continuous thromboelastography: A rapid, accurate and superior predictor of bleeding following primary coronary bypass surgery. Personal communication.
10. Wong CA, ML Franklin, LD Wade: Comparison of thromboelastography, blood loss and transfusion requirements in platelet-rich plas-

mapheresed vs non-pheresed cardiac surgery patients. Anesthesiology 77:A541, 1992.
11. Shore-Lesserson L, DL Reich, RN DePerio, G Silvay: Thromboelastographic assessment of platelet-rich plasmapheresis during cardiac reoperations. Anesthesiology 77:A138, 1992.
12. Whitten C, P Allison, T Latson, R Cochran, R Gulden, J Wiley: Autologous blood after cardiopulmonary bypass alters hemostatic and thromboelastographic parameters. Anesth Analg 74:S348, 1992.
13. Wojciechowski Z, BD Spiess, L Soltow, W Chandler: Hypo/hypercoagulability TEG index correlates with postoperative chest tube output after cardiopulmonary bypass. Society of Cardiovascular Anesthesiologists Annual Meeting, San Diego, California, 1993.
14. Spiess BD, KJ Tuman, RJ McCarthy, AD Ivankovich: Thromboelastographic diagnosis of coagulopathies in patients undergoing cardiopulmonary bypass. Anesth Analg 68:S273, 1989.
15. McCarthy R, K Tuman, M Djuric, V Rizzo, A Ivankovich: Use of heparinase to evaluate coagulation during cardiopulmonary bypass with systemic heparinization. Anesthesiology 77:A98, 1992.
16. Wall MH, BD Spiess, B Gillies, WL Chandler: Thromboelastography with added heparinase or protamine sulfate during cardiopulmonary bypass. Anesthesiology 79:A96, 1993.
17. VanRiper DF, JC Horrow, BS Osborne: Is the thromboelastograph a clinically useful predictor of blood loss after bypass? Anesthesiology 73:A1206, 1990.
18. Whitten CW, PM Allison, TW Latson, J Elmore, RH Gulden, B Burkhardt, V Hyndman: Thromboelastographic fibrinolysis does not correlate with levels of D-dimer after cardiopulmonary bypass. Anesthesiology 75:A432, 1991.
19. Spiess BD, RJ McCarthy, A Ivankovich: Primary fibrinolysis or DIC differential by viscoelastic coagulation testing. Anesthesiology 71:A415, 1989.
20. Spiess BD, W Logas, KJ Tuman, T Hughes, J Jagmin, AD Ivankovich: Thromboelastography (TEG) used for the detection of perioperative fibrinolysis: A report of four cases. J Cardiothorac Anesth 2:666–672, 1988.
21. Salzman EW, MJ Weinstein, RM Weintraub, JH Ware, RL Thurer, L Robertson, A Donovan, T Gaffuey, V Bertele, J Troll, M Smith, LE Chute: Treatment with desmopressin acetate to reduce blood loss after cardiac surgery. A double-blind randomized trial. N Engl J Med 314:1402–1406, 1986.
22. Mannucci PM: Desmopressin, a nontransfusional form of treatment for congenital and acquired bleeding disorders. Blood 72:1449–1455, 1988.
23. Millard F: Desmopressin acetate to reduce blood loss after cardiac surgery. N Engl J Med 315:834–835, 1986.
24. Sarraccino S, E Adler, PA Gibbons, R Gruppo, WW Bailey, DW Runions, TW Martin: DDAVP does not decrease postbypass bleeding in acyanotic children. Anesthesiology 71:A1057, 1989.
25. Mongan PD, MP Hosking: The role of desmopressin acetate in patients undergoing coronary artery bypass surgery. Anesthesiology 77:38–46, 1992.
26. Bashein G, ML Nessly, AL Rice, RB Counts, GA Misbach: Preoperative aspirin therapy and reoperation for bleeding after coronary artery bypass surgery. Arch Intern Med 151:89–93, 1991.
27. Trentalange MJ, LF Waltz: A comparison of thromboelastogram and template bleeding time in the evaluation of platelet function after aspirin ingestion. J Clin Anesth 3:377–381, 1991.
28. Channing Rodgers RP, J Levin: A critical reappraisal of the bleeding time. Semin Thromb Hemost 16:1–24, 1990.
29. Tuman KJ, RJ McCarthy, RV Patel, AD Ivankovich: Comparison of thromboelastography and platelet aggregometry. Anesthesiology 75:A433, 1991.
30. Shinoda T, H Arakura, M Katakura, T Shiorta, S Nakagawa: Usefulness of thromboelastography for dosage monitoring of low molecular weight heparin and unfractionated heparin during hemodialysis. Artif Organs 14:413–415, 1990.
31. Syzmonowicz M, B Lowkis: In vitro testing method of polymers candidate destined for contact with blood. Polim Med 20:43–55, 1990.

Thromboelastography in Liver Transplantation

YOOGOO KANG, M.D.

The first clinical use of thromboelastography (TEG) during liver transplantation, in 1966, was reported by von Kaulla and associates.[1] They observed a typical fibrinolytic TEG pattern after reperfusion of the engrafted liver and administered ϵ-aminocaproic acid (EACA) to treat fibrinolysis. In the next experience reported, Howland and associates[2] saw a heparin effect demonstrated by a prolonged reaction time and reversed this by administering protamine sulfate. Nevertheless, TEG was used infrequently during liver transplantation because liver transplantation was still in its infancy, clinical studies were concentrated on improvement of surgical technique and immunosuppression, the need for a darkroom facility made real-time TEG monitoring impossible, and TEG was not welcomed by many coagulationists due to its lack of specificity.

In the early 1980s, liver transplantation evolved from experimental procedures after the improvement of surgical technique using venovenous bypass and the introduction of cyclosporine.[3] At this time, difficulty in clinical monitoring and treatment of coagulation stimulated the reintroduction of TEG by anesthesiologists at the University of Pittsburgh.[4] Continuing studies by the Pittsburgh group and other centers led to a better understanding of the clinical significance of TEG monitoring and to a rational approach to coagulation therapy.[4–6] In this article the coagulation of patients undergoing liver transplantation and our experience with TEG monitoring at Presbyterian-University Hospital are described.

COAGULATION PATTERNS IN PATIENTS WITH LIVER DISEASE

Monitoring of coagulation is critically important during liver transplantation because of preexisting coagulopathy, dilutional coagulopathy associated with surgical bleeding, and pathologic coagulation.

All phases of the coagulation process depend on hepatic function and are disrupted by liver disease. The vascular phase of coagulation, vasoconstriction and the interaction between the vessel wall and platelets, may be impaired.[7] The platelet phase is adversely affected by thrombocytopenia and platelet dysfunction. Thrombocytopenia is observed in up to 70% of patients[4] and is caused by splenomegaly,[8] shortened platelet survival,[9] platelet consumption,[10] sequestration of platelets in the regenerating liver,[10] folic acid deficiency in alcoholic liver disease,[11] and toxic effects of ethanol on megakaryocytes.[12] Platelet dysfunction may be caused by decreases in production of thromboxane A_2 and adenine nucleotides[13] and impaired aggregation by small, hypofunctional platelets.[14] In addition, dialyzable plasma factors may hinder platelet function in patients with the hepatorenal syndrome. The coagulation cascade is affected by low levels of procoagulants and inhibitors produced by the liver, including coagulation factors (I, II, V, VII, VIII, IX, X, XI, XII, XIII, prekallikrein, and high molecular weight kininogen), inhibitors (antithrombin III and α_1-antitrypsin), and regulatory proteins (C1 inhibitor and α_2-macroglobulin). The fibrinogen level is generally within the normal range, but dysfibrinogenemia caused by excessive sialic acid in the fibrinogen molecule[15] interferes with the polymerization of fibrin.[16] The level of factor VIII is frequently increased because of increases in the level and activity of von Willebrand factor antigen.[17] The fibrinolytic activity is increased because of imbalances in levels of tissue plasminogen activator (t-PA), plasminogen, protein C, protein S, and α_2-antiplasmin.

Furthermore, liver disease impairs the balances between the production of procoagulants and the hepatic clearance of their activated forms and between coagulation and fibrinolysis. Therefore, patients with liver disease may exhibit all forms of coagulopathy, although the hypocoagulable state is the common denominator. For example, hypocoagulation is caused by insufficient hepatic

From the University of Pittsburgh School of Medicine, and Presbyterian-University Hospital, Pittsburgh, Pennsylvania.

Reprint requests: Dr. Kang, Professor, University of Pittsburgh School of Medicine, Director, Hepatic Transplantation Anesthesiology, Presbyterian-University Hospital, Pittsburgh, PA 15213.

synthesis of procoagulants. Disseminated intravascular coagulation (DIC) may occur when the hepatic clearance of activated clotting factors is reduced. Fibrinolysis occurs when the levels of fibrinolysis inhibitors (α_2-antiplasmin and histidine-rich glycoprotein) are low[18,19] or the hepatic clearance of t-PA is decreased.[20,21] A hypercoagulable state may be seen when levels of protein C or protein S are low.

INTRAOPERATIVE BLEEDING AND COAGULATION

Intraoperative bleeding is common during liver transplantation, with an average blood loss of 10 to 15 U each of red blood cells (RBC) and fresh-frozen plasma (FFP), and massive blood loss (more than 100 U) can occur when the procedure becomes complicated.[22] Surgical bleeding is caused by difficulties in the removal of the diseased liver and the need for reconstruction of the major blood vessels (the inferior vena cava, portal vein, and hepatic artery). Therefore, bleeding is more severe in patients with portal hypertension, which increases the pressure, number, and size of collateral capillaries. In addition, adhesions from previous abdominal surgery and fragile tissues increase the tendency to bleed. Surgical bleeding is compounded by the abnormal coagulation state.

Intraoperative changes in coagulation have been well described for the last 25 years.[1,4,6,23–25] During the preanhepatic stage, dilutional coagulopathy is superimposed on preexisting coagulopathy, resulting in generalized decreases in the levels of coagulation factors and thrombocytopenia. Changes in coagulation become more extreme during the anhepatic stage. When venovenous bypass is used and a small dose of heparin (2000 to 5000 U) is added to the priming solution, the resultant heparin effect is seen as prolonged activated partial thromboplastin time (aPTT). The heparin effect dissipates gradually within 1 hour without treatment. Dilutional coagulopathy becomes more pronounced owing to the absence of hepatic synthetic function. The absence of the hepatic clearance of activated coagulation factors results in activation of coagulation, and this is observed as gradual increases in thrombin–antithrombin III complex (TAT) and fibrin(ogen) degradation products (FDP).[26] Fibrinolysis begins to develop during this stage because of the absence of the hepatic clearance of t-PA.

A severe coagulopathy occurs on reperfusion of the engrafted liver and is demonstrated by prolonged prothrombin time (PT), aPTT, reptilase time, and thrombin time; a generalized decrease in coagulation factor levels, including factors I, V, VII, and VIII; a sudden increase in t-PA; thrombocytopenia; a shortened euglobulin lysis time (ELT); and a moderate increase in FDP and TAT. The heparin effect is seen in about one third of patients because of the release of heparin from the engrafted liver. In 40% of patients on reperfusion severe fibrinolysis occurs[4,25] when the massive release of t-PA from the engrafted liver, congested viscera, and lower extremities overwhelms the activity of plasminogen activator inhibitor (PAI).[27,28] Fibrinolysis is believed to be primary in origin because of the observed association between high t-PA levels and fibrinolysis[27,28]; a relatively steady level of antithrombin III[29]; only moderate levels of FDP and D-dimer[4,5]; an association between fibrinolysis and a selective decrease in factors I, V, and VIII[25]; no known microembolization; and its effective treatment with EACA.[5] DIC does not appear to be a major cause of coagulopathy, and inadvertent hypothermia (as low as 31°C) and ionized hypocalcemia[30] may contribute to bleeding. Coagulopathy improves as the engrafted liver begins to function: fibrinolysis and the heparin effect dissipate gradually, and coagulation factors and platelet count increase toward baseline levels. In some patients, however, bleeding or oozing may persist as a result of severe fibrinolysis or inadequate graft function.

THROMBOELASTOGRAPHIC MONITORING

The management of dynamic coagulation depends on a thorough understanding and careful monitoring of coagulation and the selection of proper therapies. A conventional coagulation profile, including the PT, aPTT, fibrinogen level, platelet count, ELT, FDP level, factor assays (I, V, VII, XII), reptilase time, and thrombin time, has been used for this purpose. Nevertheless, the coagulation profile has several drawbacks. First, its interpretation is difficult during liver transplantation. For example, PT is a sensitive test for hepatic synthetic function, and it is frequently prolonged during liver transplantation even with the transfusion of sufficient volume of FFP. The intraoperative change in aPTT is similar to that in PT, although aPTT is more prolonged when heparin is added at the onset of venovenous bypass and immediately after reperfusion of the engrafted liver. Fibrinogen level may not be meaningful in the presence of dysfibrinogenemia. Platelet count is a poor indicator of platelet function, as is evident from the lack of correlation between platelet count and bleeding time.[7] ELT determines the activity of plasminogen, but does not evaluate the net balance between plasminogen and antiplasmin. FDP becomes positive during major surgery because of resolution of blood clots in the capillaries or reabsorption of defibrinated blood from the surgical field. Bleeding time is close to that of the natural coagulation process. However, bleeding time estimates only the first half of coagulation, the results may not be reproducible, and multiple tests may not be feasible during surgery. Furthermore, most coagulation

tests require a laboratory facility, which may be unable to provide results in a timely fashion. These difficulties in using the coagulation profile were evident in our early experience at the University of Pittsburgh: coagulation profile was not recorded on the anesthesia record because of the delay in obtaining results, and blood products were administered empirically for uncontrollable coagulopathy.

TEG has several advantages for clinical monitoring of coagulation during liver transplantation. It reflects the natural coagulation process by measuring coagulability of whole blood, including the activities of cellular elements, procoagulants and their inhibitors, plasmin and antiplasmin, and the effects of biochemical and physical properties, although it does not measure the vascular phase and the interaction between platelets and vascular endothelium. This property of TEG is particularly valuable, because blood coagulability or blood clotting on the surgical field, not the quantity of coagulation elements, is the concern of clinicians. Furthermore, TEG provides a differential diagnosis of clinically important types of coagulopathy. For example, indications for replacement therapy can be obtained by comparing TEGs of plain blood with blood treated with FFP, platelets, or cryoprecipitate. More important, the type of pathologic coagulation can be identified by comparing TEGs of plain blood with those of blood treated with EACA for fibrinolysis and blood treated with protamine sulfate for the heparin effect. Another advantage of TEG is that the test can be performed in the operating room for timely monitoring of coagulation.

TEG patterns typical of several disease states have been described.[31] A normal TEG pattern is characterized by an initial fluid state (reaction time) followed by a gradual increase in fibrin shear elasticity (clot formation rate) that reaches maximum amplitude (MA) in 30 to 60 minutes (Fig. 1). The whole blood clot lysis index (A60/MA × 100) remains above 85%. In patients with hemophilia, the reaction time is prolonged and the clot formation rate is decreased because of the delayed formation of thrombin associated with insufficient activity of factor VIII. However, MA (platelet function) is within the normal range. A similar TEG pattern is observed in patients with ionized hypocalcemia (less than 0.6 mmol/L) or hypothermia (less than 34°C). Ionized hypocalcemia prolongs the reaction time and clot formation rate because the calcium ion is a cofactor in the coagulation cascade. Hypothermia delays coagulation because coagulation is an enzymatic process involving many proteinases. MA is decreased with quantitative and qualitative defects of platelets. In addition, the reaction time is prolonged and the clot formation rate is decreased because of the role of platelets in the activation of coagulation factors. In fibrinolysis, a rapid decrease in amplitude is accompanied by a prolonged reaction time and a decreased MA. These changes are caused by a net decrease in the amount of fibrin, because coagulation and fibrinolysis are simultaneously activated. Hypercoagulation is characterized by a short reaction time and increased clot formation rate and MA.

REPLACEMENT THERAPY

Clinical use of TEG in the Pittsburgh center began in 1983. The TEG patterns of the first patient are shown in Figure 2. During liver transplantation, the attending anesthesiologist was blind to the TEG results in the management of the patient. Poor coagulation improved gradually with the administration of FFP and platelets in the beginning of the procedure. However, severe bleeding on the surgical field was compounded by massive intragastric bleeding during the anhepatic stage (1000 mL every 30 minutes), and gastrectomy was planned after reperfusion of the engrafted liver. A straight line was observed on TEG at this time, and this finding was relayed to the anesthesiologist because such coagulopathy may affect the outcome of surgery. Aggressive replacement therapy improved TEG within 2 hours and stopped intragastric bleeding.

After this dramatic experience, all patients were monitored by TEG and a clinical study was carried out in 66 consecutive patients.[4] In this study, coagulation tests were performed 8 to 12 times during liver transplantation. TEG variables included reaction time (R minutes); MA (millimeters); amplitude 60 minutes after MA (A60, millimeters); coagulation time R plus coagulation [K] (minutes); clot formation rate (a, degrees); fibrinolysis time (F, minutes); and whole blood clot lysis index (A60/MA × 100, %). The coagulation profile included measurement of PT; aPTT; thrombin time; reptilase time; levels of factors I, II, V, VII, VIII, IX, X, XI, and XII; FDP level; ELT; and platelet count. Clinical protocol included an infusion of a fluid mixture (RBC:FFP:PlasmaLyte-A = I unit: I unit: 250 mL) yielding hematocrit of 27 vol% and clotting factors of 20 to 50% of normal to maintain steady hematocrit and coagulation factors above critical levels. Additional blood products were administered: 2 U of FFP when the reaction time was longer than 15 minutes, 10 U of platelets when the MA was less than 40 mm, and 6 U of cryoprecipitate containing fibrinogen and factor VIII when the clot formation rate was persistently less than 40°.

The preoperative coagulation profile was abnormal in large proportions of patients: PT in 61%, aPTT in 71%, fibrinogen level in 14%, factor V level in 59%, factor VII level in 56%, factor VIII level in 3%, and platelet count in 70%. ELT was less than 2 hours in 24% and

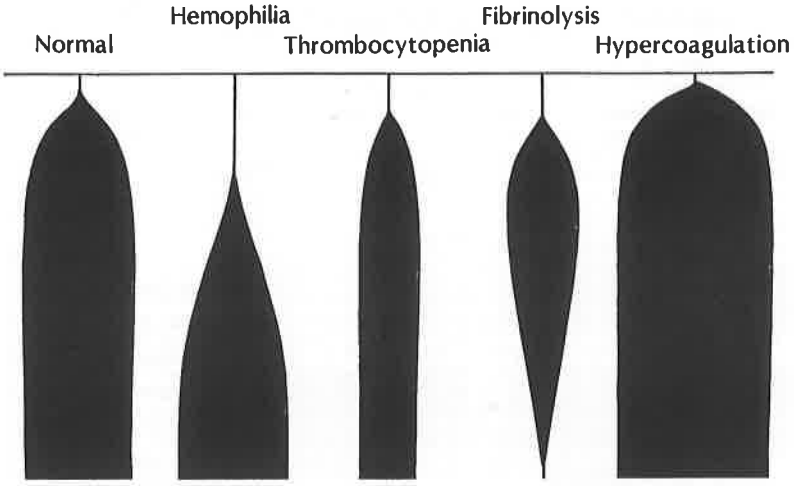

FIG. 1. Thromboelastographic patterns of normal and disease states. (Reprinted with permission from Kang et al.[30])

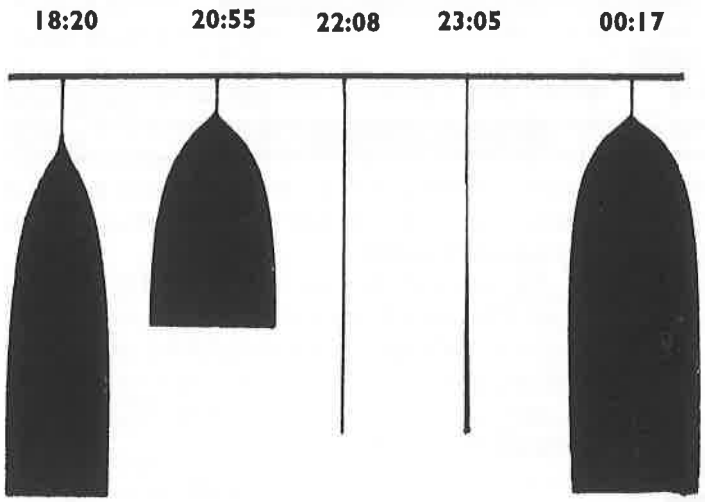

FIG. 2. Thromboelastographic patterns of the first patient with thromboelastographic monitoring during liver transplantation.

FDP was positive in 24% of patients. The intraoperative coagulation profile and TEG variables of patients with primary biliary cirrhosis are shown in Table 1. During the preanhepatic stage, PT and aPTT remained prolonged, and all the coagulation factor levels and platelet count decreased progressively. Significant changes seen during the anhepatic stage were further prolongation of aPTT and a decrease in ELT. On reperfusion, PT and aPTT were remarkably prolonged, and all coagulation factor levels were very low. The coagulation profile improved gradually and returned to clinically acceptable levels at the end of surgery. On the contrary, changes in TEG patterns were less remarkable except for the significant inhibition of coagulation on reperfusion of the engrafted liver and fibrinolytic activity during the anhepatic stage and the early phase of the neohepatic stage. The correlation between TEG variables and coagulation profile variable was generally poor, except for a clot formation rate with aPTT and MA with platelet count, indicating poor relationship between the coagulation profile and blood coagulability. TEG-guided replacement therapy appeared to be effective: 10 U of platelets increased platelet count by 40,200 ± 31,400/mm^3 and MA by 13.2 mm, and 6 U of cryoprecipitate increased fibrinogen level by 37 mg/dL, decreased aPTT by 5.7 seconds, and increased clot formation rate by 9.4°. Interestingly, blood usage in this group of patients was significantly less than that in the historic control group of patients: RBC, 17.0 versus 26.7 U; FFP, 18.3 versus 26.7 U; platelets, 20.8 versus 14.1 U; and cryoprecipitate, 12.2 versus 3.9 U.

The TEG patterns and coagulation profile of one of the studied patients with fulminant hepatic failure are shown in Figures 3 and 4. The baseline TEG pattern showed a prolonged reaction time, decreased MA, and decreased clot formation rate, indicating a generalized decrease in coagulation factor levels and platelets. Ad-

TABLE 1. Intraoperative Changes in Coagulation of Patients with Primary Biliary Cirrhosis*†

	Before Operation	*Stage I*	*Stage II*		*Stage III*			
		120 min	*5 min*	*30 min*	*5 min*	*30 min*	*120 min*	*End*
PT (s)	13.9 ± 1.7	13.7 ± 1.0	14.0 ± 1.3	13.8 ± 1.2	15.4 ± 1.6‡	15.7 ± 1.5‡	15.0 ± 1.4	14.6 ± 1.4
aPTT (s)	41.0 ± 8.7	40.2 ± 7.7	46.0 ± 12.9	51.2 ± 16.0	75.2 ± 39.2‡	61.5 ± 23.6‡	46.0 ± 9.7	38.4 ± 3.8
Platelets (1000/mm^3)	181 ± 199	150 ± 122	149 ± 120	154 ± 122	124 ± 63‡	132 ± 61	134 ± 82	143 ± 74
Fibrinogen (mg/dL)	323 ± 166	288 ± 100	252 ± 113	235 ± 88	194 ± 93‡	195 ± 101‡	191 ± 90‡	210 ± 92
Factor V (U/mL)	0.54 ± 0.27	0.43 ± 0.22	0.35 ± 0.19	0.38 ± 0.19	0.26 ± 0.18‡	0.22 ± 0.13‡	0.21 ± 0.11‡	0.23 ± 0.13‡
Factor VII (U/mL)	0.83 ± 0.35	0.71 ± 0.37	0.62 ± 0.31	0.65 ± 0.27	0.52 ± 0.23‡	0.52 ± 0.18‡	0.53 ± 0.18	0.51 ± 0.17
Factor VIII (U/mL)	2.03 ± 0.73	1.81 ± 0.86	1.51 ± 0.88	1.49 ± 0.75	1.11 ± 0.70‡	0.82 ± 0.53‡	0.87 ± 0.44‡	0.93 ± 0.35‡
ELT (min)	215 ± 77	134 ± 93	140 ± 88	101 ± 77‡	68 ± 53‡	108 ± 45	155 ± 63	185 ± 70
Reaction time (min)	8.5 ± 2.4	8.2 ± 3.6	6.6 ± 3.4	7.5 ± 2.3	11.2 ± 5.0‡	9.7 ± 2.7	8.8 ± 3.0	8.0 ± 2.9
Coagulation time (min)	12.0 ± 2.7	12.0 ± 6.3	9.5 ± 2.5	11.0 ± 2.2	23.5 ± 16.4‡	15.0 ± 6.1	12.5 ± 4.5	10.0 ± 2.6
Clot formation rate	55.5 ± 8.5	53.8 ± 12.2	53.6 ± 10.4	53.1 ± 7.4	39.1 ± 17.5‡	48.6 ± 10.5	50.6 ± 12.0	55.1 ± 8.0
MA (mm)	54.6 ± 11.2	57.3 ± 9.4	52.3 ± 11.0	51.3 ± 9.4	38.2 ± 14.5‡	51.7 ± 8.9	51.5 ± 11.9	52.1 ± 10.8
A_{60}/MA × 100 (%)	83.0 ± 7.8	76.4 ± 14.5	73.9 ± 27.0	65.5 ± 35.5	75.8 ± 34.4	79.8 ± 29.3	92.2 ± 8.6‡	91.7 ± 6.7‡

* Eleven patients; values are mean ± SD. aPTT: activated partial thromboplastin time; ELT: euglobulin lysis time; MA: maximum amplitude; PT: prothrombin time.
† Reprinted with permission from Kang et al.[4]
‡ Significantly different from the corresponding preoperative value ($p < 0.05$).

ministration of 10 U of platelets improved MA to close to normal range, but mild fibrinolysis began to develop. Transfusion of 6 U of cryoprecipitate did not improve clot formation rate, possibly by continuous deterioration of coagulation. The anhepatic stage was characterized by pronounced fibrinolysis. On reperfusion, a severe coagulopathy was noted: prolonged reaction time, decreased clot formation rate, decreased MA, and signs of fibrinolysis. Reperfusion coagulopathy improved somewhat 30 minutes after reperfusion, although fibrinolysis was still detectable. TEG improved, with no fibrinolysis 2 hours after reperfusion, and administration of platelets and cryoprecipitate resulted TEG becoming normal by the end of surgery, even in the presence of a mild abnormality in the coagulation profile.

The results of this study suggest that TEG monitoring and TEG-guided replacement therapy are clinically effective in maintaining blood coagulability and reducing blood loss. However, the pathologic coagulation, fibrinolysis, and the heparin effect observed by TEG were disturbing phenomena.

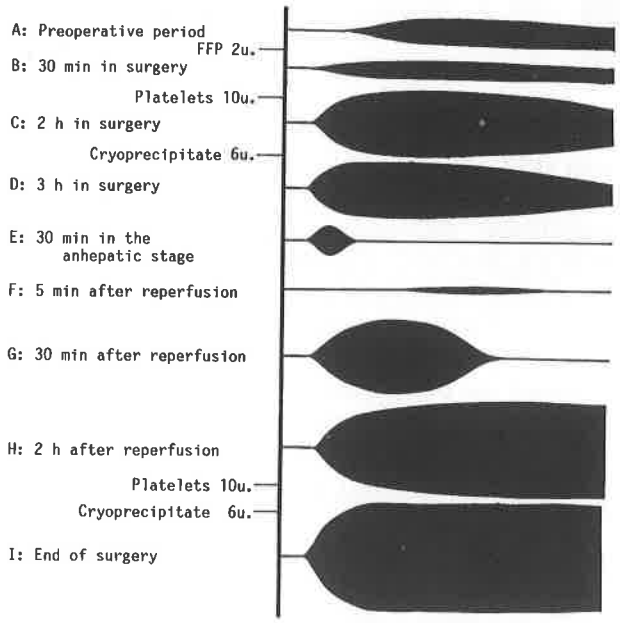

FIG. 3. Thromboelastographic patterns of a patient with fulminant hepatic failure during liver transplantation. (Reprinted with permission from Kang et al.[4])

PHARMACOLOGIC THERAPY

The role of pathologic coagulation in a patient undergoing liver transplantation is demonstrated in Figure 5. The TEG pattern 5 minutes before reperfusion was close to normal. However, TEG performed 5 minutes after reperfusion showed fibrinolysis and severe inhibition of coagulation. On the other hand, TEG of blood treated with EACA (0.03 mL of 1% solution in 0.33 mL of blood) showed improved coagulation and complete inhibition of fibrinolysis. TEG of blood treated with protamine sulfate (0.03 mL of 0.01% solution in 0.33 mL of blood) showed improved reaction time and MA. Similar observations in following patients clearly indicated that reperfusion coagulopathy could be treated with antifibrinolytic therapy and protamine sulfate. However, the clinical use of pharmacologic agents was delayed because of controversies surrounding antifibrinolytic therapy. The beneficial effect of antifibrinolytic therapy was recognized in the early era of liver transplantation,[24] and von Kaulla and

Coagulation profile (LW)

	Preanhepatic Stage		Anhepatic Stage		Neohepatic Stage		End
Platelet (K/mm3)	28	109	92	85	107	110	125
PT (sec)	27.7	18.5	19.1	15.3	14.6	14	13.5
aPTT (sec)	47.1	43.4	72.9	41.3	40.9	39.3	33.3
Thrombin time (sec)	28.2	21.4	34.2	21.7	20.3	18.8	20.9
Reptilase time (sec)	33.5	24.1	24.6	24.4	23.4	21.6	23.1
Fibrinogen (mg/mL)	95	130	130	175	225	230	250
Factor II (U/mL)	0.17	0.24	0.24	0.31	0.33	0.35	0.43
Factor V (U/mL)	0.12	0.18	0.16	0.25	0.29	0.29	0.41
Factor VII (U/mL)	0.04	0.09	0.09	0.22	0.29	0.34	0.44
Factor VIII (U/mL)	2.75	1.95	1.75	1.9	0.15	1.25	1.4
Factor IX (U/mL)	0.31	0.36	0.33	0.52	0.62	0.58	1.15
Factor X (U/mL)	0.17	0.22	0.25	0.23	0.33	0.38	0.49
Factor XI (U/mL)	0.44	0.52	0.78	0.58	0.66	0.6	0.84
Factor XII (U/mL)	0.52	0.52	0.52	0.6	0.6	0.33	0.4
FDP (ug/mL)	0	0	0	0	0	0	20
ELT (h)	2	1	1	1	2.5	2.75	4

FIG. 4. Coagulation profile of a patient with fulminant hepatic failure during liver transplantation.

associates[1] administered EACA to three patients (5 g loading dose followed by 1 g/h) during liver transplantation. These patients, however, developed serious hemorrhagic and thrombotic complications, and the authors suggested that the fibrinolysis during liver transplantation was a self-limiting process and did not require pharmacologic manipulation, which might be harmful. However, the common occurrence of coagulopathy associated with fibrinolysis and its reversal by EACA in vitro led to the clinical use of EACA (Fig. 6). In this patient, fibrinolysis noted on TEG was accompanied by severe oozing during the dissection of the diseased liver. A TEG of blood treated with EACA showed an absence of fibrinolysis, and EACA (1 g, single dose) was administered to the patient. The next TEG showed complete control of fibrinolysis within 20 minutes, and, more remarkably, the

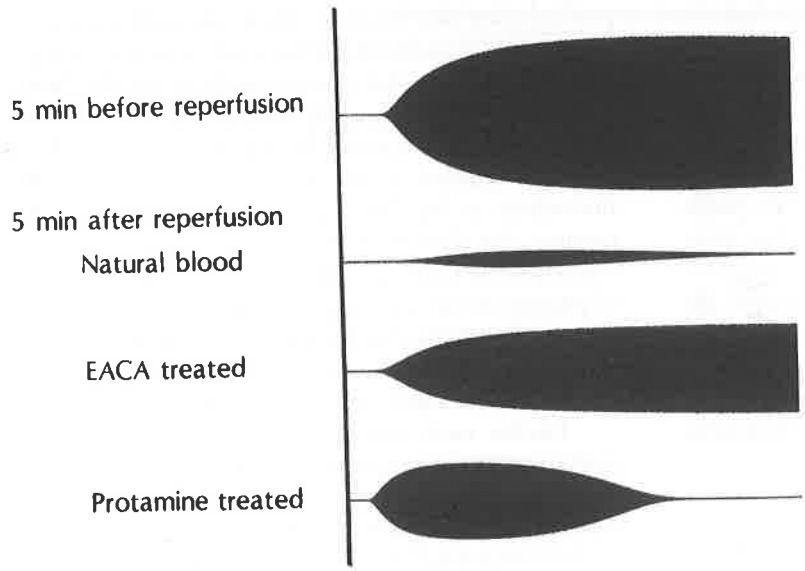

FIG. 5. Effects of pharmacologic agents in blood of a patient undergoing liver transplantation. (Reprinted with permission from Kang et al.[30])

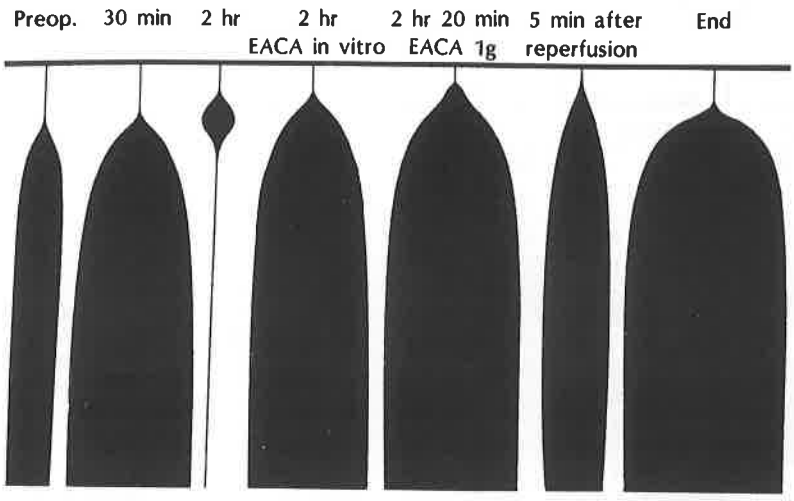

FIG. 6. Thromboelastographic patterns of the first patient whose fibrinolysis was treated with ϵ-amino-caproic acid (1 g).

surgeon was surprised to observe a sudden decrease in oozing within 5 minutes after the drug therapy.

This successful case was followed by a clinical study investigating the role of EACA in treating fibrinolysis in 79 patients undergoing liver transplantation.[6] Times and types of coagulation monitoring were the same as in the study just described. Fibrinolysis was defined as present when fibrinolysis time was less than 120 minutes or when whole blood clot lysis index was less than 80%. When fibrinolysis was observed, or 5 minutes after reperfusion of the engrafted liver, TEGs of plain blood and blood treated with EACA were compared. Intraoperatively, 82.5% of patients showed fibrinolysis. Of these patients, fibrinolysis was most severe during the preanhepatic stage in 26.2%, during the anhepatic stage in 23.8%, and during the nonhepatic stage in 50%. The mean duration of fibrinolysis, the interval between its appearance and disappearance, was 216 ± 136 minutes. TEG variables and coagulation profiles of the preoperative stage and the fibrinolytic stage are shown in Table 2. The significant TEG changes were decreases in MA (from 44.8 ± 14.3 to 32.6 ± 15.6 mm), clot formation rate (from 42.1 ± 16.4 to 36.6 ± 18.3°), and whole blood clot lysis index (from 80.9 ± 17.4 to 23.9 ± 27.7%). At the same time, all coagulation factor levels and platelet counts decreased. Intraoperative changes in fibrinolysis time, ELT, FDP, and fibrin monomers indicated a gradual increase in fibrinolytic activity. Fibrinolysis was not observed in any sample of blood treated with EACA in vitro, and EACA treatment increased whole blood clot lysis index from 49 to 94%. A single, 1 g dose of EACA was administered to the 20 patients who had fibrinolysis (less than 120 minutes) and fibrinolysis was effectively treated by EACA in vitro. EACA improved MA, whole blood clot lysis index, and fibrinolysis time in all patients, but it did not improve their coagulation profiles except for an increase in ELT (Table 3). No patient who received EACA developed hemorrhagic, thrombotic, or renal complications.

The results of this study changed the concept of antifibrinolytic therapy. First, the presence of fibrinolysis and indications for antifibrinolytic therapy were defined by TEG monitoring, which reflects the overwhelming plasmin activity over antiplasmin. Previously, EACA was used in cases of severe oozing with short ELT. However, generalized bleeding is not necessarily caused by fibrinolysis, and ELT does not reflect the net balance between plasminogen activators and its inhibitors, as seen in the lack of correlation between ELT and fibrinolysis time. Second, surveillance of coagulation after the antifibrinolytic therapy is possible with TEG monitoring. It is unclear whether the hemorrhagic or thrombotic complications seen by von Kaulla and associates[1] were related to antifibrinolytic therapy, because follow-up coagulation profiles were not reported. Third, the dose of EACA was reduced dramatically in this study. The conventional EACA dosage schedule is based on the recommendation of McNicol et al[32]: a priming dose of 4 to 5 g, followed by 1 g/h to achieve a plasma level of 13 mg/dL. However, this dose schedule is based on complete inhibition of fibrinolysis in vitro and must be adjusted for clinical practice. For patients undergoing liver transplantation, fibrinolysis is transient, although severe, and inhibition of plasmin in the early stage of fibrinolysis is sufficient to stop fibrinolysis. Furthermore, EACA is almost completely eliminated in urine within 6 hours, and no residual effect of EACA is anticipated at the end of or after surgery.

Further modifications have been made in TEG-guided antifibrinolytic therapy. Early diagnosis of fibrinolysis is made when significant improvement in TEG variables (reaction time and clot formation rate) is observed in blood treated with EACA compared with plain

TABLE 2. Thromboelastographic Variables and Coagulation Profile in the Preoperative Period and in the Most Active Fibrinolytic Stage in 80 Patients with Fibrinolysis*†

Variable	*Normal Values*	*Preoperative Period*	*Fibrinolytic Stage*
Reaction time (min)	6–8	9.88 ± 7.66	10.71 ± 12.1
Coagulation time (min)	10–12	16.7 ± 12.3	14.1 ± 7.0
Maximum amplitude (mm)	50–70	44.8 ± 14.3	32.6 ± 15.6‡
Clot formation rate (°)	>50	42.1 ± 16.4	36.6 ± 18.3‡
Whole blood clot lysis index (%)	>80	80.9 ± 17.4	23.9 ± 27.7‡
Prothrombin time (s)	11–13	14.7 ± 3.7	15.5 ± 3.4‡
Activated partial thromboplastin time (s)	26–34	46.5 ± 21.8	60.2 ± 32.5‡
Thrombin time (s)	13–18	26.8 ± 11.6	29.7 ± 13.1
Platelet count (1000/mm^3)	150–450	122.4 ± 128.8	92.8 ± 58.7‡
Fibrinogen (mg%)	150–450	198.0 ± 103.3	129.3 ± 66.0‡
Factor II (U/mL)	0.5–1.5	0.58 ± 0.25	0.50 ± 0.17‡
Factor V (U/mL)	0.5–1.5	0.47 ± 0.29	0.30 ± 0.15‡
Factor VII (U/mL)	0.5–1.5	0.47 ± 0.32	0.38 ± 0.21‡
Factor VIII (U/mL)	0.5–1.5	1.79 ± 0.87	1.22 ± 0.70‡

* Values are means ± SD.
† Reprinted with permission from Kang et al.[5]
‡ $p < 0.05$ compared with preoperative values.

blood in the first 10 to 15 minutes, instead of waiting to observe complete fibrinolysis on TEG. Even a smaller dose of EACA (250 to 500 mg) was found to be effective in treating most types of fibrinolysis, although a second dose may be necessary when severe bleeding reduces the plasma level of EACA or when an extremely high t-PA level persists for a prolonged period. Early antifibrinolytic therapy appears to be beneficial because uninhibited plasmin may selectively destroy factors I, V, and VIII. Therefore, early treatment of fibrinolysis reduces the need for FFP and cryoprecipitate by maintaining the levels of these factors, decreases the need for RBC by reducing bleeding, and minimizes warm ischemia of the engrafted liver by reducing the surgical hemostasis time. The prophylactic use of EACA is not recommended, however, to avoid unnecessary treatment.[33]

The heparin effect was documented in the 1960s, and a marked heparin effect is seen in about one third of cases after reperfusion and may last for 2 hours.[34] It is diagnosed by a severe prolongation of aPTT, thrombin time, and reptilase time, but the comparison of TEGs of plain blood and blood treated with protamine sulfate is a more reliable test. Shortening of reaction time of blood treated with protamine sulfate within the first 10 to 15

TABLE 3. Thromboelastographic Variables and Coagulation Profile in Patients Who Received ε-Aminocaproic Acid (EACA, 1 g)*†

Variable	*Before EACA*	*10 Min After EACA*
Reaction time (min)	11.1 ± 11.3	8.3 ± 3.8
Coagulation time (min)	21.1 ± 22.9	14.2 ± 6.4
Maximum amplitude (mm)	35.9 ± 12.8	49.8 ± 5.7‡
Clot formation rate (°)	37.6 ± 19.4	44.8 ± 13.6
Whole blood clot lysis index (%)	28.5 ± 29.5	94.8 ± 7.4‡
Whole blood clot lysis time (min)	75.7 ± 34.6	>180§
Prothrombin time (s)	16.0 ± 1.9	16.3 ± 1.7
Activated partial thromboplastin time (s)	62.5 ± 23.1	57.6 ± 15.0
Thrombin time (s)	30.4 ± 10.9	30.1 ± 10.9
Platelet count (1000/mm^3)	132.6 ± 80.5	113.1 ± 36.5
Fibrinogen (mg%)	128.2 ± 48.1	124.2 ± 38.9
Factor II (U/mL)	0.46 ± 0.11	0.45 ± 0.11
Factor V (U/mL)	0.22 ± 0.08	0.20 ± 0.06
Factor VII (U/mL)	0.33 ± 0.10	0.34 ± 0.09
Factor VIII (U/mL)	0.98 ± 0.67	0.91 ± 0.62

* Values are mean ± SD.
† Reprinted with permission from Kang YG, JH Lewis, A Navalgund.[5]
‡ $p < 0.05$ compared with the corresponding values before EACA.
§ Whole blood clot lysis time was greater than 180 min in all occasions.

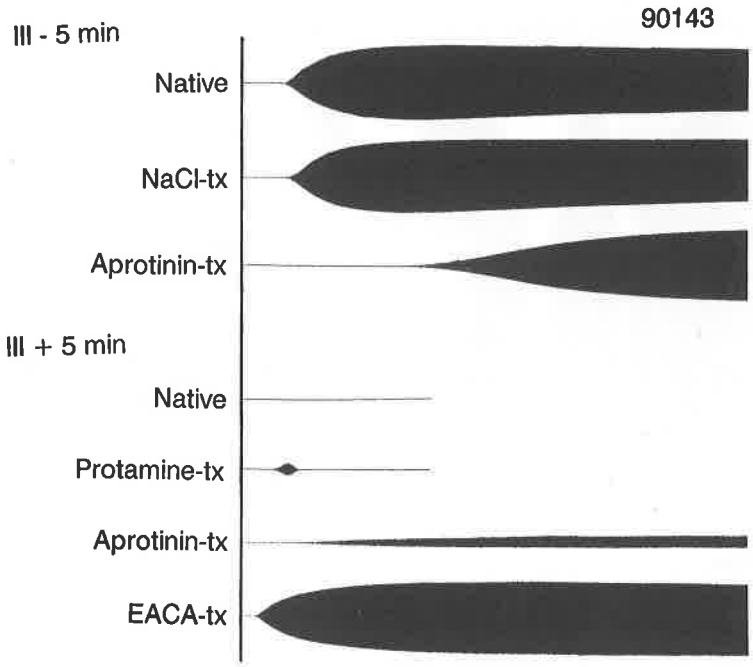

FIG. 7. Thromboelastographic patterns of blood treated with aprotinin. (Reprinted with permission from Kang et al.[40])

minutes suggests the need for the reversal of heparin by the administration of protamine sulfate (25 to 50 mg).

Effects of other pharmacologic agents, such as aprotinin and desmopressin, on coagulation of patients undergoing liver transplantation have been studied. Aprotinin, a potent inhibitor of plasmin and serine proteases, has been reported to reduce blood loss during cardiac surgery and liver transplantation,[35–38] possibly by inhibiting fibrinolysis by the decreasing t-PA level[39] and improving adhesion and aggregation of platelets.[35] To identify the effects of aprotinin, TEG was performed twice in 55 patients undergoing liver transplantation.[40] Three different preparations of blood samples taken during the anhepatic stage were tested: plain blood (0.36 mL), blood treated with sodium chloride (NaCl)(0.03 mL of 0.9% NaCl in 0.33 mL of blood), and blood treated with aprotinin (125 kIU in 0.03 mL in 0.33 mL of blood). Blood treated with aprotinin demonstrated a prolonged reaction time and smaller clot formation rate compared with the plain blood samples in all cases (Fig. 7), with an average difference of 13.5 mm and 13.0°, respectively. Whole blood clot lysis index was always greater than 93% and no fibrinolysis was seen in blood samples with aprotinin. Five preparations of blood samples taken after reperfusion were tested: plain blood, blood treated with NaCl, blood treated with aprotinin, blood treated with EACA (0.03 mL of 1% EACA in 0.33 mL of blood), and blood treated with protamine (0.03 mL of 0.01% of protamine sulfate in 0.33 mL of blood). Generalized coagulopathy was seen in plain blood and blood treated with NaCl at this time, indicated by a prolonged reaction time and decreased maximum amplitude, clot formation rate, and whole blood clot lysis index. Fibrinolysis time was less than 180 minutes in 50% of patients (59.5 ± 49.3 minutes). Blood treated with protamine had increased MA and clot formation rate, but the degree of fibrinolysis was similar to that in plain blood and blood treated with NaCl. Blood treated with EACA had improved MA and clot formation rate, and fibrinolysis was not seen in any case. Blood treated with aprotinin demonstrated a significantly prolonged reaction time and inhibition of fibrinolysis in all cases. The results of this study indicate that the antifibrinolytic activity of aprotinin in vitro is similar to but less than that of EACA, possibly by the inhibition of t-PA activity. However, it appears that aprotinin inhibits coagulation by inhibiting kallikrein, a potent activator of factor XII, as well as other coagulation factors.

Desmopressin acetate, a synthetic analogue of 8-arginine vasopressin, increases the levels of factor VIII, von Willebrand factor (vWF:Ag), and plasminogen. It has been used in patients undergoing cardiac surgery[41] and in uremic patients[42] with platelet dysfunction to improve coagulation by increasing vWF: Ag and by promoting the endothelial release of factor VIII.[43] Desmopressin may have a similar beneficial role in patients with liver disease and was studied in 29 patients undergoing liver transplantation.[44] TEGs were performed using plain blood (0.36 mL) and blood treated with desmopressin (0.03 mL of 0.0016% desmopressin in 0.33 mL of blood) three times during surgery. During the preanhepatic stage and

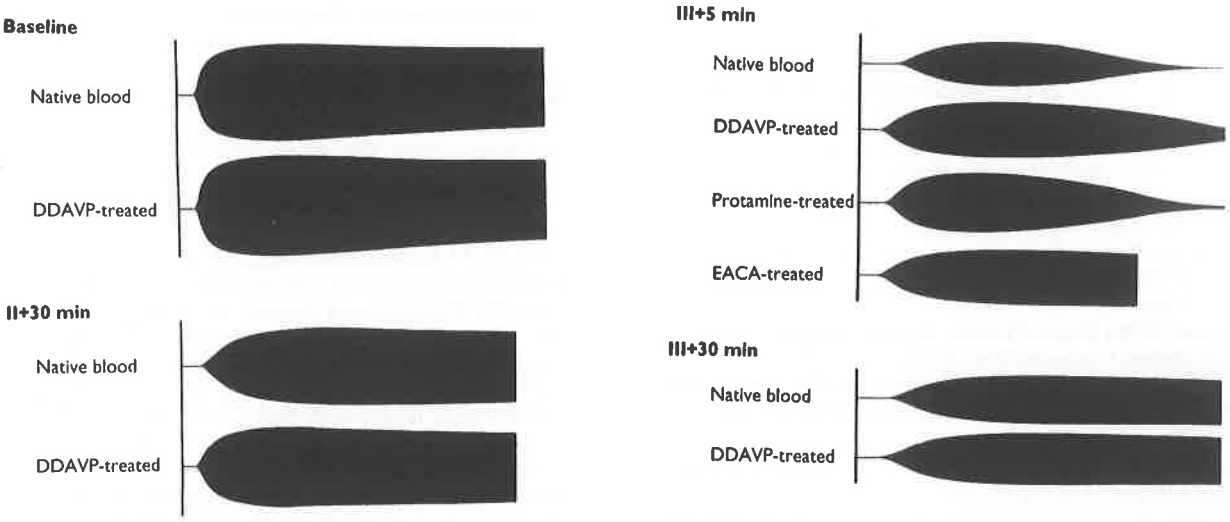

FIG. 8. Thromboelastographic patterns of untreated and desmopressin-treated blood. (Reprinted with permission from Kang et al.[44])

30 minutes after reperfusion, blood treated with desmopressin showed only a moderate increase in clot formation rate. However, blood treated with desmopressin improved most TEG variables immediately after reperfusion: reaction time decreased by 2.9 minutes, clot formation rate increased by 11°, and MA increased by 9 mm (Fig. 8). These results demonstrate that desmopressin improves blood coagulability in vitro during liver transplantation, possibly by activating coagulation factors and platelets.

PEDIATRIC LIVER TRANSPLANTATION

Coagulation in children undergoing liver transplantation follows the same course as seen in adults.[45] However, intraopeative changes in the coagulation profile and TEG patterns of children appear to be less marked than those of adults; severe thrombocytopenia did not occur, heparin effect was seen in 50% of children compared with 70% of adults, fibrinolysis in 62.5% of children compared with 82.5% of adults, and antifibrinolytic therapy was indicated in 12.5% of children compared with 21% of adults. These differences may be caused by the better hepatic reserve in pediatric patients, who have a preponderance of cholestatic disease, a shorter duration of disease, and possibly better graft function.

CONCLUSION

TEG has been found to be a reliable tool for monitoring and treatment of coagulation and has been indispensable for the liver transplantation program of the University of Pittsburgh. As Starzl has stated, "Now cautious correction of coagulation defects is an integral part of liver transplantation, greatly diminishing the hemorrhages of nightmare proportions that were common."[46] TEG is analogous to a 5-lead electrocardiogram, which monitors clinically important information, such as conduction abnormalities, ischemia, or other pathologic conditions. Certainly, specific diagnosis of cardiac pathology can be made only by elaborate studies with the involvement of a cardiologist. However, the concern of clinicians involved in the acute care setting is not the type of disease, but the level of cardiac output and tissue perfusion. Similarly, TEG provides clinicians caring for patients undergoing liver transplantation with critical information: does blood clot?; what is the potential cause of coagulopathy?; and what is the proper treatment?

REFERENCES

1. Von Kaulla KN, H Kaye, E Von Kaulla, TL Marchioro, TE Starzl: Changes in blood coagulation, before and after hepatectomy or transplantation in dogs and man. Arch Surg 92:71–79, 1966.
2. Howland WS, GM Ryan, RE Bettigole, JG Fortner: Coagulation abnormalities associated with liver transplantation. Surgery 68:591–596, 1970.
3. Starzl TE, S Iwatsuki, DH Van Thiel, JC Gartner, BJ Zitelli, JJ Malatack, RR Schade, BW Shaw Jr, TR Hakala, JT Rosenthal, KA Porter: Evolution of liver transplantation. Hepatology 2:614–636, 1982.
4. Kang YG, DJ Martin, J Maquez, JH Lewis, FA Bontempo, BW Shaw Jr, TE Starzl, PM Winter: Intraoperative changes in blood coagulation and thromboelastographic monitoring in liver transplantation. Anesth Analg 64:888–896, 1985.

5. Kang Y, JH Lewis, A Navalgund, MW Russell, FA Bontempo, LS Niren, TE Starzl: Epsilon-aminocaproic acid for treatment of fibrinolysis during liver transplantation. Anesthesiology 66:766–773, 1987.
6. Owen CA Jr, SR Rettke, EJW Bowie, TL Cole, CC Jensen, RH Wiesner, RA Krom: Hemostatic evaluation of patients undergoing liver transplantation. Mayo Clin Proc 62:761–772, 1987.
7. Ballard HS, AJ Marcus: Platelet aggregation in portal cirrhosis. Arch Intern Med 136:316–319, 1976.
8. Aster RH: Pooling of platelets in the spleen: Role in the pathogenesis of "hypersplenic" thrombocytopenia. J Clin Invest 45:645–657, 1966.
9. Canoso RT, RA Hutton, D Deykin: The hemostatic defect of chronic liver disease. Gastroenterology 76:540–547, 1979.
10. Stein SF, LA Harker: Kinetic and functional studies of platelets, fibrinogen, and plasminogen in patients with hepatic cirrhosis. J Lab Clin Med 99:217–230, 1982.
11. Lindenbaum J: Folate and vitamin B12 deficiencies in alcoholism. Semin Hematol 17:119–129, 1980.
12. Cowan DH: Effect of alcoholism on hemostasis. Semin Hematol 17:137–147, 1980.
13. Owen JS, RA Hutton, RC Day, KR Bruckdorfer, N McIntyre: Platelet lipid composition and platelet aggregation in human liver disease. J Lipid Res 22:423–430, 1981.
14. Karpatkin KS, ML Freedman: Hypersplenic thrombocytopenia differentiated from increased peripheral destruction by platelet volume. Ann Intern Med 89:200–203, 1978.
15. Martinez J, JE Palascak, D Kwasniak: Abnormal sialic acid content of the dysfibrinogenemia associated with liver disease. J Clin Invest 61:535–538, 1978.
16. Green G, JM Thomson, IW Dymock, L Poller: Abnormal fibrin polymerisation in liver disease. Br J Haematol 34:425–439, 1976.
17. Green AJ, OD Ratnoff: Elevated antihemophilic factor (AHF, factor VIII) procoagulant activity and AHF-like antigen in alcoholic cirrhosis of the liver. J Lab Clin Med 83:189–197, 1974.
18. Knot EAR, HR Drijfhout, JW ten Cate, E De Jong, AH Iburg, LH Kahle, R Grijm: α_2-Antiplasmin inhibitor metabolism in patients with liver cirrhosis. J Lab Clin Med 105:353–358, 1985.
19. Saito H, LT Goodnough, JM Boyle, N Heimburger: Reduced histidine-rich glycoprotein levels in plasmas of patients with advanced liver cirrhosis: Possible implications for enhanced fibrinolysis. Am J Med 73:179–182, 1982.
20. Fletcher AP, O Biederman, D Moore, N Alkjaersig, S Sherry: Abnormal plasminogen-plasmin system activity (fibrinolysis) in patients with hepatic cirrhosis: Its cause and consequences. J Clin Invest 43:681–695, 1964.
21. Tytgat G, D Collen, RR DeBreker, M Verstraete: Investigatious on the fibrinolytic system in liver cirrhosis. Acta Haematol (Basel) 40:265–274, 1968.
22. Bontempo FA, JH Lewis, DH Van Thiel, JA Spero, MV Ragni, P Butler, L Israel, TE Starzl: The relation of preoperative coagulation findings to diagnosis, blood usage, and survival in adult liver transplantation. Transplantation 39:532–536, 1985.
23. Groth CG, L Pechet, TE Starzl: Coagulation during and after orthotopic transplantation of the human liver. Arch Surg 98:31–34, 1969.
24. Flute PT, MO Rake, R Williams, MJ Seaman, RY Calne: Liver transplantation in man-IV, haemorrhage and thrombosis. Br Med J 3:20–23, 1969.
25. Lewis JH, FA Bontempo, SA Awad, YG Kang, JE Kiss, MV Ragni, JA Spero, TE Starzl: Liver transplantation: Intraoperative changes in coagulation factors in 100 first transplants. Hepatology 9:710–714, 1989.
26. Kratzer MAA, J Dieterich, H Denecke, M Knedel: Hemostatic variables and blood loss during orthotopic human liver transplantation. Transplant Proc 23:1906–1911, 1991.
27. Porte RJ, FA Bontempo, EAR Knott, JH Lewis, YG Kang, TE Starzl: Systemic effects of tissue plasminogen activity-associated fibrinolysis and its relation to thrombin generation in orthotopic liver transplantation. Transplantation 47:978–984, 1989.
28. Virji MA, S Aggarwal, Y Kang: Alterations in plasminogen activator and plasminogen activator inhibitor levels during liver transplantation. Transplant Proc 21(Suppl 3):3540–3541, 1989.
29. Lewis JH, FA Bontempo, MV Ragni, TE Starzl: Antithrombin III during liver transplantation. Transplant Proc 21:3543–3544, 1989.
30. Kang YG: Monitoring and treatment of coagulation. *In*: Winter PM, YG Kang (Eds): *Hepatic Transplantation: Anesthetic and Perioperative Management.* Praeger, New York, 1986, pp 151–173.
31. Zuckerman L, L Cohen, JP Vagher, E Woodward, JA Caprini: Comparison of thromboelastography with common coagulation tests. Thromb Haemostas 46:752–756, 1981.
32. McNicol GP, AP Fletcher, N Alkjaersig, S Sherry: The absorption, distribution, and excretion of ϵ-aminocaproic acid following oral or intravenous administration to man. J Lab Clin Med 59:15–24, 1962.
33. Rake MO, PT Flute, G Parnell, R Williams: Intravascular coagulation in acute hepatic necrosis. Lancet 1:533–537, 1970.
34. Bellani KG, JA Estrin, NL Ascher, JS Najarian, J Bushman, JJ Buckley: Reperfusion coagulopathy during human liver transplantation. Transplant Proc 19 (Suppl 3):71–72, 1987.
35. Royston D: Aprotinin prevents bleeding and has effects on platelets and fibrinolysis. J Cardiothorac Anesth 5 (Suppl 1):18–23, 1991.
36. Neuhaus P, WO Bechstein, B Lefebre, G Blumhardt, K Slama: Effect of aprotinin on intraoperative bleeding and fibrinolysis in liver transplantation. Lancet 2:924–925, 1989.
37. Mallett SV, D Cox, AK Burroughs, K Rolles: Aprotinin and reduction of blood loss and transfusion requirements in orthotopic liver transplantation. (Letter.) Lancet 336:886–887, 1990.
38. Grosse H, W Lobbes, M Frambach, O von Broen, B Ringe, M Barthels: The use of high dose of aprotinin in liver transplantation: The influence of fibrinolysis and blood loss. Thromb Res 63:287–297, 1991.
39. Himmelreich G, B Kierzek, P Neuhaus, KJ Slamer, H Riess: Fibrinolytic changes and the influence of the early perfusate in orthotopic liver transplantation with intraoperative aprotinin treatment. Transplant Proc 23:1936–1937, 1991.
40. Kang Y, AM De Wolf, S Aggarwal: In vitro study of the effects of aprotinin on coagulation during orthotopic liver transplantation. Transplant Proc 23:1934–1935, 1991.
41. Anderson TLG, JO Solem, L Tengborn, E Vinge: Effects of desmopressin acetate on platelet aggregation, von Willebrand factor, and blood loss after cardiac surgery with extracorporeal circulation. Circulation 81:872–878, 1990.
42. Mannucci PM, V Vicente, L Vianello, M Cattaneo, I Alberca, MP Coccato, E Faioni, D Mari: Controlled trial of desmopressin in liver cirrhosis and other conditions associated with a prolonged bleeding time. Blood 67:1148–1153, 1986.
43. Mannucci PM: Desmopressin: A nontransfusional form of treatment for congenital and acquired bleeding disorders. Blood 72:1449–1455, 1988.
44. Kang Y, V Scott, A DeWolf, J Roskoph, S Aggarwal: In vitro effects of DDAVP during liver transplantation. Transplant Proc 25:1821–1822, 1993.
45. Kang Y, LM Borland, J Picone, LK Martin: Intraoperative coagulation changes in children undergoing liver transplantation. Anesthesiology 71:44–47, 1989.
46. Starzl TE, CG Groth, L Makowka: *Clio Chirurgica: Liver Transplantation.* Silvergirl, Austin, 1988, p 167.

Thromboelastography and Liver Transplantation

BRUCE S.A. GILLIES, M.D.

Liver transplantation is currently thought to be the optimum treatment for a number of causes of end-stage liver disease (ESLD). Significant improvements over the last 10 years with immunosuppression, organ preservation, and operative technique have improved perioperative morbidity and mortality. However, a number of problems continue to have a major impact on outcome. Bleeding and massive blood loss are two of the major challenges facing the anesthesia and surgical teams during the intraoperative management of these patients. Plasma clotting factor deficiencies are usually present due to altered hepatic synthetic function or malnutrition. In addition, hypofibrinogenemia, platelet dysfunction, thrombocytopenia, and ongoing bleeding may all be present in the patient with ESLD. Surgical dissection, massive transfusion, or inadequate replacement, dilutional changes, fibrinolysis, hypothermia, and poor graft function may augment this preexisting coagulopathy intraoperatively.

Continuous and aggressive monitoring of coagulation is necessary for a number of reasons. Blood components are a precious and limited resource. Coagulation monitoring can facilitate a rational approach to replacement therapy, which is necessary to correct the appropriate clotting abnormalities, minimize unnecessary transfusion, and avoid transfusion risk. Thromboelastography (TEG), a whole blood viscoelastic test of clot formation, strength, and elasticity has been used with liver transplantation since the early 1980s as a rapid and reliable method of monitoring the dynamic coagulation changes associated with this procedure.[1] The efficacy of the TEG as a clinically useful monitor has been established with a number of operative procedures, including liver transplantation and cardiac surgery.[2–4] It provides a measure of the speed and strength of clot formation and an overall assessment of the platelet-coagulation protein cascade interaction. Specific deficiencies in coagulation factors, fibrinogen, or the amount of platelet dysfunction are not quantitatively measured with TEG, but each of these abnormalities shows a qualitative change in the thromboelastogram. The entire process of clot formation to clot retraction or clot lysis, if present, may be observed with TEG. The initial information generated from the TEG is rapidly obtained and may be useful to guide replacement therapy.

The test involves a small amount of blood placed in a standard cuvette. A piston is placed in the blood sample. The cuvette rotates at a standard speed and around a standard angle with eventual rotation of the piston as clot formation occurs. The rotation of the piston correlates with the viscoelastic strength of the clot formed and can be measured and recorded as a function of time giving rise to the characteristic TEG tracing (Fig. 1).[1,3] Four parameters are commonly obtained from the TEG tracing. Briefly, the reaction time, or R value, is functionally the time to onset of clot formation. It correlates with initial fibrin formation and is related to the activity of clotting factors and inhibitors, such as heparin, if present. The K value represents the clot formation time and is a measure of fixed clot strength. The K value correlates with fibrin cross-linking. The α angle is a measure of the speed of clot formation. It is primarily related to fibrinogen concentration and the fibrin-platelet interaction. The MA value represents the maximum amplitude of the TEG tracing. It is related to the absolute clot strength and is a direct measure of the dynamic properties of platelets and fibrin. This value is usually affected by platelet abnormalities. As noted, the TEG is also useful in the diagnosis of fibrinolysis. If fibrinolysis is present, it will be reflected in the TEG as a loss of piston rotation. The degree of lysis may be quantified by the clot lysis index (CLI). This index is obtained from the TEG by taking the quotient of the amplitude 60 minutes (A60) after the MA and the MA, expressed as a percent.

TEG is frequently used in conjunction with several routine coagulation and hematologic tests during liver transplantation, including the prothrombin time (PT), par-

From Transplant Anesthesia, University of Washington, Seattle, Washington.

Reprint requests: Dr. Gillies, Assistant Professor, Chief, Transplant Anesthesia, University of Washington, Seattle, WA 98195.

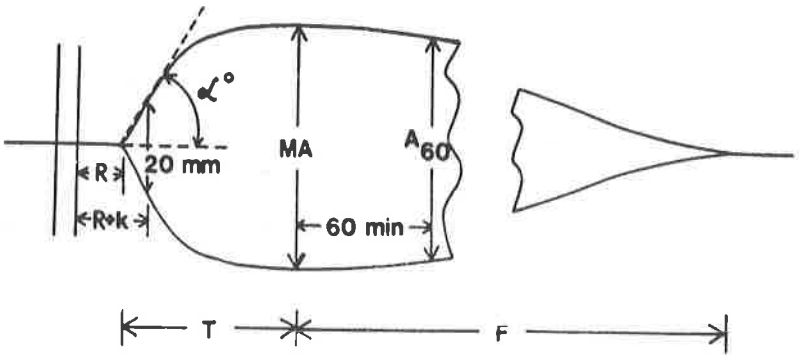

FIG. 1. Variables and normal values measured by thromboelastography. R: reaction time, 6 to 8 minutes; R + K: coagulation time, 10 to 12 minutes; α: clot formation rate, >50°; MA: maximum amplitude, 50 to 70 mm; A60: amplitude 60 minutes after MA; A60/MA × 100 = whole blood clot lysis index, more than 85%; and F: whole blood clot lysis time, more than 300 minutes. (Reprinted with permission from Kang et al.[3])

tial thromboplastin time (PTT), thrombin time (TT), fibrinogen level, platelet count, and fibrin(ogen) degradation products (FDP). The fibrinogen level provides a quantitative test of fibrinogen concentration. The platelet count only gives information about the actual platelet number. It is not a measure of the platelet function or dysfunction that may be present in patients with liver disease. FDP are often performed as a part of a routine coagulation screen as an indirect measure of fibrinolysis. The information gained from a FDP measurement is unclear as all patients undergoing surgery will show some elevated levels of FDP in the blood as clot formation occurs. This may be the case even without evidence of fibrinolysis. The PTT, PT, and TT are tests on isolated centrifuged plasma. These tests only provide information about specific aspects of the coagulation cascade. They are measurements of the time to onset of clot formation and reveal little about the important platelet–protein coagulation cascade interface.

In addition, the time it takes to obtain information from the routine coagulation profile may range anywhere from 45 minutes to 1 hour. Substantial changes in the patient's coagulation status may have occurred, rendering the information less useful. A complete TEG will take approximately 70 minutes; however, the initial information obtained from the TEG may be available in approximately 15 to 25 minutes if the TEG tracing is generated on site in the operating room laboratory or if it is sent to the main coagulation laboratory. The University of Washington Medical Center uses a closed circuit television system for real-time observation of the TEG tracing. This is described elsewhere in this issue. The thromboelastograph instruments are located in the central laboratories primarily for quality control assurance. Each liver transplant room is equipped with two television monitors capable of showing two TEG tracings, each in real-time.

PERIOPERATIVE MONITORING

Coagulation function in patients with ESLD presenting for liver transplantation varies with type and severity of disease. Most patients have generalized defects in their coagulation profiles, resulting in an overall hypocoagulable state. Concentrations of protein coagulation factors are usually low except for factor VIII and fibrinogen, which are often normal or even elevated. Kang et al[3,4] have characterized the perioperative coagulation changes in several studies of liver transplant patients. They found that the routine coagulation function test profiles are usually abnormal with prolongations of the PT and PTT in most patients. Patients with disease states characterized by hepatocellular destruction show the most significant derangements. Patients with primary neoplasms of the liver often had normal coagulation profiles. Thrombocytopenia due to hypersplenism is common in most patients with ESLD and is often accompanied by platelet dysfunction. Evidence for primary fibrinolysis has been shown to be present in as many as 15 to 30% of patients.[5,6] The typical preoperative changes in TEG are shown in Figure 2.[7] The TEG shows the expected prolongation of the reaction time and the clot formation rate characteristic of low protein clotting factors and platelets. The MA is often decreased, reflecting either the presence of thrombocytopenia, platelet dysfunction, or both.

Liver transplantation has been described as occurring in three major phases[3]: (1) preoperatively to removal of the diseased liver; (2) the anhepatic phase to reperfusion; and (3) after recirculation. As noted previously, the preexisting coagulopathy may become markedly worse during the operative proceduure. The dynamic changes in coagulation function are multifactorial. Bleeding from surgical dissection, dilutional effects from blood loss and replacement, fibrinolysis, and metabolic defects are the most common insults augmenting the coagulopathy. The routine coagulation profile is markedly abnormal at this point and does not change significantly until well after reperfusion. Its utility with the exception of fibrinogen levels and platelet counts are unclear. A typical sequence of coagulation changes occurring over the course of liver transplantation, as measured by TEG, is shown in Figure 3.[8] The marked changes in the TEG due to the multiple pathologic changes should be noted. The effects of the preexisting coagulopathy are described before. The

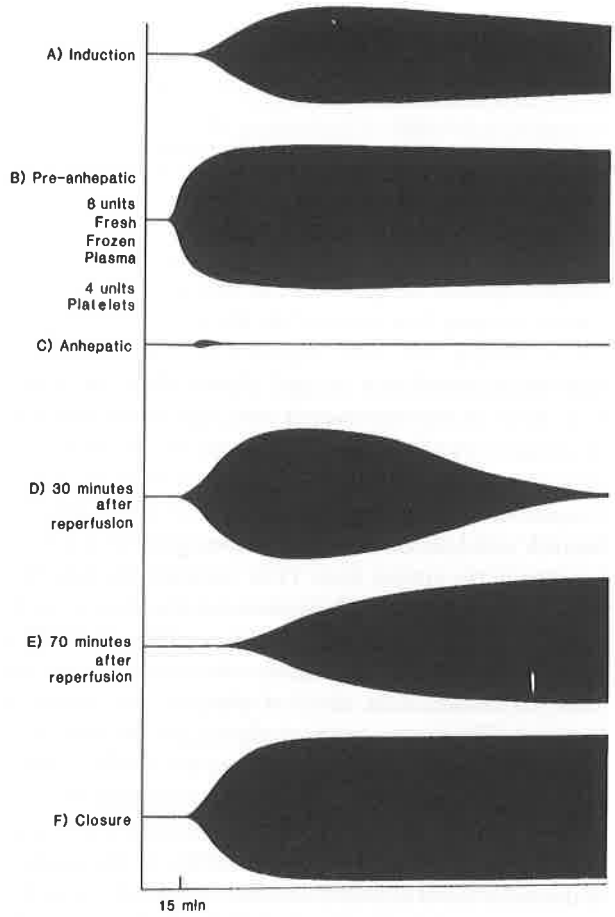

FIG. 2. The coagulation changes during liver transplantation. A: Hypocoagulability on entrance to the operating room. B: Improved coagulation after therapy. C: Fibrinolysis occurs during the anhepatic phase. D: The fibrinolysis begins to resolve after the new graft is revascularized. E and F: Coagulation function normalizes by closure. (Reprinted with permission from Spiess and Ivankovich.[7])

initial phase of transplantation is marked by significant, persistent blood loss primarily due to surgical dissection. Volume replacement often exacerbates the coagulopathy with dilutional changes in coagulation factors and platelets. This is manifest in the TEG as a further prolongation of the reaction time speed of clot formation and a decrease in the MA.

TEG has been used to guide replacement therapy. Replacement therapy consisting of fresh-frozen plasma (FFP), platelets, and cryoprecipitate based on specific TEG criteria has been shown to be useful in minimizing dilutional effects on coagulation.[3] Kang et al[3] compared replacement of plasma, fibrinogen, and platelets guided by TEG with a historical control group in which factor and platelet replacement was based on results from routine coagulation tests and clinical judgment.[3] Although the study suffers from the choice of a historical control, it is noteworthy that the overall volume administered to the TEG group was reduced by approximately 33%. The TEG group received considerably fewer units of plasma and received more transfusions of cryoprecipitate and platelets. There was no difference in the overall donor exposure in the two groups. A number of other factors may have played a role. Surgical technical advances such as experience and venovenous bypass probably contributed to improved hemostasis and a reduction in blood loss. Venovenous bypass results in a reduction in blood loss by decompression of the portal venous and systemic venous circulation below the clamping of the infrahepatic vena cava.

Data from 124 liver transplants performed at the University of Washington Medical Center between January 1990 and December 1992 concerning blood product utilization are shown in Table 1. Blood product replacement therapy and coagulation management were guided primarily by TEG, fibrinogen level, and platelet count.

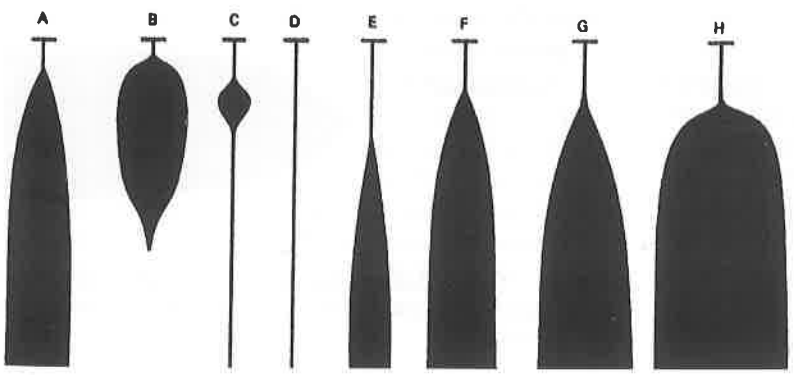

FIG. 3. Typical sequence of thromboelastography (TEG) traces during orthotopic liver transplant (OLT). A: Baseline TEG: low in clotting factors and platelets. B: Anhepatic plus 10 minutes: fibrinolysis developing. C: Anhepatic plus 45 minutes: severe fibrinolysis. D: Reperfusion plus 5 minutes: straight line TEG, no clot formation. E: Reperfusion plus 15 minutes: after tranexamic acid. F: Reperfusion plus 30 minutes: some spontaneous correction in coagulation. G: Reperfusion plus 90 minutes: additional fresh-frozen plasma and platelets given. H: Reperfusion plus 120 minutes: normal TEG apart from prolonged reaction time (international normalized ratio, 2.3). (Reprinted with permission from Mallett and Cox.[8])

TABLE 1. Blood Product Utilization

Blood product	*1990 (n = 29)*	*1991 (n = 51)*	*1992 (n = 44)*	*Total 1990–1992 (n = 124)*
Whole blood	9.8 (12.1)	5.1 (5.2)	7.4 (6.6)	7.0 (7.9)
Packed red blood cells	11.0 (10.3)	10.4 (11.2)	9.1 (8.3)	10.1 (10.0)
Red blood cells	20.8 (20.1)	15.5 (13.0)	16.5 (12.7)	17.1 (14.9)
Fresh-frozen plasma	14.0 (11.0)	13.0 (11.3)	15.8 (11.1)	14.2 (11.2)
Platelets	22.5 (16.8)	9.9 (10.1)	10.6 (11.1)	13.1 (13.3)
Cryoprecipitate	14.0 (11.0)	6.5 (11.1)	11.6 (13.1)	10.1 (12.1)
Cell saver	6.2 (9.1)	9.5 (10.1)	12.4 (11.1)	9.8 (10.4)

Overall mean red cell and blood product utilization was consistent with the TEG-monitored group in the Kang et al[3] study. The reduction in platelet use from 1990 to 1992 probably reflects our concern for a propensity to hypercoagulability and possible graft thrombosis, which can occur in the postoperative period.

Active fibrinolysis may be seen in a number of patients. Activators of plasminogen such as tissue plasminogen activator (t-PA) may be present as a result of tissue injury or bleeding. FDPs or cofactors such as protein C may also contribute to fibrinolysis. With further dissection and isolation of the diseased liver, inadequate hepatic clearance of activators may result. Circulating levels of inhibitors of plasminogen such as α_2-antiplasmin are markedly reduced. The increased proportion and imbalance of activators to inhibitors of coagulation and metabolic changes including acidosis that occur in the preanhepatic and anhepatic phases results in the so-called explosive fibrinolysis, as shown in the TEG traces in Figure 3.[3,8] The propensity to fibrinolysis is manifested as a marked reduction in the CLI. A number of studies from liver transplantation and cardiac surgery have looked at different pharmacologic regimens to treat fibrinolysis, using TEG as a measure of response.[6,7,9] Prior to studies with TEG, therapy was empirical and therapeutic endpoints were undefined. Both ϵ-aminocaproic acid (EACA) and aprotinin have been used with considerable success with liver transplantation.[6,10] Not only did both therapies show a significant reduction in the degree of fibrinolysis, but they also had considerable impact on the degree of bleeding in the post-recirculation phase. Because the use of antifibrinolytic agents could also promote prothrombotic complications, routine use awaits the results of controlled clinical trials.

At recirculation, there is usually further deterioration of the coagulopathy primarily due to fibrinolysis, but also due to humoral substances released from the washout of the new liver, acidosis, severe hypocalcemia, and, if present, hypothermia. Blood loss may be considerable at this point and dilutional effects may be superimposed due to the large amounts of fluid needed for resuscitation. Frequent TEGs are obtained with each therapeutic intervention to minimize unnecessary transfusion and to direct appropriate transfusion therapy. Both Groth[11] and Kang et al[3] have shown that overall coagulation function and fibrinolysis usually begin to improve at approximately 30 to 45 minutes following reperfusion. Levels of protein coagulation factors in the blood begin to rise except for factor V and VIII corresponding to recovery of synthetic function of the grafted liver. TEG variables reaction time (R), α, MA and A60 all show considerable improvement. Platelet transfusion may have a marked effect on the TEG result. TEG recordings with severe thrombocytopenia and after transfusion with 10 U of platelets are shown in Figure 4.[12] In the presence of significant thrombocytopenia due to hypersplenism, however, minimal long-term benefit is achieved with continuous platelet transfusions. In this case, transfusion with FFP or cryoprecipitate may manifest more of an effect on the TEG. In the absence of uncontrolled or massive bleeding, it is usually unnecessary to transfuse more than 6 to 8 U of platelets in the post-recirculation phase.

Continual improvement in coagulation function occurs with clearance of humoral factors and with improvement in the function of the newly transplanted liver. Improvement in the routine coagulation tests begin to occur in the post-recirculation phase but may remain significantly abnormal. As the coagulation profile begins to return to normal, the TEG may be useful in distinguishing between surgical and coagulopathic causes of bleeding.

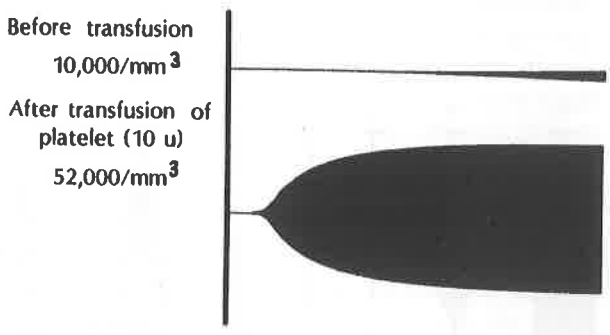

FIG. 4. Thromboelastographic patterns before and after transfusion of platelets (10 U). (Reprinted with permission from Kang.[12])

In the absence of significant untreated abnormalities in the TEG tracing a surgical cause of bleeding should be presumed.

Not all patients presenting for liver transplantation have major derangements in coagulation function. As already noted, patients with hepatocellular injury have the most severe changes in their coagulation profiles. A number of diseases may present with minimal changes or even normal function. In fact, there are several diseases, Budd-Chiari syndrome and patients with primary hepatocellular tumors, for example, who may present with a hypercoagulable profile. Hypercoagulability can be easily detected using TEG. Short reaction times, fast speed of clot formation, and markedly increased MA are hallmarks of hypercoagulability. Routine coagulation tests are usually ineffective in making the diagnosis. TEG may be useful if these patients require anticoagulant therapy after transplantation.[13]

Postoperatively, continued bleeding from coagulopathy is usually due to inadequate graft function. Typical TEG traces and routine coagulation tests show deficiencies in protein coagulation factors and thrombocytopenia. Replacement therapy, if necessary, may easily be gauged from the TEG. Ongoing bleeding, requiring continuous red cell transfusions, is usually due to surgical causes. A number of investigators have shown that a propensity to a hypercoagulable state may occur in the postoperative period.[13–15] Thrombosis of the hepatic arterial anastomosis may be devastating to the survival of the transplanted graft. Continuous monitoring of the TEG may help in the diagnosis and prevention of this complication.

SUMMARY

TEG has played an integral part in the growth of liver transplantation. The group at the University of Pittsburgh early on realized that coagulation dysfunction during liver transplantation would be both severe and dynamic. Each phase of the operation appears to have both predictable and unexpected changes in clot dynamics. The routine coagulation profile, although of great use, does not provide an overview of the interaction of stimulators, inhibitors, and available procoagulants to effect a final process, the production of a solid clot. The TEG is a unique gross test of clot strength perfectly suited to the changes during liver transplantation. The initial pioneering work during liver transplantation has inspired the work of others in related surgical fields to explore its utility. There is little doubt that its full utility has not yet been realized. Many questions still remain with regard to liver transplantation. New medications such as aprotinin will be applied to this procedure over the next few years. What effect these new medications will have on hemorrhage or thrombosis of vascular anastomoses is yet to be adequately explored. A new awareness appears to be arriving that normal or excessively hypercoagulable states could contribute to such thromboses. TEG as a technology will certainly contribute to a number of future studies and clinical care, which will enhance the conduct of liver transplantation in the future.

REFERENCES

1. Kang Y: Anesthesia for liver transplantation. *In*: Benumuf JL, AS Wheeler (Eds): *Anesthesiology Clinics of North America. Anesthesia and New Surgical Procedures.* WB Saunders, Philadelphia: 1989, pp 551–580.
2. Tuman KJ, BD Spiess, RJ McCarthy, AD Ivankovich: Comparison of viscoelastic measures of coagulation after cardiopulmonary bypass. Anesth Analg 69:69–75, 1989.
3. Kang Y, DJ Martin, J Marquez, JH Lewis, FA Bontempo, BW Shaw, TE Starzl, PM Winter: Intraoperative changes in blood coagulation and thromboelastographic monitoring in liver transplantation. Anesth Analg 64:888–896, 1985.
4. Kang Y, LM Borland, J Picone, LK Martin: Intraoperative coagulation changes in children undergoing liver transplantation. Anesthesiology 71:44–47, 1989.
5. Fletcher AP, O Biederrnan, D Moore, N Alkjaersig, S Sherry: Abnormal plasminogen-plasmin system activity (fibrinolysis) in patients with hepatic cirrhosis. J Clin Invest 43:681–684, 1964.
6. Kang Y, JH Lewis, A Navalgund, MW Russell, FA Bontempo, LS Niren, TE Starzl: Epsilon-aminocaproic acid for treatment of fibrinolysis during liver transplantation. Anesthesiology 66:766–773, 1987.
7. Spiess BD, AD Ivankovich: Thromboelastography: A coagulation monitoring technique applied to cardiopulmonary bypass. *In*: Ellison N, D Jobes (Eds): *Effective Hemostasis in Cardiac Surgery.* W.B. Saunders, Philadelphia: 1988, pp 163–181.
8. Mallett SV, DJA Cox: Thromboelastography. Br J Anaesth 69:307–313, 1992.
9. Kang Y, AM De Wolf, S Aggarwal, E Campbell, LR Martin: In vitro study of the effects of aprotinin on coagulation during orthotopic liver transplantation. Transplant Proc 23:1934–1935, 1991.
10. Mallett S, K Rolles, D Cox, A Burroughs, B Hunt: Intraoperative use of aprotinin (Trasylol) in orthotropic liver transplantation. Transplant Proc 23:1931–1932, 1991.
11. Groth CG: Changes in coagulation. *In*: Starzl TE (Ed): *Experience in Hepatic Transplantation.* WB Saunders, Philadelphia: 1969, pp 159–175.
12. Kang YG: Monitoring and treatment of coagulation. *In*: Winter PM, YG Kang (Eds): *Hepatic Transplantation. Anesthetic and Perioperative Management.* Praeger, New York, 1986, pp 151–173.
13. Howland WS, O Schweizer, P Gould: A comparison of intraoperative measurements of coagulation. Anesth Analg 53:657–663, 1974.
14. Stahl RL, A Duncan, MA Hooks, JM Henderson, WJ Millikan, WD Warren: A hypercoagulable state follows orthotopic transplantation. Hepatology 12:553–558, 1990.
15. Howland WS, EB Castro, JG Fortner: Hypercoagulability. Thromboelastographic monitoring during extensive hepatic surgery. Arch Surg 108:605–608, 1974.

Application of Thromboelastography in Other Medical and Surgical States

CLARA I. TRAVERSO, M.D., Ph.D., JOSEPH A. CAPRINI, M.D., F.A.C.S., and JUAN I. ARCELUS, M.D., Ph.D.

Since thromboelastography (TEG) has been applied to the study of numerous fields, it is not feasible to dedicate one article to each particular issue in this review. Accordingly, this article will recapitulate the role of TEG in studying those clinical states that are not included in other articles of this special issue of *Seminars*.

MALIGNANCIES

There is no doubt regarding the role of TEG in detecting increased coagulability, which is considered the most important advantage of this test system.[1,2] Based on this characteristic, TEG has often been utilized in conditions that either could be followed by hypercoagulability or in which hypercoagulability already exists. Patients with malignancies constitute such a situation, and so cancer has been the center of numerous thromboelastographic studies.

We reported that TEG is able to not only identify individuals with accelerated coagulability, but also to discriminate between normal, healthy populations and others with proven malignancies.[3] Using the celite-activated thromboelastographic technique introduced by us, which combines native whole blood TEG and celite-activated TEG, 141 normal persons on no medication were compared to 121 cancer patients. Results were excellent, showing a 100% specificity and 97% sensitivity of TEG. In a parallel experimental study, we have found TEG to reflect qualitatively the presence of carcinoma.[4] Analyzing childhood cancer, Hathaway and Hays[5] had previously reported that accelerated coagulability, as detected by TEG, was more often seen in those cases in which the disease was disseminated, as well as in relapsed brain cancer.

In another study,[6] we compared the findings of Hathaway and Hays with results found by Raby and Pieptea, who used decalcified whole blood and native whole blood, respectively, for the thromboelastographic identification of accelerated coagulability in the same two populations, i.e., healthy individuals versus cancer patients. From that comparison, it was concluded that the different thromboelastographic parameters proposed by the three separate groups of investigators measured the same biologic process and were able to identify accelerated coagulability in a high percentage of the analyzed cases. Nevertheless, celite-activated TEG provided more useful information than did native whole blood TEG in all cases. Using celite-activated TEG, but using decalcified whole blood instead of native whole blood, Aznar and associates[7] studied 198 patients with proven gynecologic malignancies. They reported TEG to be strongly useful for detecting hypercoagulable states in cancer patients, which agrees with other authors' data.[8–11] However, they did not find TEG to be a specific test for detecting cancer. The main point is that TEG detects changes in coagulability, regardless of the etiology, as there are several pathologic states that are able to generate hypercoagulability. In this direction, we have analyzed celite-activated TEG performed in native whole blood as a test for preoperatively detecting cancer in patients entering the hospital for breast biopsies,[12] that is, two well-defined populations were not compared in this case, which differs from the previously described findings. Results were less optimal than those previously reported, showing a 94% sensitivity (48 of 51) and a 74% sensitivity (50 of 68). Consequently, it was concluded that TEG is not specific for detecting

From the Department of Surgery and Related Specialties, University of Granada Hospital, Granada, Spain (C.I.T.), Department of Surgery, The Glenbrook Hospital and Northwestern University Medical School, Glenview, Illinois (J.A.C.), and Department of Surgery, Hospital of the Axarquía, Vélez-Málaga (Málaga), Spain (J.I.A.).

Reprint requests: Dr. Traverso, Department of Radiation Oncology, "La Paz" Hospital, 28016 Madrid, Spain.

cancer, although it is a tool that may help to identify that state.

HYPERCOAGULABLE STATES

TEG has been widely used for studying hypercoagulable states developed in patients undergoing several surgical procedures. Although this condition of increased coagulability is one of the most often investigated, it is reviewed in other articles of this issue of *Seminars* and therefore will not be covered here.

Fisch and Freedman[13] analyzed data from 4315 women taking oral contraceptives and reported a more rapid fibrin formation and increased clot firmness as measured by TEG performed in whole blood. These data agree with previous thromboelastographic results.[14–16] Findings were not related to dosage, type of drug, or duration of the exposure. Interestingly, reversibility in the hemostatic condition was recorded when ceasing the treatment. De Nicola[17] reported that adding cortisone in vitro to plasma increased the blood coagulability as well. The same author recorded a tendency to hypercoagulability after the ingestion of fatty meals.[17] The existence of hypercoagulable states has also been reported in overweight patients,[18] as well as in end-stage renal failure.[11,19,20] Finally, with regard to TEG and hypercoagulability, Confino and associates,[21] using the celite-activated TEG technique, did not find lupus anticoagulant levels and TEG to be interchangeable as predictors of the development of hypercoagulable states. However, Raby[19] found TEG able to differentiate between those cases of acute disseminated lupus associated with hypercoagulability and those associated with hypocoagulability, and proposed TEG for the management of these two groups of patients.

DISSEMINATED INTRAVASCULAR COAGULATION

Disseminated intravascular coagulation (DIC) is a complex clinical state that requires special care in patients who already are critically ill and whose surveillance is difficult because of the underlying disease itself. Hathaway[22] observed that in neonates with respiratory failure, DIC was often diagnosed when overt clinical bleeding occurred. Findings like this support the fact that any tool that may help to display promptly the development of DIC would be useful for avoiding grave complications.

In this direction, Watkins et al[23] analyzed the role of celite-activated TEG and other hemostatic tests for diagnosing DIC in newborns. Thirteen neonates requiring ventilation for respiratory failure were studied. Good results were reported for the thromboelastographic analysis, plasminogen levels, and fibrin(ogen) split product levels. Additionally, the thromboelastographic index had a prognostic value, since it monitored the progressive tendency to hypocoagulability, which continued to increase until the patients died. In some of them, tracings were straight lines, indicating complete loss of coagulability. Another interesting finding was related to the fact that thromboelastographic parameters remained within normal ranges until the first 12 hours of age, after which they became progressively more hypocoagulable and were pathologic at 48 hours of age. On the other hand, in the two survivors the thromboelastographic values fluctuated near the normal ranges despite their critical condition.

A prognostic value of the thromboelastographic index has also been reported by Zuckerman et al[24] when studying thermal injuries, where the index discriminated between patients who survived and those who did not. Raby[19] proposed TEG, performed in decalcified whole blood and in platelet-rich plasma, for diagnosing and treating DIC in the emergency room.

LOCAL INTRAVASCULAR COAGULATION

In cases of local intravascular coagulation (LIC), a role for TEG has been reported as well. By histologic analyses, LIC has been implicated in acute rejections following cardiovascular transplants. Raby[19] applied TEG, performed in decalcified whole blood, to the study of hemostatic changes associated with that condition. He found that there is a "dynamic hypocoagulability," as exhibited by increased values of the thrombodynamic potential index (TPI), together with either a "kinetic iso- or hypercoagulability" or a "kinetic hypocoagulability," as shown by prolonged values of both longitudinal parameters, the reaction time and the clot formation time. The knowledge of the biologic significance of these changes allows not only a better understanding of the underlying state, but also, and more importantly, its treatment by heparin or other therapy. LIC is also responsible for both thromboembolic and bleeding complications that may simultaneously develop following a hiatal hernia. Raby[19] also proposed TEG for the study and management of heparin therapy in these patients.

BLEEDING

TEG provides worthwhile information regarding bleeding complications that may be presented following other disorders. Bleeding related specifically to surgical procedures is reviewed in other articles of this issue of *Seminars*. An especially difficult condition to be managed is hemorrhage following portal hypertension, which was

studied by Raby and Couinaud.[25] By combining the information provided by thromboelastographic studies performed in decalcified whole blood (analyzing the TPI) and in platelet-rich plasma (analyzing reaction time), the authors identified the ongoing stages that a patient with portal hypertension endures, from hyper- to hypocoagulability, as well as adjusted the treatment to each state, under circumstances that are frequently difficult to handle. Twenty-nine of 38 patients studied bled; in all cases, the coagulation state and the treatment of bleeding complications were controlled by the thromboelastographic study with excellent results.

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REFERENCES

1. Audier M, A Serradimigni: *Savoir interpreter un thrombo-elastogramme ou l'exploration precise de la coagulation sanguine.* Paris, Maloine, 1962, pp 135.
2. Raby C: Exploración del tiempo trombodinámico. *In*: Hemorragias y Trombosis. Toray-Masson, Barcelona, 1968, pp 190–211.
3. Caprini JA, L Zuckerman, A Cohen, JP Vagher, V Lipp: The identification of accelerated coagulability. Thromb Res 9:167–180, 1976.
4. Haid M, L Zuckerman, JA Caprini, S Kurtides, JP Vagher: Thromboelastographic changes in carcinoma: an animal model. J Med 7:189–216, 1976.
5. Hathaway WE, T Hays: Hypercoagulability in childhood cancer. J Pediatr Surg 10:893–899, 1975.
6. Cohen E, JA Caprini, L Zuckerman, JP Vagher, B Robinson: Evaluation of three methods used to identify accelerated coagulability. Thromb Res 10:587–604, 1977.
7. Aznar J, F Soto, P Villa, L Lonjedo, P Bulufer: Valoración tromboelastográfica de la hipercoagulabilidad plasmática. Sangre 24: 1–8, 1979.
8. Gormsen J: Thrombelastographic findings in surgery. Acta Chir Scand 122:466–470, 1961.
9. Traverso CI: Estudio de las modificaciones tromboelastográficas inducidas por la heparina a bajas dosis y el dextrano-70 como profilaxis de la enfermedad venosa postoperatoria. Postgraduate Thesis. University of Granada School of Medicine (Spain), 1986.
10. Zyryanov BN, II Tutrin, VV Udut: Risk evaluation of thromboembolic diseases according to functional test data in patients with advanced malignancies. Neoplasma 34:353–360, 1987.
11. Edwards CM, PK Donnelly: Comparison of thrombelastography with conventional assays of coagulation. Br J Haematol 80 (Suppl 1):32, 1992.
12. Caprini JA, L Zuckerman, P Vagher, J Mitchell, E Cohen: Coagulation changes in patients with breast lesions: A possible test for detecting cancer. *In*: *Neoplasm Immunity: Experimental and Clinical.* Elsevier, North Holland, 1980, pp 181–186.
13. Fisch IR, SH Freedman: Oral contraceptives, ABO blood groups, and in vitro fibrin formation. Obstet Gynecol 46:473–479, 1975.
14. Poller L, JM Thompson, W Thomas: Oestrogen/progestogen oral contraception and blood clotting: a long-term follow-up. Br Med J 4:648–650, 1971.
15. Lorram J, P Harel: Effects of certain contraceptive hormones on blood coagulation. Fertil Steril 23:422–427, 1972.
16. Siro B, M Misz, J Bazsó: Thromboelastographic studies in oral contraception. Am J Obstet Gynecol 116:1167–1168, 1973.
17. De Nicola P: *Thromboelastography.* Charles C Thomas, Springfield, IL, 1957, pp 110.
18. García-Monteavaro M, M Rodriguez, M Diez, A Navarro: Thromboelastographic assays of the clotting process in situations of obesity and caloric restriction. Rev Esp Fisiol 42:57–62, 1986.
19. Raby C: *Coagulaciones intravasculares diseminadas y localizadas.* Toray-Masson, Barcelona, 1976, pp 255.
20. Holloway DS, JP Vagher, JA Caprini, NM Simon, LF Mockros: Thromboelastography of blood from subjects with chronic renal failure. Thromb Res 45:817–825, 1987.
21. Confino E, A El-Roeiy, J Friberg, N Gleicher: The thromboelastogram and circulating lupus anticoagulant. J Reprod Med 4:289–291, 1989.
22. Hathaway WE: Coagulation problems in the newborn infant. Pediatr Clin North Am 17:929–942, 1970.
23. Watkins MN, S Swan, JA Caprini, TH Gardner, I Zuckerman, JP Vagher: Coagulation changes in the newborn with respiratory failure. Thromb Res 17:153–175, 1980.
24. Zuckerman L, JA Caprini, V Lipp, JP Vagher: Disseminated intravascular multiple systems activation (DIMSA) following thermal injury. J Trauma 18:432–439, 1978.
25. Raby C, C Couinaud: Les trubles de la coagulation dans les hemorragies des hypertensions portales. Chirugie 102:186–196, 1976.

Thromboelastographic Modifications Induced by Intravenous and Subcutaneous Heparin Administration

CLARA I. TRAVERSO, M.D., Ph.D., JOSEPH A. CAPRINI, M.D., F.A.C.S., JUAN I. ARCELUS, M.D., Ph.D., and IGNACIO M. ARCELUS, M.D., Ph.D.

Hartert introduced thromboelastography (TEG) in clinical practice in 1951. The original use that he proposed was to monitor anticoagulant therapy and detect thrombosis. Since then, TEG has been widely used for studying drugs influencing the hemostatic mechanism, particularly heparin.

De Nicola[1] was the first to describe the thromboelastographic modifications induced by heparin. The standard thromboelastographic response to this anticoagulant agent was characterized by a lengthening of the reaction time, and the clot formation time, and a decrease of the maximal amplitude (MA). This response was dependent on the degree of anticoagulation, that is, on the dosage and route of heparin administration, as well as the patient's response to the drug.

As shown in Figure 1, the thromboelastogram following intravenous heparin administration has a more peculiar appearance than the tracing recorded when the subcutaneous route is used, with very marked modifications of the reaction and coagulation times (R and K) and MA. The thromboelastogram has a typical shape of a spindle, as in hemophilia (in which R and K values are prolonged and MA is normal or increases later), but it is narrow, as in thrombocytopenia. This reflects the relatively low degree of stiffness of the clot generated because of a slow clotting process. The difference between the thromboelastogram following heparin administration and the tracing of acquired or congenital circulating anticoagulants is on MA, which is always normal in the latter case.

Thromboelastographic analysis of the intravenous administration of heparin has been widely investigated. In the past, TEG was especially used for monitoring heparin therapy;[2–6] in the current decade, TEG as a method for monitoring intravenous heparin was primarily applied in cardiopulmonary bypass, having proven its role in this surgical procedure.[7–9]

INTRAVENOUS HEPARIN MONITORING

It has been stated that the most informative tests to monitor anticoagulant drugs are those that record all phases of the coagulation-fibrinolytic system and, at the same time, evaluate the different components involved in the clot formation.[5,10,11] This is the case with TEG; when it is performed in whole blood, it resembles what occurs in the patient's blood by reflecting the interaction of all components of the clotting process.[4,12–14] For this reason, TEG has been reported as an excellent technique for monitoring heparinized patients.[3–6] Native whole blood, not plasma, is thus the specimen of choice for the thromboelastographic monitoring of heparin therapy. Additionally, it is known that the fibrinolytic response of the endothelium changes following the injection of an intravenous bolus of heparin,[15] which can be best analyzed if TEG is performed in whole blood.

TEG records the outcome of fibrin formation in a graphic way not measured by most other hemostatic tests and reflects the biologic effectiveness of any anticoagulant treatment.[2–4] Furthermore, the MA is able to provide an important clue to platelet function.[16,17] TEG becomes an especially valuable monitor of intravenous heparin

From the Department of Surgery and Related Specialties, University of Granada Hospital, Granada, Spain (C.I.T., I.M.A.), Department of Surgery, The Glenbrook Hospital and Northwestern University Medical School, Glenview, Illinois (J.A.C.), and Department of Surgery, Hospital of the Axarquía, Vélez-Málaga (Málaga), Spain (J.I.A.).

Reprint requests: Dr. Traverso, Department of Radiation Oncology, "La Paz" Hospital, 28016 Madrid, Spain.

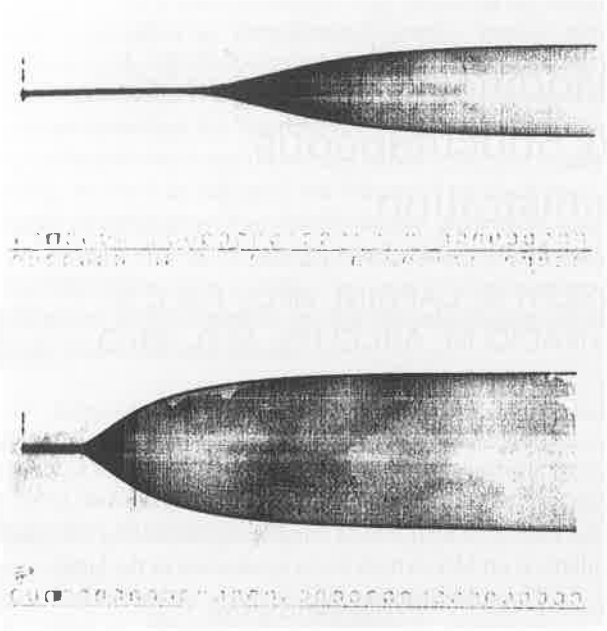

FIG. 1. Thromboelastographic modifications induced by heparin. Tracing at the top registers intravenous heparin administration. At the bottom, thromboelastogram after subcutaneous administration of the drug. (Authors' tracings.)

administration in patients who develop heparin-related complications that are known to affect platelets.[18,19] Using the TEG, Caprini et al have reported successful prediction of some heparin-associated complications in patients who were administered heparin therapy for thromboembolic events. The clinical application of this thromboelastographic aspect followed several experimental studies in dogs in which Caprini and associates analyzed the heparin interference on platelet function.[20,21]

Another reason for advocating TEG use in intravenous heparin monitoring is the fact that the test is able to detect small therapeutic dosages, as small as in 1000 IU.[22,23] Howland and associates[22] applied this singular thromboelastographic characteristic to the treatment of hypercoagulable states, which developed from extensive hepatic surgery, with low doses of heparin. Based on this thromboelastographic aspect and the original idea of De Takats,[24] Traverso[25] implemented a heparin tolerance test using TEG. When analyzing Hartert's parameters, that is, R, K and MA, this heparin tolerance test allowed for the assessment of the patient's response to a single 2500 IU intravenous bolus of heparin (Figs. 2 and 3). The measurement of R every 2 minutes during the 10 minutes after a 1000 IU intravenous bolus of heparin allows, according to Lee and associates,[26] evaluation of the patient's sensitivity to heparin administration. Vagher et al[23] utilized this thromboelastographic attribute to study differences between heparin salts when administered to healthy volunteers.

The wide variation in the heparin response among patients and within each patient, as well as the unpredictable kinetics of heparin,[27-29] necessitates individual management of patients to avoid heparin therapy failure. As already described, the optimal heparin dosage for a given patient can be achieved by a thromboelastographic adjustment,[4-6,30] which is especially important in heparin-resistant patients.[5,25] Heparin therapy should be monitored most closely on the first few days after the acute thromboembolic event, since heparin requirements are usually greater at this time.[31] In addition, the highest risk of bleeding derived from the use of heparin occurs on the third day of therapy, especially in severely ill women who, in some cases, have been administered acetylsalicylic acid.[32] Regarding bleeding, the information TEG provides on platelets by the MA analysis is important because of the lack of a clear relationship between heparin interference with platelet function and bleeding.

Although it is clear that the role of TEG for monitoring intravenous heparin therapy is established, there remain controversies about which should be the thromboelastographic parameter selected. Lee and associates[33] proposed R values in native whole blood TEG to be prolonged to 1.5 to 2 times the baseline values. According to Raby,[34] the thrombodynamic potential index (TPI) calculated on a decalcified whole blood thromboelastogram was the best parameter to adjust heparin administration because it describes the patient's tolerance to heparin treatment. To Eliot and associates[2] both longitudinal parameters (R and K) were adequate; however, they measured K at 5 mm, instead of at 20 mm, and administered therapeutic heparin subcutaneously.

In our experience, Caprini's thromboelastographic index which consists of native whole blood TEG plus celite-activated TEG (see Spiess in this issue of *Seminars*), ranging from -8 to -15, interpreted together with the activated partial thromboplastin time (aPTT) and platelet count, is a suitable monitor for heparin therapy. Similarly, our experience using decalcified whole blood TEG has been excellent, but limited. We recommend a ratio of anticoagulant/blood equal to 0.5/2, that is, a dilution equal to 1/5 (instead of 1/20 proposed by Raby[4]), as well as the measurement of K and MA, since R is more affected by manipulations when decalcified whole blood TEG is used.[35]

All authors agree, however, that it is imperative to compare TEG results to the baseline values for each patient instead of using predetermined ranges. In other words, normal ranges for each laboratory need to be established. To adjust heparin dosages individually, the thromboelastographic parameter values should be brought within the TEG baseline ranges of that patient.

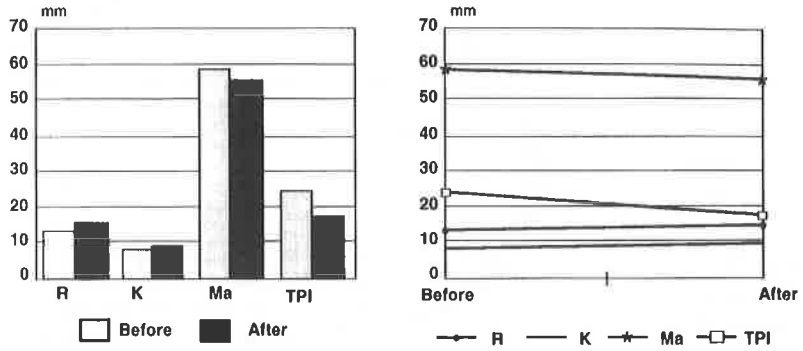

FIG. 2. Thromboelastographic response to a 2500 IU intravenous bolus of sodium heparin (n = 100). Graphic to the right illustrates modifications. R: reaction time; K: clot formation time; MA: maximal amplitude; TPI: thrombodynamic potential index. **:$p < 0.001$. (Authors' study.)

By doing so, the risk of both clotting and bleeding complications is low.

SUBCUTANEOUS HEPARIN MONITORING

TEG has been less utilized as a monitor of subcutaneous heparin administration than as a monitor of heparin when the intravenous route is used. Subcutaneous low-dose heparin (LDH) is primarily administered to avoid the development of a hypercoagulable state. Even though many authors have studied the hypercoagulable state by TEG, especially in the perioperative period, only limited data are available regarding the thromboelastographic analysis of subcutaneous LDH effects on coagulation.

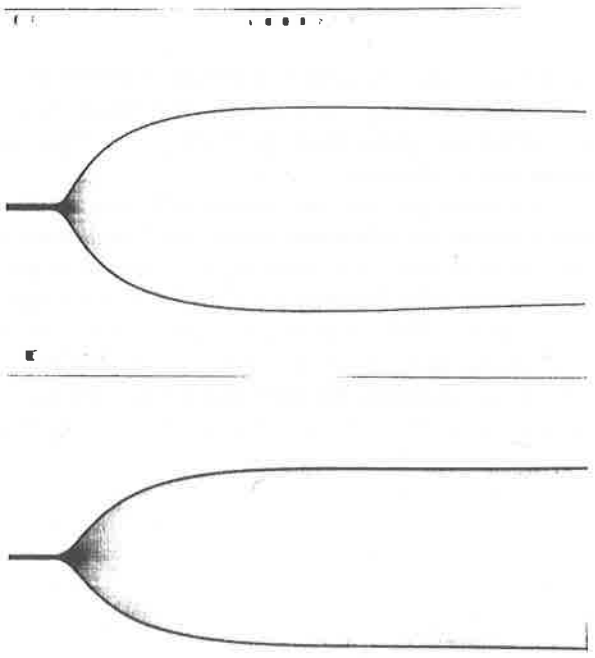

FIG. 3. Heparin tolerance test performed by thromboelastography (TEG) on a 59-year-old patient. Cholelithiasis. TEG baseline at the top. At the bottom, TEG at 120 minutes following an intravenous bolus of heparin. TEG baseline values: R: 14.5; K: 8.0; MA: 56.5; TPI: 16.2. TEG after heparin: R: 16.0; K: 10.0; MA: 49.5; TPI: 9.8. (Authors' tracings.)

Most authors agree that whole blood is the best specimen to be used for monitoring LDH administered subcutaneously.[4,36,37] As already mentioned, the thromboelastogram following subcutaneous LDH administration presents the same modifications as those after intravenous heparin, that is a lengthening of R and K, and a decrease of MA, but these changes are much less marked. In most cases, however, if prophylactic doses of heparin are administered, all TEG parameters can be measured (Fig. 1).

Torras-Barba and associates[37] reported that the TEG was unable to detect postoperative modifications induced by prophylactic heparin on coagulation of patients who underwent surgery for trauma or orthopedic problems. However, it should be kept in mind that their conclusions could be somehow biased, since the authors compared the patients' data to the data from a control group of healthy volunteers. As emphasized before, TEG results of any population studied need to be compared to TEG baseline values obtained from that specific population; only in this case, can one consider that the results are being compared to a reliable control group, that is, the baseline tracings.

In doing so, we have reported excellent results in general surgical patients, after studying 30 patients older than 40 years whose surgical procedures required at least 1 hour under general anesthesia, that is, patients who were at risk of developing thromboembolism.[38] We studied tracings preoperatively, the third and the seventh postoperative days, measuring reaction time, coagulation times, R, K, MA, TPI, MA/R + K, and MA/K 120 minutes after the subcutaneous injection of prophylactic heparin. In one-third of the patients we performed another thromboelastogram immediately prior to surgery, in the operating room, about 120 minutes after the preoperative heparin dose was given. The results were correlated with postop-

erative bleeding complications. As illustrated in Figure 4, the thromboelastographic study properly depicted the coagulation-anticoagulation state throughout the perioperative period; consequently, it allowed us to assess whether or not heparin prophylaxis was adequate.

We concluded that heparin prophylaxis prevented the development of the postoperative hypercoagulable state, as detected by TEG, which has been observed in untreated general surgical patients by using TEG as well.[25,39,40] Furthermore, by using TEG to monitor heparin dosing, hemorrhagic complications were avoided. This agrees with the conclusions of Sagar and associates.[41]

Evaluating the effects of LDH in other hypercoagulable states, some authors have reported good results.[42,43] In contrast, Vernese and associates[44] found that 800 IU of subcutaneous heparin administered experimentally to five dogs had no effect on the hemostatic parameters as measured by TEG 60 minutes after the heparin injection, and this dosage did not prevent the accelerated coagulation associated with surgery. The explanation for this may be that the dosage was too low, especially for subcutaneous administration, to induce detectable thromboelastographic modifications. Additionally, the blood sample was withdrawn earlier than the blood levels following subcutaneous injection.

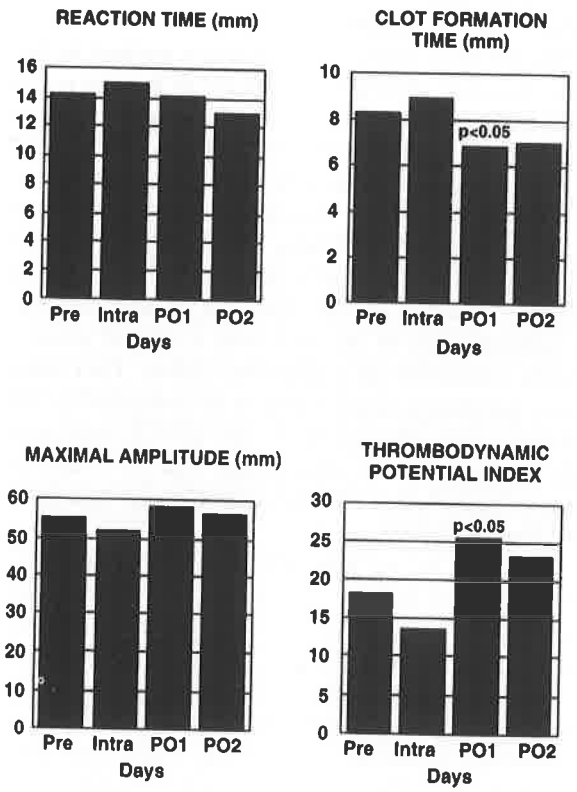

FIG. 4. Thromboelastographic analysis of general surgical patients who were administered prophylactic heparin subcutaneously. Pre: preoperative period; Intra: intraoperative period; PO1: third postoperative day; PO2: seventh postoperative day. (Authors' study.)

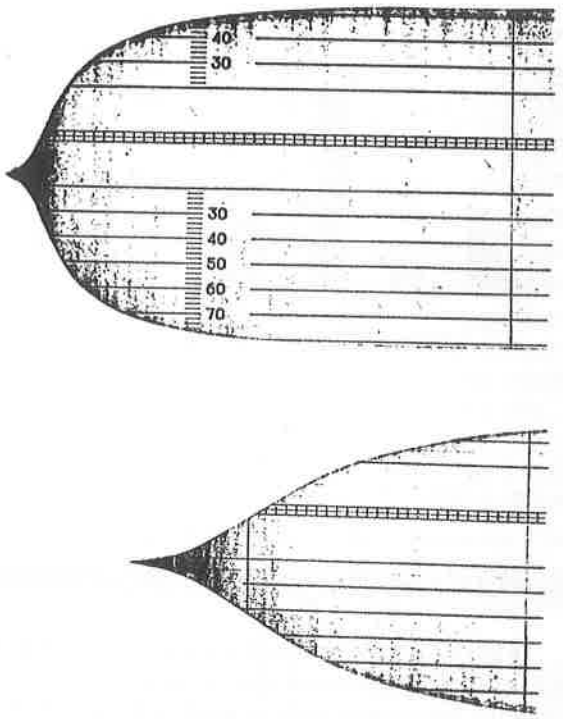

FIG. 5. Celite-activated thromboelastography as a monitor of adjusted subcutaneous heparin administration. (Authors' tracing.)

In clinical practice, the standard LDH is not considered to require any laboratory monitoring.[45] However, the adjustment of LDH is recommended in patients at high risk of developing thromboembolism.[46,47] In our experience, Caprini's TEG index in association with the aPTT, is a suitable monitor for that high-risk population. In general, our guideline for LDH prophylaxis consists of keeping the aPTT at 1.3 to 1.5 times the control value with a negative TEG index ranging from -2 to -7 (Fig. 5). Although our results are optimal, it seems reasonable to underscore the issue that more prospective studies, evaluating thromboelastography or any other test, are needed to establish definitively the laboratory monitoring of subcutaneous heparin administration in high-risk patients, as well as in the heparin-resistant group.

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REFERENCES

1. De Nicola P: *Thromboelastography*. Charles C Thomas, Springfield IL, 1957, pp 110.
2. Eliot RS, KN von Kaulla, G Blount: Thromboelastographic studies with a twenty-four-hour schedule for subcutaneous heparin. Circulation 24:1206–1214, 1961.
3. Audier M, A Serradimigni: *Savoir interpreter un thrombo-elastogramme ou l'exploration precise de la coagulation sanguine*. Maloine, Paris, 1962, pp 135.
4. Raby C: Exploración del tiempo trombodinámico. *In: Hemorragias y trombosis*. Toray-Masson, Barcelona, 1968, pp 190–211.
5. Lee BY, FS Trainor, D Kavner, WJ McCann: Monitoring of heparin therapy with thromboelastography. Surg Gynecol Obstet 149:843–846, 1979.
6. Caprini JA, JP Vagher, SJ Rabidi, JE Mitchell: Laboratory monitoring of continuous intravenous heparin therapy. Thromb Res 29:91–94, 1983.
7. Spiess BD, KJ Tuman, RJ McCarthy, GA Delaria, R Schilo, AD Ivancovich: Thromboelastography as an indicator of post-cardiopulmonary bypass coagulopathies. J Clin Monit 3:25–30, 1987.
8. Tuman KJ, BD Spiess, RE Schoen, AD Ivankovich: Use of thromboelastography in the management of von Willebrand's disease during cardiopulmonary bypass. J Cardiothorac Anesth 1:321–324, 1987.
9. Gillies BSA, BD Spiess: Cardiopulmonary bypass coagulation management, education, and transfusion practice at the University of Washington. Am Society of Anesthesiology, October, 1991 (Abst).
10. Soloway HB, SP Cox: In vitro comparison of the thrombin time and activated partial thromboplastin time in the laboratory control of heparin therapy. Am J Clin Pathol 60:648–650, 1973.
11. Bird R, B Hall, KEF Hobbs, D Chapman: New haemocompatible polymers assessed by thromboelastography. J Biomed Eng 11:231–234, 1989.
12. Caprini JA, JB Eckenhoff, JM Ramstack, L Zuckerman, LF Mockros: Contact activation of heparinized plasma. Thromb Res 5:379–400, 1974.
13. Lee BY, WR Thoden, LRM Del Guercio, *et al.*: Monitoring coagulation dynamics with thromboelastography. Contemp Surg 24:19–24, 1984.
14. Coppola L, R Giunta, R Spiezia, V Nappo, C Scognamiglio, A Tirelli: Accentuata ed irregolare deflessione postmassimale nei tracciati tromboelastografici: Incidenza in varie patologie. Boll Soc It Biol Sper 61:263–270, 1985.
15. Jespersen J: Pathophysiology and clinical aspects of fibrinolysis and inhibition of coagulation. Danish Med Bull 35:1–33, 1988.
16. De Gaetano G, J Vermylen: Effect of aspirin on the thrombelastogram of human blood. Thromb Diath Haemorrh 30:494–498, 1973.
17. Tuman KJ, RJ McCarthy, RV Patel, RB Patel, AD Ivankovich: Comparison of thromboelastography and platelet aggregometry. (Abst.) Anesthesiology 75:A433, 1991.
18. Laster J: The heparin induced thrombocytopenic syndrome: An update. Surgery 102:763–770, 1987.
19. Copeland JG, LA Harker, JH Joist, WC De Vries: Circulatory support 1988. Bleeding and anticoagulation. Ann Thorac Surg 47:87–95, 1989.
20. Zuckerman L, JM Ramstack, JP Vagher, JA Caprini, LF Mockros: Neutralization of heparin by cellular blood elements. Thromb Res 7:149–159, 1975.
21. Ramstack JM, L Zuckerman, LF Mockros, JA Caprini, JP Vagher: Effect on platelet count and hematocrit on the coagulability of heparinized blood. Res Commun Chem Pathol Pharmacol 21:327–341, 1978.
22. Howland WS, B Castro, J Fortner, P Gould: Hypercoagulability, thrombelastographic monitoring during extensive hepatic surgery. Arch Surg 108:605–608, 1974.
23. Vagher JP, JA Caprini, L Zuckerman: Multi-system analysis of intravenous calcium and sodium heparin. Thromb Res 14:881–900, 1979.
24. De Takats G: Heparin tolerance. A test of the clotting mechanism. Surg Gynecol Obstet 77:31–39, 1943.
25. Traverso CI: Valoración del test de tolerancia a la heparina intravenosa realizado con tromboelastografia en la predicción de la enfermedad tromboembólica venosa tras cirugía general electiva. Granada University School of Medicine, Spain, doctoral thesis, 1989.
26. Lee BY, FS Trainor, WR Thoden, D Kavner: Monitoring coagulation dynamics: Thromboelastography. *In: Handbook of Noninvasive Diagnostic Techniques in Vascular Surgery*. Appleton-Century-Crofts, New York, 1981, pp 266–286.
27. Bull HS, WN Huse, FS Brauer, RA Korpman: Heparin therapy during extracorporeal circulation. The use of a dose-response curve to individualize heparin and protamine dosage. J Thorac Cardiovasc Surg 69:685–689, 1975.
28. Friesen RH, AJ Clement: Individual responses to heparinization for extracorporeal circulation. J Thorac Cardiovasc Surg 72:875–879, 1976.
29. Whitfield L, J Schentag, G Levy: Relationship between concentration and anticoagulant effect of heparin in plasma of hospitalized patients: magnitude and predictability of interindividual differences. Clin Pharmacol Ther 32:503–516, 1982.
30. Mockros LF, SD Hirsch, L Zuckerman, JA Caprini, WP Robinson, JP Vagher: Anticoagulation kinetics following bolus injection of heparin. Thromb Haemostas 42:1248–1260, 1979.
31. Hyers TM, RD Hull, JG Weg: Antithrombotic therapy for venous thromboembolic disease. Chest 95:37s–51s, 1989.
32. Walker A, H Jick: Predictors of bleeding during heparin therapy. JAMA 244:1209–1212, 1980.
33. Lee B, S Taha, F Trainor, D Kavner, M McCann: Monitoring heparin therapy with thromboelastography and activated partial thromboplastin time. World J Surg 4:232–239, 1980.
34. Raby C: *Coagulaciones intravasculares diseminadas y localizadas*. Toray-Masson, Barcelona, 1976, pp 255.
35. Traverso CI: Estudio de las modificaciones tromboelastográficas inducidas por la heparina a bajas dosis y el dextrano 70 como profilaxis de la enfermedad tromboembólica venosa postoperatoria. Granada University School of Medicine, Spain, medical degree thesis, 1986.
36. Aznar J, F Soto, P Villa, L Lonjedo, P Bulufer: Valoración tromboelastográfica de la hipercoagulabilidad plasmática. Sangre 24:1–8, 1979.
37. Torras-Barba J, J Duran, A Pales, J Alloza, J Monasterio, A Triginer: Valoración tromboelastográfica de la hipercoagulabilidad en pacientes traumatológicos sometidos a profilaxis con heparina cálcica. Sangre 25:35–44, 1980.
38. Arcelus JI, CI Traverso, M López-Cantarero, *et al.*: Estudio de las modificaciones tromboelastográficas inducidas por la heparinizción profiláctica en cirugía general. Cir Esp 43:518–526, 1988.
39. Butler MJ: Thromboelastography during and after elective abdominal surgery. Thromb Haemostas 39:488–495, 1978.
40. Fano R, H Gonzalez: Valor de la tromboelastografia en la detección

de la hipercoagulabilidad plasmática. Barcelona Quir 26:288–294, 1983.
41. Sagar S, J Massey, JM Sanderson: Low-dose heparin prophylaxis against fatal pulmonary embolism. Br Med J 4:257–261, 1975.
42. Raby C, C Couinaud: Les trubles de la coagulation dans les hemorragies des hypertensions portales. Chirugie 102:186–196, 1976.
43. Duraffourd P: Les conditions de la fiabilite et les avantages du thromboelastogramme. Coeur 12:447–455, 1981.
44. Vernese NA, CR Spillert, CD Franco, EJ Lazaro: Thromboelastographic evaluation of mini-dose heparin in minor surgical procedures. Am Surg 2:112–113, 1986.
45. Kakkar VV, TA Corrigan, DP Fossard: Prevention of fatal postoperative pulmonary embolism by low doses of heparin. Reappraisal of results. International multicenter trial. Lancet 1:567–571, 1977.
46. Leyvraz PF, J Richard, F Bachmann, G VanMelle, J-M Treyvaud, J-J Livio, G Candardjis: Adjusted versus fixed-dose heparin in the prevention of deep-vein thrombosis after total hip replacement. N Engl J Med 309:954–958, 1983.
47. Taberner DA, L Poller, JM Thomson, G Lemon, FJ Weighill: Randomized study of adjusted versus fixed low dose heparin prophylaxis of deep vein thrombosis in hip surgery. Br J Surg 76:933–935, 1989.

Role of Thromboelastography in Evaluating Other Anticoagulant Agents: Warfarin and Heparin-Dihydroergotamine

CLARA I. TRAVERSO, M.D., Ph.D., JUAN I. ARCELUS, M.D., Ph.D., and JOSEPH A. CAPRINI, M.D., F.A.C.S.

The study of prothrombin-depressing agents, primarily derivatives of 4-hydroxycoumarin, was one of the original uses of thromboelastography (TEG) proposed by Hartert in 1951 when he introduced the test into clinical practice. At that time, there was no effective way to monitor this type of anticoagulant agent. Because of the risk to patients, sometimes fatal, of the unstable coagulation-anticoagulation state associated with the use of these drugs, a prompt solution was required. The risks of clotting and hemorrhage were balanced by the prothrombin time (PT), which most clinicians considered to be indicative of the degree of anticoagulation and which was used for managing the therapeutic warfarin dosage. Several authors, however, were not so confident about the value of the PT; they considered the test unable to reflect the correct degree of anticoagulation, given the fact that it provided only partial information on the coagulation cascade. For these reasons, and knowing the kind of information provided by TEG, during the early years several European groups combined the use of the PT and TEG plasma for warfarin monitoring.

EFFECTS ON THROMBOELASTOGRAPHY

As reported by Audier and Serradimigni,[1] the following three thromboelastrographic patterns can be recorded after warfarin administration: (1) A tracing that resembles one obtained following intravenous heparin administration, characterized by a lengthening of the reaction plus coagulation (R + K) parameters (which result from adding the reaction time, R, and the clot formation time, K) and a decrease of the maximal amplitude (MA) (Fig. 1); (2) a lengthening of R + K, with an unchanged MA; (3) no modification when compared with tracings obtained prior to drug administration. The first result indicates that hypoprothrombinemia, achieved under the therapeutic dosage of the drug, is adequate to neutralize all coagulation factors. The second result reflects a delay in clot generation that, according to Leroux and associates,[2] reflects to a greater degree the amount of thromboplastin generated rather than the speed of its formation; for this reason, R + K values are increased. However, the fact that MA values are not modified means that neither the speed of fibrin formation nor the amount of fibrin formed are influenced by the drug. In other words, the clot formed under warfarin administration is quantitatively unchangeable, as displayed by the MA. Finally, the third result reflects the ineffectiveness of warfarin, which, therefore, indicates the need to administer heparin instead of the prothrombin-depressing agent. This is so if PT values, analyzed together with TEG results, have been brought to the proper ranges of anticoagulation.

The thromboelastographic pattern that is most frequently recorded in clinical practice and deserves additional comments is the second result described, that is, tracings characterized by an unchanged or slightly modified MA despite a significant lengthening of R + K. Although these modifications depend on dosage, they have even been recorded when administering experimentally high doses of warfarin to rabbits.[3] In patients, studies conclude that prothrombin-depressing agents reduce the overall coagulability of the blood to a very limited extent;

From the Department of Surgery and Related Specialties, University of Granada Hospital, Granada, Spain (C.I.T.), Department of Surgery, Hospital of the Axarquía, Vélez-Málaga (Málaga), Spain (J.I.A.), and Department of Surgery, The Glenbrook Hospital and Northwestern University Medical School, Glenview, Illinois (J.A.C.).

Reprint requests: Dr. Traverso, Department of Radiation Oncology, "La Paz" Hospital, 28016 Madrid, Spain.

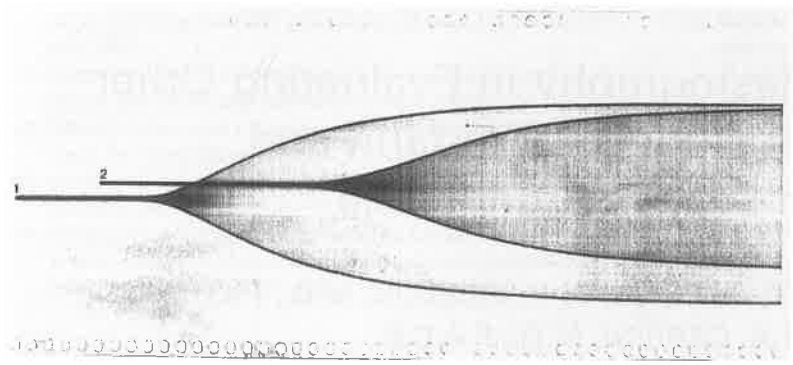

FIG. 1. Thromboelastograms under effects of warfarin resembling tracings after intravenous heparin administration. (Authors' tracings.)

in other words, these drugs modify clotting factors but do not necessarily influence fibrin generation, as depicted by TEG.[4-6] Additionally, those cases in which hypercoagulability was recorded before drug administration showed a trend of being reversed following treatment, with ensuing prolonged R + K values.[3]

One may ask which factor or factors explain the persistence, under the effects of the drug, of increased MA values in cases in which R + K values are sufficiently prolonged. The answer could be found either in platelets or in fibrinogen levels, since MA essentially depends on both factors. According to the experience of Marchal and associates,[7] hyperfibrinogenemia could explain this dissociation in 75% of cases, whereas an exaggerated platelet function was responsible in the remaining 25% of cases. In contrast, Raynaud and associates[4] thought that platelet function was responsible for the findings in practically all cases, creating what they call "dissociated hypercoagulability." In any case, the analysis in platelet-rich plasma (PRP) versus platelet-poor plasma (PPP) allows one to comprehend this observation better.

CLINICAL USE

It is more important to establish some criteria that can be applied in clinical practice. R + K are considered to reflect the degree of anticoagulation induced by the warfarin-like drug.[1,7,8] Thus, R + K values ranging between 30 and 50 mm according to Audier and Serradimigni,[1] or from 35 to 60 mm according to Marchal and associates,[7] indicate efficacy of the anticoagulant treatment. If R + K values are less than 30 mm, increasing the dosage is probably necessary, although additional thromboelastographic analyses should be performed before adjusting the treatment. If values are greater than 50 mm, tracings are very hypocoagulable, and MA modifications may occur. In fact, according to Audier and Serradimigni,[1] an R + K value greater that 55 mm is followed by an abrupt decrease of MA. However, as already mentioned, MA may remain increased, and it is difficult to unequivocally set the exact R + K value from which the decrease in MA value should be expected.

After analyzing tendencies of R + K and MA under the effects of warfarin in PRP, that is plotting R + K and MA values on the same graph, as well as establishing that if MA values are greater than R + K values, there is neither risk of clotting nor hemorrhage (safety zone), Audier and Serradimigni[1] empirically formulated the MA/R + K ratio. Although this is not a mathematical equation, the authors reported excellent results in monitoring prothrombin-depressing agents when using the ratio. Treatment is considered adequate and safe when the ratio ranges between 1 and 2. We have obtained useful information by applying this ratio for monitoring several anticoagulant drugs, as well as for depicting hypercoagulable states, when using decalcified whole blood.[9-11] Fano and González[12] have also reported excellent results for detecting hypercoagulable states by this ratio.

To establish the protocol of administration of warfarin-like anticoagulant agents, Audier and Serradimigni[1] recommended evaluating the PT and TEG together. In addition to this, it should be kept in mind that the body's response to these drugs, that is, the coagulation-anticoagulation state, fluctuates at the beginning of the treatment, as well as the fact that the response is different for different patients. Therefore, before adjusting the dosage, one should make sure that it is truly necessary.

If levels of prothrombin diminish as expected in relation to the administered dosage, but TEG exhibits a hypercoagulable state, that means that the treatment with anticoagulant-depressing agents is insufficient, and thus it is necessary to administer intravenous heparin. In contrast, if levels of prothrombin do not decrease as much as expected for the administered dosage, but TEG depicts a hypocoagulable state, the dosage should not be increased.[1] In clinical practice, generically speaking, Audier and Serradimigni[1] advocate the use of TEG for monitoring treatment, and the PT for checking if a dosage could become toxic to the patient. Accordingly, they recommend looking at the ratio MA/R + K in the following way: If the ratio's value is less than 1, the dosage should

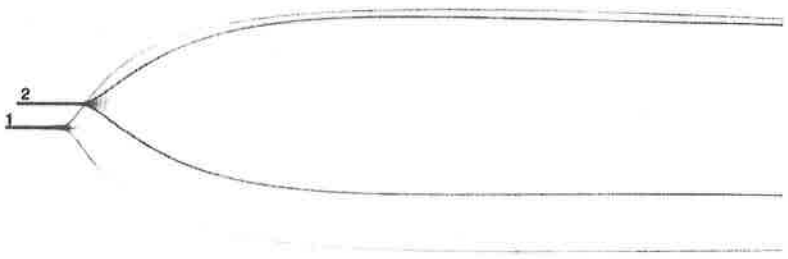

FIG. 2. Thromboelastographic response to prophylactic heparin-dihydroergotamine administration. (Authors' tracing.)

be decreased; otherwise, there is a risk of developing hemorrhagic complications. If the ratio's value is greater than 2, thromboelastographic studies should be repeated, because the body's response may be delayed. In that case, a slow yet progressive increase of the ratio's value will be noted in the following tracings. If after several studies the ratio's value is greater than 3, increasing the dosage should be planned. The authors underscore the fact that the dosage should not be modified if there is not a justified reason.

Translating this into clinical practice, two situations may take place. First, take a thromboelastographic reading of a patient who also shows clinical signs; in this case, adjustment of dosage is the rule. Second, the thromboelastographic findings are isolated, that is, there are no clinical signs. Before adjusting the treatment, ensure that patients are following their treatment, check that they did not take drugs that are known to increase the effects of anticoagulant-depressing agents, and repeat the thromboelastographic analysis after 24 hours.[1] If findings coincide with previous results, adjustment of treatment should be planned. Further work needs to be done to compare these parameters to the international normalized ratio system.

CLINICAL STUDY

Data from 65 patients were analyzed in our study. All patients were older than 40 years and their surgical procedures required at least 1 hour under general anesthesia. Patients were administered a combination of 5000 IU of heparin and 0.5 mg of dihydroergotamine subcutaneously, 2 hours prior to the surgical procedure and every 12 hours in the postoperative period for at least 1 week. TEG was performed on decalcified whole blood (Fig. 2) using a ratio of blood/anticoagulant equal to 2/0.5 based on our previous experience with such a dilution.[9,10] Thromboelastograms were performed before surgery and on the third and seventh postoperative days, at a time ranging between 2 and 2.5 hours after administration of the drug. Thromboelastographic parameters analyzed were R, K, MA, and the thrombodynamic potential index (TPI). Results are compiled in Table 1. All thromboelastographic parameters except R indicated a trend to hypercoagulability on the seventh postoperative day, and statistically significant differences were found when compared with preoperative tracings ($p < 0.05$). These differences were also found for K and TPI values on the third postoperative day.

In similar studies carried out by us, statistically significant differences were not found between postoperative and preoperative tracings when using low-dose heparin and dextran 70 as thrombosis prophylaxis, whereas postoperative hypercoagulable states were recorded in untreated patients.[9,10] The trend to develop hypercoagulable states that was registered in patients who were administered heparin-dihydroergotamine was well correlated with the low number of bleeding complications, but was

TABLE 1. Thromboelastographic Values Registered Pre- and Postoperatively in Patients Who Were Administered Heparin-Dihydroergotamine Prophylaxis

	Reaction Time (R)	*Clot Formation Time (K)*	*Maximal Amplitude (MA)*	*Thrombodynamic Potential Index (TPI)*
Preoperative				
Median	12.3	8.4	54.9	17.7
Standard deviation	3.1	2.9	7.2	9.0
Third postoperative day				
Median	12.4	6.4*	58.2	24.2
Standard deviation	2.6	1.8	3.6	10.7
Seventh postoperative day				
Median	11.8	6.8*	58.7*	23.9*
Standard deviation	2.1	1.1	3.7	10.4

* Indicates $p < 0.05$ (Arcelus and Traverso, 1989).

not related to the rate of deep vein thrombosis, which was low. We see another example of normalization of a hypercoagulable TEG pattern using a specific drug. Further studies are necessary to correlate TEG graphs with the actual occurrence of venous thrombosis.

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REFERENCES

1. Audier M, A Serradimigni: *Savoir interprèter un thromboèlastogramme ou l'exploration prècise de la coagulation sanguine.* Maloine, Paris, 1962, pp 135.
2. Leroux ME, B Jamain, J Cheymol: La Thrombodynamographie (thrombèlastographie) en obstètrique et en gynècologie. I. Principe et signification biologuique des examens thrombodynamographies. Rev Fr Gynecol Obstet 53:25–28, 1958.
3. De Nicola P: *Thromboelastography.* Charles C Thomas, Springfield, IL, 1957, pp 110.
4. Raynaud R, M Bochier, P Griger: Le controle des traitements anticoagulants par la thrombèlastographie. Bull Mem Soc Med Hop Paris 20:587, 1958.
5. Escudero J, E McDevitt, IS Wright: Dicumarol, Coumadin, Marcumar, and Tromexan. Comparative study of their action on the clot as registered by the thromboelastogram. Circulation 20:405–409, 1959.
6. Eliot RS, KN von Kaulla, G Blount: Thromboelastographic studies with a twenty-four-hour schedule for subcutaneous heparin. Circulation 24:1206–1214, 1961.
7. Marchal G, ME Leroux, M Samama: La Thrombodynamographie (Thrombèlastographie). Sa place actuelle en clinique. Presse Med 68:1919–1922, 1960.
8. Bosson P, R Dechamboux: Colloque sur les anticoagulants en pathologie cardiovasculaire. Pathol Biol 8:939–1007, 1960.
9. Traverso CI: Estudio de las modificaciones tromboelastográficas inducidas por la heparina a bajas dosis y el dextrano 70 como profilaxis de la enfermedad tromboembólica venosa postoperatoria. Granada University School of Medicine, Spain, medical degree thesis, 1986.
10. Arcelus JI, CI Traverso, M Lopez-Cantarero: Estudio de las modificaciones trombelastográficas inducidas por la heparinización profiláctica en cirugía general. Cir Esp 43:518–526, 1988.
11. Traverso CI: Valoración del test de tolerancia a la heparina intravenosa realizado con tromboelastografía en la predicción de la enfermedad tromboembólica venosa tras cirugía general electiva. Granada School of Medicine, Spain, doctoral thesis, 1989.
12. Fano R, H González: Valor de la tromboelastografía en la detección de la hipercoagulabilidad plasmática. Barcelona Quir 26:288–294, 1983.

Thromboelastographic Study of Fibrinolytic Agents

LOUIS SUMMARIA, Ph.D.

Plasminogen activators have been used as thrombolytic agents in the treatment of thromboembolic disorders for more than 30 years.[1–3] The two agents that were initially used were streptokinase (SK) and urokinase (UK), both of which have been extensively characterized.[4–7] Neither of these activators has specificity for fibrin and activate both the circulating and the fibrin-bound plasminogen, leading in many cases to bleeding complications.[8,9] Although it was recommended that the dosage of these agents be determined by a dose titration technique and tailored to the antifibrinolytic status of the individual patient,[10] this recommendation was never implemented. The primary reason for such resistance was that the procedures available for estimating the loading dose of SK and UK required a great deal of laboratory control and were quite complex. Consequently, the technique adopted was the use of a fixed dose followed by a sustained infusion to maintain a specified level of systemic clot-lysing activity. Unfortunately, this kind of schedule is associated with considerable variability in plasma thrombolytic activity. Nevertheless, early trials indicated fixed dosage schedules were effective, and in the late 1970s, these two drugs were approved by the Food and Drug Administration for the treatment of deep vein thrombosis and pulmonary embolism.

Also in the late 1970s, newer techniques were developed for locally administering thrombolytic agents, which have resulted in more rapid clot lysis in a greater percentage of treated patients.[11–14] This technique has been reported to establish reperfusion in 60 to 80% of myocardial infarction patients, leading to improved cardiac function.[15–17] However, this type of treatment was available to only a small percentage of affected patients.

This limitation stimulated interest, on the one hand, in the use of short-term, high-dose intravenous SK therapy and, on the other hand, for the use of more specific and effective thrombolytic agents. Human tissue plasminogen activator (t-PA) was first isolated from melanoma cell cultures.[18] It is fibrin specific[19,20] and is now produced by recombinant DNA technology.[21] It has been shown to be a very effective agent for treating coronary thrombosis in a variety of clinical trials carried out during the past few years.[22] However, large quantities of this drug are required, and it is extremely expensive. An acylated plasminogen-SK complex (APSAC) has also been prepared, which is partly fibrin specific and has been evaluated in animals and patients.[23,24] It, too, is very expensive. Other derivatives of SK have been prepared, such as the B-chain-SK complex and the miniplasminogen-SK complex, but they have not been clinically evaluated. However, they have been extensively characterized biochemically,[25–28] have been shown to be effective in producing in vitro clot lysis, and have been evaluated in animal clot lysis studies.[29] Their properties have recently been reviewed extensively.[30] A pro-UK, which is a single chain precursor of UK has also been isolated.[31] It is fibrin specific, although this fibrin specificity differs from that of t-PA.[32] It has been shown to be effective clinically in several small-scale clinical trials.[33]

Although effective in a large percentage of patients, all of these drugs fail to achieve reperfusion in at least 20% of patients, and an early reocclusion occurs in up to 25% of cases. Part of the reocclusion problem is due to the role that platelets play, the morphologic features of the thrombus, the short half-life of thrombolytic agents, and the thrombogenic properties of the thrombus surface. Efforts to reduce reocclusion have led to combined therapies utilizing anticoagulants, antiplatelet agents, aspirin, and thrombin inhibitors in combination with thrombolytic drugs. The main risk in using these combined strategies is that they will result in increased bleeding. This is all the more reason for developing methodology that will evaluate the patient's hemostatic system, both before and

From Armour Pharmaceuticals, Kankakee, Illinois.

Reprint requests: Dr. Summaria, Armour Pharmaceuticals, P.O. Box 511, Kankakee, IL 60901.

during treatment. It is also important to have a system that can be used to compare different thrombolytic agents. There have been very few published studies, both in vivo and in vitro, in which direct comparisons of these various activators have been made.

A system that has been in existence for more than 40 years was used more than 15 years ago to determine anti-SK levels in thrombosis patients as well as to monitor patients undergoing fibrinolytic treatment.[34] That method is thromboelastography and although this rheologic technique has been used almost exclusively to measure parameters of coagulation, a study was carried out by our group in 1986 using this instrument to measure parameters of fibrinolysis.[35] We found that thromboelastography not only could be used to quantitatively compare plasminogen activators, but could also be used to determine the antifibrinolytic content of whole blood more rapidly and more accurately than other methods now being used.

THROMBOELASTOGRAPHY

For our study, blood was drawn by the two-syringe technique from patients who were candidates for thrombolytic therapy and from laboratory personnel with normal coagulation-fibrinolytic parameters. Thromboelastography was initiated within 6 minutes after drawing the blood. For preparing plasma, blood was anticoagulated with 0.1 M sodium citrate and the plasma was removed after centrifugation at 1200 rpm for 10 minutes and stored at −70°C. For platelet-rich plasma (PRP), the sample was centrifuged in an IEC centrifuge at 900 rpm for 10 minutes at room temperature. For platelet-poor plasma, the sample was centrifuged in a Sorvall RC-5 instrument at 5000 rpm for 10 minutes at 5°C.

If the fibrinolytic system is activated, the clotted sample in the thromboelastography cuvette will lyse and the two recorded symmetrical branches that normally remain apart will recede again to a straight line. The parameters that can be measured are the maximum amplitude, minimum amplitude, percent lysis, and lysis time. By adding aliquots of plasminogen activators, such as SK, B-chain-SK complex, and plasmin-SK complex to each cuvette containing freshly drawn blood, we were able to measure the degree of lysis produced by each quantity of activator. By measuring the amplitude at different time periods after the addition of each activator, we were able to titrate accurately the level of anti-SK activity for SK activators, and the level of inhibitory activity of the fibrinolytic system present in each sample. This thromboelastography method was used to compare SK, B-chain-SK complex, plasmin-SK complex, miniplasminogen-SK complex, urokinase, and t-PA. Analyses were carried out using whole blood, pooled normal plasma (PNP), and PRP.

Figure 1 is a thromboelastographic pattern showing the parameters of coagulation and fibrinolysis. The reaction time is the distance from the start of the pattern until an amplitude of 2 mm is recorded and is a measure of the clotting time. The instrument is designed so that 2 mm is equivalent to 1 minute. The max A is the maximum amplitude (MA) attained (stiffest clot), the min A is the minimum amplitude or point of lysis. Coagulation occurs until the MA is reached and fibrinolysis occurs after the MA is reached until a straight line is observed. Since the fibrinolytic system is activated during the coagulation phase (especially when activators are added), the lysis time is calculated from the initiation of coagulation to the min A. The percent lysis can be calculated at any interval between MA and min A, by the formula: 100% − (min A/MA)%.

ACTIVATOR ACTIVITIES OF PLASMINOGEN ACTIVATORS

The plasminogen activator activity of each activator preparation used was determined in a caseinolytic assay using Glu-plasminogen as the substrate.[36] A standard curve was determined using a standard clinical SK preparation (Kabikinase) with an activity of 100,000 IU/vial. All activator activities of SK derivatives were expressed relative to the SK standard.

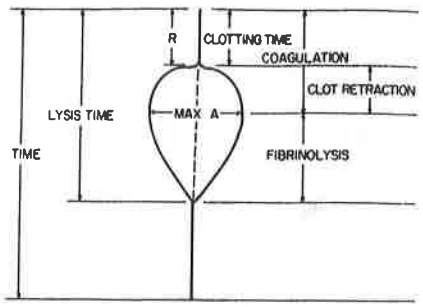

FIG. 1. Thromboelastographic pattern with coagulation-fibrinolytic parameters. The pattern is obtained using 300 μL of whole blood and 30 μL of activator. The reaction (R) time is the clotting time, the maximum amplitude is the stiffest clot attained, the point of lysis is at the minimum amplitude and the lysis time is calculated from the initiation of coagulation to the minimum amplitude. The amplitude and lysis time are both functions of the amount of activator added to the system.

TABLE 1. Plasminogen Activator Activities of Six Activators Determined by a Caseinolytic Assay

Activator	*International Units per Vial on Label*	*International Units Determined*	*Specific Activity IU/mg Protein*
Streptokinase* (SK)	100,000	100,000	100,000†
Plasmin-SK	—	—	32,500
Miniplasminogen-SK	—	—	54,000
Plasmin-B-Chain-SK	—	—	66,000
Urokinase‡	358,000	376,000	N.D.
t-PA§	1,000	86‖	100,000¶

* SK was a standard Kabi preparation.
† All plasminogen activator activities were determined relative to 100,000 IU of SK as the standard.
‡ Urokinase was an Abbott Clinical Tissue Preparation.
§ Tissue plasminogen activator (t-PA) was a standard melanoma cell culture preparation with an activity of 1000 IU/mL.
‖ The casein assay with t-PA was carried out in the absence of fibrin.
¶ The specific activity of this preparation of t-PA was reported to be 100,000 IU/mg protein.[18]

For comparative purposes, all of the activators were assayed by a fibrin plate method[37] using bovine fibrinogen (Pentex), bovine thrombin (Pentex), and human LYS-plasminogen (Kabi), at a concentration of 2.2 IU per plate.

The specific activity per milligram of protein or the activity per milliliter of six activators is shown in Table 1. The determined specific activities of the plasmin–SK complex, the mini-plasminogen-SK complex, and the B-chain-SK complex were 32,500 IU/mg protein, 54,000 IU/mg protein, and 66,000 IU/mg protein, respectively. The activity of the clinical urokinase preparation was determined to be 376,000 IU per vial, which was within experimental error of the units (358,000) on the label. Solutions of 100,000 IU/mL of these five activators were prepared as stock solutions and were stored at −70°C at this concentration. The activity of the t-PA preparation was determined in this assay system to be 86 IU/mL and this preparation was stored at −70°C at this concentration. This activity was determined in the absence of fibrin, which accounts for the low activity of this preparation, since fibrin has been shown to enhance considerably the activity of t-PA.

THROMBOELASTOGRAPHIC COMPARISON OF PLASMINOGEN ACTIVATORS USING WHOLE BLOOD

From the stock solution of each activator, dilutions were prepared in a 25% glycerol/0.05 M Tris buffer, pH 8.0, to yield solutions of 1000, 800, 600, 400, 200, 150, 100, 50, 25, 12.5, and 6.2 IU/mL. After preliminary analyses, intermediate dilutions of some activator preparations were prepared and tested. All activator dilutions were stored at −70°C and thawed in ice before use. For a specific analysis, 30 μL of each activator solution was added to 330 μL of whole blood using a 50 μL syringe. The thromboelastograph pin was moved up and down 10 times to ensure complete mixing and the degree of lysis was then measured in each sample. The patterns shown in Figure 2 indicate the different thromboelastographic responses obtained when increasing amounts of SK were added to whole blood.

The pattern in position 1 is the normal pattern measuring coagulation and contained no SK. Positions 2, 3, 4, 5, and 6 contained SK aliquots at 12.5, 25, 50, 100, and 150 μ/mL. The concentrations correspond to 1.0 to 12.5 IU of SK/mL of blood. Coagulation followed by clot lysis occurred in patterns 2, 3, 4, and 5, whereas the

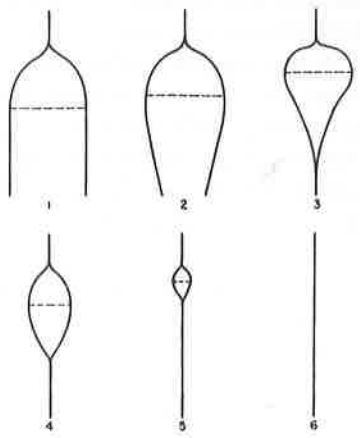

FIG. 2. Thromboelastographic analysis of whole blood clot-lysis using streptokinase (SK). Aliquots of SK corresponding to 1.0 IU SK/mL of blood to 12.6 IU SK/mL of blood were added to generate patterns 2 through 6. Different lysis patterns occur with different maximum amplitudes reached and progressively shorter lysis times until complete inhibition of coagulation is reached in pattern 6.

straight line in pattern 6 indicated complete inhibition of coagulation. The MA in pattern 2 is similar to that in pattern 1, but lysis begins after the MA is reached. Patterns 3, 4, and 5 show that a smaller MA is reached followed by shorter and shorter lysis times with increasing amounts of SK.

Preliminary experiments were carried out using many different activator dilutions so that the optimal ones could be compared. These experiments were carried out using fresh blood drawn from patients scheduled to undergo thrombolytic therapy. With each of these patients, SK was always evaluated as a control, and one other activator was analyzed and compared to SK. With this method and these patients, we were able to determine accurately the level of anti-SK antibody, (or SK resistance), the amount of antifibrinolytic activity, the optimal dose of activator to lyse an in vitro clot, and the proper activator to be used in treating a patient. However, since the anti-SK antibody titer showed some degree of variation in this group of patients, one laboratory employee with an average level of anti-SK antibody (75,000 anti-SK units) was enlisted as a blood donor so that all six activators could be compared on one blood sample.

As seen in Figure 2, by using MA measurements at various time intervals to calculate the percent lysis, the rate of fibrinolysis could be calculated and the time for complete lysis could be determined with each concentration of activator. However, these determinations did not prove to be as accurate for calculating the inhibitor content of whole blood as using the lowest concentration of activator that produced complete inhibition of coagulation (straight-line thromboelastographic pattern). Multiplying the units of activator per milliliter of blood times the blood volume of a patient yielded the inhibitor content of whole blood. The series of patterns obtained with each plasminogen activator appeared to be very similar, except with t-PA. With t-PA, increasing amounts produced a less dramatic change in the MA and lysis time than did other activators, but complete inhibition of coagulation was eventually obtained. This was not anticipated, since the unique quality of t-PA is that t-PA is not expected to produce fibrinolysis until fibrin is formed, but inhibition of coagulation was obtained with 7.3 IU of t-PA per milliliter of whole blood.

Table 2 shows the comparative data for six plasminogen activators using thromboelastographic analyses on whole blood from one individual. The most efficient activator found was the B-SK complex. It was four times as efficient as SK, requiring only one-fourth as many units as SK to prevent completely formation of a clot in the thromboelastograph cup. In this blood sample, the anti-B-SK titer was 18,600 compared with 75,000 for SK. The mini-plasminogen SK complex was almost as efficient as the B-SK complex and three times more efficient than SK. The plasmin-SK complex was only slightly more efficient than SK and was three times less efficient than B-SK. t-PA was 1.73 times as efficient as SK, but was less than half as efficient as B-SK. The anti-t-PA-fibrinolytic titer of whole blood was 43,800 IU compared with 75,000 anti-SK inhibitory units. The poorest activator was UK. The anti-UK-fibrinolytic titer was 300,000 IU and it was only one-fourth as efficient as SK and only one-sixteenth as efficient as B-SK. These same relative efficiencies were determined on many blood samples when each activator was compared individually against SK.

THROMBOELASTOGRAPHIC DETERMINATION OF ANTI-FIBRINOLYTIC INHIBITORY ACTIVITY OF WHOLE BLOOD

Nearly all patients contain anti-SK antibodies as well as naturally occurring plasmin inhibitors and plasminogen activator inhibitors in their blood. There are several clot lysis assays presently being used to determine this inhibitor content.[38,39] We used the thromboelastography method to determine the antifibrinolytic content of blood in 28 patients who were candidates for thrombolytic therapy. Since the thromboelastograph displayed a continual readout of the amplitude, it was not necessary to wait until the analysis was completed, which would have required approximately 2 hours. Readings of the amplitude were determined every 5 minutes, and a calculation was made as to the proper dose of activator and which particular activator to use, within 20 minutes from the time the blood was drawn. Shown in Table 3 are the values recorded for the amplitude on a patient's blood sample comparing SK and UK.

With this patient and SK added at 4.2 IU/mL of blood, the largest amplitude was recorded at 20 minutes followed by partial lysis at 30 minutes. With 8.4 IU/mL of SK, lysis began at 15 minutes and was complete by 25 minutes. Complete inhibition of coagulation occurred with 16.8 IU/mL of SK. With the same patient, 16.8 IU/mL of UK produced no discernible lysis. With 50 IU/mL of UK, a much smaller MA was reached, followed by lysis at 20 minutes, but even with 67 IU/mL, complete inhibition of coagulation was not quite reached. However, with this technique a judgment could be made within 15 minutes as to how much SK or UK should be administered and which thrombolytic agent should be chosen.

Using this thromboelastograph method, we determined the anti-SK inhibitory activity in 28 patients and the anti-UK inhibitory activity in 14 patients subsequently treated with thrombolytic therapy. Table 4 shows the data generated by thromboelastographic analysis of these patients.

TABLE 2. Relative Efficiencies of Six Plasminogen Activators Using Thromboelastography and Whole Blood

*Activator**	*Concentration That Inhibits Coagulation (IU/mL of Blood)*	*Anti-Streptokinase Antibody*	*Anti-Fibrinolytic Titer*	*Relative Efficiency*
Streptokinase (SK)	12.5	75,000	75,000	1.00
Plasmin-SK	9.4	56,400	56,400	1.33
Miniplasminogen-SK	4.2	25,200	25,200	3.00
B-SK	3.1	18,600	18,600	4.00
Tissue plasminogen activator	7.3	—	43,800†	1.73
Urokinase	50.0	—	300,000	0.25

* The relative efficiency of each activator is expressed with SK as the standard.
† The value for tissue plasminogen activator was determined theoretically in the absence of fibrin.

TABLE 3. Comparison of Thromboelastography Amplitudes on Whole Blood with Streptokinase and Urokinase

	Thromboelastography Amplitude (mm) Time (Minutes)					
Activator	*5*	*10*	*15*	*20*	*25*	*30*
Streptokinase (IU/mL of blood)						
0	0	19	43	54	55	55
2.1	0	16	40	49	51	54
4.2	0	15	30	43	40	36
8.4	0	15	10	3	0	0
12.6	0	14	2	0	0	0
16.8	0	0	0	0	0	0
Urokinase (IU/mL of blood)						
0	0	18	40	52	54	54
8.4	0	17	42	53	56	56
16.8	0	18	37	45	52	55
33.6	0	16	31	35	36	32
50.4	0	14	24	16	8	2
67.2	0	8	0	0	0	0

TABLE 4. Thromboelastographic Titration of Streptokinase and Urokinase Inhibitor Levels of Whole Blood in Thrombolytic Therapy Patients

Parameter	*Streptokinase*	*Urokinase*
No. patients	28	14
Inhibitor (IU)*		
Range	30,000–360,000	270,000–1,100,000
Average	127,000 ± 75,000	520,000 ± 108,000
Patient distribution inhibitor level		
30,000 to 60,000	2	0
60,000 to 90,000	8	0
90,000 to 180,000	8	0
180,000 to 270,000	5	0
270,000 to 360,000	4	4
360,000 to 540,000	1	9
540,000 to 1,100,000	0	1
Total patients	28	14

* The values are the averages ± standard deviation (in IU of streptokinase SK).

The average anti-SK inhibitory activity was 127,000 ± 75,000 IU of SK and the range was 30,000 to 360,000 IU. The average anti-UK inhibitory activity was 520,000 + 108,000 and the range was 270,000 to 1,100,000. Twenty-three of the 28 patients had titers below 270,000 IU of SK, whereas none of the 14 patients tested had titers below 270,000 IU of UK.

A standard SK resistance clot lysis was also used to determine the anti-SK inhibitory activity in several patients using plasma. In this assay, dilutions of activator were added to plasma, and a fibrinogen-plasminogen solution was clotted with thrombin with the activator incorporated into the clot. Although this assay was only qualitative and involved extrapolation to zero time, the critical dose determined for SK in five patients tested was similar to that determined using the thromboelastographic analysis.

THROMBOELASTOGRAPHIC COMPARISONS OF PLASMINOGEN ACTIVATORS USING PLASMA

PNP from a pool of 20 donors and individual plasma samples were analyzed for anti-SK antibody titers and antifibrinolytic activity so that comparisons between plasma and whole blood could be made. Clotting times with plasma that were comparable to those of whole blood were obtained by adding 285 μL of plasma to the thromboelastographic cup, followed by 30 μL of activator, and 45 μL of 0.645% calcium chloride. If no activator was to be used, 30 μL of 0.1 M phosphate buffer, pH 7.0, was used. Figure 3 shows thromboelastographic lysis patterns with SK added to whole blood, PNP and PRP from one individual.

The most significant coagulation difference observed was the twofold greater MA of whole blood compared to PNP. The smaller amplitude of PNP could not be increased by initiating coagulation with higher calcium concentrations or using thrombin. Fresh plasma showed

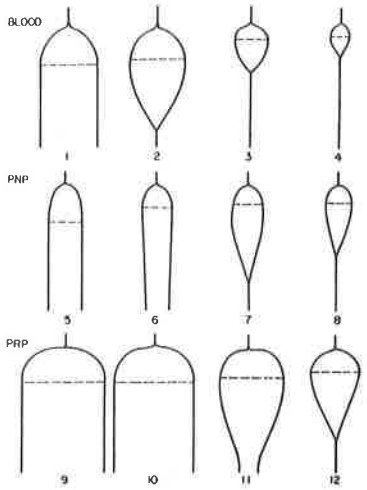

FIG. 3. Comparison of clot lysis with whole blood, pooled normal plasma, and platelet-rich plasma using streptokinase (SK). Plasma clot lysis was carried out using 285 μL plasma, 30 μL of activator, and 45 μL of 0.645% calcium chloride. The patterns in positions 1, 5, and 9 contained no SK. Patterns 2, 3, 4, 6, 7, and 8 and 10, 11, and 12 were generated using 2.1, 4.2, and 8.4 IU of SK/mL of blood or plasma.

no greater MA than frozen and thawed plasma. PRP was found to have a greater MA than whole blood. The platelet count of the PRP was found to be 1.75 times the number of platelets in whole blood. The platelet count of PNP was determined to be 3000/mL. Since clot retraction is one factor contributing to the MA, the greater amplitude observed may be due to this platelet function. By adjusting the PRP with PNP to the same platelet count as whole blood, the MA was reduced to less than that of PRP but was still found to be greater than whole blood.

The most significant difference in fibrinolysis between whole blood, PRP, and PNP was the rate of lysis. With the same concentration of SK, the lysis time for PRP and PNP was approximately twice as long as for whole blood. The anti-SK antibody titer was found to be approximately 100,000 SK units for PRP and PNP compared with 75,000 units for whole blood. This slight difference in titer was found with all the activators when PNP was used and compared to whole blood. The ratios expressing relative efficiencies of different activators were the same regardless of whether PNP or whole blood was used to make the comparisons.

COMPARISON OF PLASMINOGEN ACTIVATORS USING THE FIBRIN PLATE ASSAY

A standard fibrin plate assay method was also used to compare these six plasminogen activators. The degree of lysis was estimated after 18 hours by squaring the diameter of the zone of lysis. Table 5 shows the results of the fibrin plate assays. Four quantities of each activator were added to a fibrin plate. SK was used as the standard preparation. The same number of international units of each activator was assayed using the activities of each activator shown in either column 2 or 4 of Table 1. Thus, the designated international units per vial was used for SK, UK, and t-PA, whereas the determined specific activities of plasmin-SK, miniplasminogen-SK, and B-SK were used to prepare stock solutions from which further dilutions were prepared. The slope of the plot of the area of lysis versus international unit per assay was calculated for each activator and the ratios of the slopes were expressed relative to SK as the standard.

The results of the fibrin plate assay were completely different from the results using thromboelastographic clot lysis analyses. In the fibrin plate assay, UK was the most efficient activator, being approximately 3.42 times as efficient as SK. The plasmin-SK complex was similar in efficiency to SK, whereas the miniplasminogen-SK complex, B-SK complex, and t-PA were all equivalent and approximately twice as efficient as SK, but only 0.6 as efficient as UK. The relationships between SK and plasmin-SK, and B-SK and miniplasminogen-SK were similar in both assays; however, the difference between UK and SK in the two assays showed a nearly 14-fold variation.

CONCLUSIONS

Although thrombolytic therapy has been used to treat thromboembolic disorders for more than 30 years, there is still a need for more effective thrombolytic agents that will achieve thrombolysis faster and in a higher percentage of patients, for better schedules of administration, and for better methods of evaluating new agents.

The whole blood clot lysis method used for these analyses was first introduced in 1951. The thromboelastograph measures the dynamics and kinetics of whole blood coagulation beginning with the fluid state until the solid clot is formed and provides graphic representation of the processes of clot retraction and fibrinolysis. We used thromboelastography to compare the efficacy of various plasminogen activators in lysing human whole blood clots and plasma clots and in determining the antifibrinolytic inhibitory activity of whole blood. We found that the B-SK complex and the miniplasminogen-SK complex were the most efficient activators, were nearly equivalent, and were three to four times more efficient than SK. The plasmin-SK complex had nearly the same efficiency as SK. B-SK and miniplasminogen-SK were nearly twice as efficient as t-PA and approximately 16 times as effi-

TABLE 5. Relative Efficiencies of Six Plasminogen Activators Using the Fibrin Plate Assay

	Area of Lysis (mm^2)*					
IU/Assay	*SK*	*UK*	*Plasmin-SK*	*Miniplasminogen-SK*	*B-SK*	*tPA*
0.06	18 ± 6	70 ± 14	12 ± 6	30 ± 11	49 ± 12	62 ± 10
0.12	31 ± 7	126 ± 18	35 ± 10	74 ± 12	86 ± 18	82 ± 11
0.24	59 ± 9	196 ± 28	70 ± 12	97 ± 16	103 ± 21	122 ± 14
0.48	107 ± 20	310 ± 31	116 ± 18	174 ± 22	179 ± 22	160 ± 20
Relative efficiency†	1.00	3.42	1.13	1.89	2.09	2.07

* B-SK: B-chain streptokinase; t-PA: tissue plasminogen activator; UK: urokinase. The values are the averages of three experiments ± standard deviation.
† The relative efficiencies were calculated by comparing the slopes of the plot of the log of each activator relative to a standard SK preparation containing 100,000 IU/mg protein.

cient as UK. The differences we observed using whole blood may be due partly to the different catalytic efficiencies of each activator and partly to the differences in sensitivities to the anti-SK antibody. The role of plasma inhibitors was dramatized by comparing the thromboelastographic results with the fibrin plate assay. Since the fibrin plate assay has no inhibitor component, it cannot be considered a true reflection of thrombolytic efficacy. In the absence of inhibitors, UK was found to be nearly 1.5 times as efficient as B-SK and t-PA, and 3.4 times as efficient as SK.

Very few studies have been reported comparing SK or its derivatives with t-PA, and by the fibrin plate assay there is at most a twofold variation; by thromboelastographic analyses using whole blood, we also found approximately the same variation. B-SK and miniplasminogen-SK were 1.7 to 2.3 times more efficient than t-PA, whereas t-PA was slightly more efficient than SK and seven times more efficient than UK. One deficiency with the thromboelastographic method is that it is a static and confined system and does not measure fibrin specificity; consequently, it is possible that t-PA may be more efficient relative to SK if another system were used to measure these two agents.

Progressive changes in the pattern of fibrinolysis can be monitored by this technique as increasing concentrations of an activator are added to whole blood before it clots. Many parameters relating to fibrinolysis can be generated from these analyses. We used this thromboelastographic method to pretitrate 28 patients who were subsequently treated with thrombolytic therapy. In these patients, local infusion of the SK or UK dose determined by this method in nearly all cases produced a very slight systemic fibrinolytic response.

The thromboelastographic titration method works with PNP as well as with whole blood. Using PNP, the comparative efficiencies of the six activators were the same as with whole blood, and this was true for both fresh and frozen PNP. However, differences were noted between PNP and blood both in terms of MA differences and the rate of fibrinolysis. Blood has an approximately twofold greater amplitude than PNP, whereas PRP has an even greater amplitude than blood. This is probably due to the clot retraction produced by the platelets. With the same concentration of activator, we found that complete lysis took approximately twice as long with PNP as with whole blood. This would indicate that red cells may play a role in fibrinolysis by releasing a factor that aids the lytic process. However, the red cell effect may only be one of inhibiting the formation of a tight fibrin clot by being trapped in the fibrin strand network. Another explanation may be that there was a greater content of inhibitor in PNP than whole blood, since the amount of PNP tested contained more plasma than was present in the whole blood sample.

Somewhat different results were observed during the thromboelastographic analyses using t-PA. Although various comparable amounts of t-PA produced lysis patterns similar to SK, the rate at which lysis occurred was slower with t-PA. Since t-PA is fibrin-specific and, if it is assumed that some fibrin had to form before t-PA could activate plasminogen, this might account for this delayed lysis. However, with increasing amounts of t-PA, we did reach a point of complete inhibition of coagulation, indicating systemic activation of plasminogen and fibrinogen degradation. This occurred at a relatively low antifibrinolytic titer of 43,800 IU. With a specific activity of 100,000 IU/mg, this would indicate a concentration of 0.1 to 0.2 μg of t-PA/mL of blood would produce systemic activation, making t-PA only slightly less efficient in producing systemic activation than SK.

However, in the case of t-PA, it is not as easy to extrapolate from an in vitro system to an in vivo system, due to the fact that there is a fast reacting inhibitor in blood specific for t-PA, and there are receptor sites in the liver that rapidly remove t-PA.[40,41] Both of these factors help to account for the extremely short half-life of infused t-PA.[42,43]

This study shows that the thromboelastographic method can provide insight into the fibrinolytic activity of different activators and supplements the knowledge obtained by other assay methods. Clinical use of pretitered activator dosage may further reduce bleeding complications while providing adequate thrombolytic effects. This method would allow rapid individualization of agent and dosage for each patient, which may be a better protocol than giving all patients the same dose and the same activator, regardless of patient age, weight, sex, and other characteristics. This method also appears to be a useful research tool correlating in vitro titration with known in vivo thrombosis models.

REFERENCES

1. Fletcher AP, N Alkaersig, FE Smyrniotis, S Sherry: The treatment of patients suffering from early myocardial infarction with massive and prolonged streptokinase therapy. Trans Assoc Am Physicians 71:287–296, 1958.
2. Hansen PF, M Jorgensen, NO Kjeldgaard, J Ploug: Urokinase—an activator of plasminogen from human urine. Experiences with intravenous application on twenty-two patients. Angiology 12:367–371, 1961.
3. Fletcher AP, N Alkaersig, S Sherry, E Genton, J Hirsh, F Bachmann: The development of urokinase as a thrombolytic agent. Maintenance of a sustained thrombolytic state in man by its intravenous infusion. J Lab Clin Med 65:713–731, 1965.
4. Urokinase–streptokinase pulmonary embolism trials. JAMA 229: 1606–1626, 1974.
5. White WF, GH Barlow, MM Mozen: The isolation and characterization of plasminogen activators (urokinase) from human urine. Biochemistry 5:2160–2169, 1966.
6. European Cooperative Study Group for Streptokinase Treatment in Acute Myocardial Infarction: Streptokinase in acute myocardial infarction. N Engl J Med 301:779–785, 1979.
7. Summaria L, L Arzadon, P Bernabe, KC Robbins: The interactions of streptokinase with human, cat, dog and rabbit plasminogens. The fragmentation of streptokinase in the equimolar plasminogen-streptokinase complex. J Biol Chem 249:4760–4769, 1974.
8. Marder VJ: Guidelines for thrombolytic therapy of deep-vein thrombosis. Prog Cardiovasc Dis 21:327–332, 1979.
9. Bell WR, AG Meek: Guidelines for the use of thrombolytic agents. N Engl J Med 301:1266–1270, 1979.
10. Sherry S: History and development of thrombolytic therapy. In: Camerota AJ (Ed): *Thrombolytic Therapy.* Grune & Stratton, New York, 1988.
11. Rentrop KP, H Blanke, KR Karsch: Acute myocardial infarction: Intracoronary application of nitroglycerine and streptokinase in combination with transluminal recanalization. Clin Cardiol 2:354–363, 1979.
12. Ganz W, N Buchbinder, H Marcus, A Mondkar: Intracoronary thrombolysis in evolving myocardial infarction. Am Heart J 101:4–13, 1981.
13. Mathey DG, KH Kuck, V Tilsner, HJ Krebber, W Bleifield: Nonsurgical coronary artery recanalization in acute transmural myocardial infarction. Circulation 63:489–497, 1981.
14. Markis JE, M Molagold, A Parker, KJ Silverman: Myocardial salvage after intracoronary thrombolysis with streptokinase in acute myocardial infarction. N Engl J Med 305:77–82, 1981.
15. Sheehan FH, DG Mathey, J Schofer, HJ Krebber, HT Dodge: Effect of interventions in salvaging left ventricular function in acute myocardial infarction: A study of intracoronary streptokinase. Am J Cardiol 52:431–438, 1983.
16. Bergman SR, RA Lerch, KAA Fox: Temporal dependence of beneficial effects of coronary thrombolysis characterized by position tomography. Am J Med 73:573–581, 1982.
17. Kennedy JW, JL Ritchie, KB Davis, JK Fritz: Western Washington randomized trial of intracoronary streptokinase in acute myocardial infarction. N Engl J Med 309:1477–1482, 1983.
18. Collen D, DC Rijken, J Van Damme, A Billiau: Purification of human tissue-type plasminogen activator in centigram quantities from human melanoma cell culture fluid and its conditioning for use in vivo. Thromb Haemostas 48:294–296, 1982.
19. Matsuo O, DC Rijken, D Collen: Thrombolysis by human tissue plasminogen activator and urokinase in rabbits with experimental pulmonary embolus. Nature 291:590–598, 1981.
20. Korninger C, O Matsuo, R Suy, JM Stassen, D Collen: Thrombolysis with human extrinsic (tissue-type) plasminogen activator in dogs with femoral vein thrombosis. J Clin Invest 69:573–580, 1982.
21. Collen D, EJ Topol, AJ Tiefenbrun: Coronary thrombolysis with recombinant human tissue-type plasminogen activator: A prospective, randomized placebo-controlled trial. Circulation 70:1012–1017, 1984.
22. Van de Werf F, PA Ludbrook, SR Bergman, AJ Tiefenbrunn, KAA Fox, H de Geest, M Verstraete, D Collen, BE Sohel: Coronary thrombolysis with tissue-type plasminogen activator in patients with evolving myocardial infarction. N Engl J Med 310:609–613, 1984.
23. Dupe RJ, PD English, RAG Smith, J Green: The activity of an acylated streptokinase-plasminogen complex (BRL 26921) in dog models of thrombosis. *In:* Davidson JF, F Bachmann, CA Bouvier, EK Kruithof (Eds.) *Progress in Fibrinolysis,* vol. VI. Churchill Livingstone, Edinburgh, 1983.
24. Walker JD, JF Davidson, AP Rae, I Hutton, TDV Lawrie: Acylated streptokinase-plasminogen complex in patients with acute myocardial infarction. Thromb Haemostas 51:204–206, 1984.
25. Summaria L, KC Robbins: Isolation of a human plasmin-derived functionally active, light (B) chain capable of forming with streptokinase an equimolar light (B) chain-streptokinase complex with plasminogen activator activity. J Biol Chem 251:5810–5813, 1976.
26. Wohl RC, L Summaria, KC Robbins: Kinetics of activation of human plasminogen by different activator species at pH 7.4 and 37°C. J Biol Chem 255:2005–2013, 1980.
27. Wohl RC, L Summaria, L Arzadon, KC Robbins: Steady state kinetics of activation of human and bovine plasminogens by streptokinase and its equimolar complexes with various activated forms of human plasminogen. J Biol Chem 253:1402–1407, 1978.
28. Robbins KC, RC Wohl, L Summaria: Activation of human plasminogen in both purified systems and plasma by streptokinase and urokinase activator species. In: Davidson JF, V Cepelak, MM Samama, PC Desnoyers (Eds.) *Progress in Chemical Fibrinolysis and Thrombolysis,* vol IV. Churchill Livingstone, Edinburgh 1979, pp 330–338.
29. Summaria L, IG Boreisha, L Arzadon, KC Robbins: The dissolution of human cross-linked plasma fibrin clots by the equimolar human plasmin-derived light (B) chain-streptokinase complex. The acceleration of clot lysis by pretreatment of fibrin clots with the light (B) chain. Thromb Res 11:377–389, 1977.
30. Summaria L: The plasmin B-chain-streptokinase complex. In: Camerota AJ (Ed.) *Thrombolytic Therapy.* Grune & Stratton, New York, 1988.
31. Gurewich V: Tissue plasminogen activator and pro-urokinase. In:

AJ Camerota (Ed.) *Thrombolytic Therapy.* Grune & Stratton, New York, 1988.
32. Gurewich V, R Pannell: Fibrin-specificity and efficacy of proteolysis induced by single chain urokinase (SC-UK) in plasma. Thromb Haemostas 50:1018–1025, 1983.
33. Van de Werf F, M Nobuhara, D Collen: Coronary thrombolysis with human single-chain urokinase-type plasminogen activator (pro-urokinase) in patients with acute myocardial infarction. Ann Intern Med 104:345–351, 1986.
34. Martin M: *Streptokinase in Chronic Arterial Disease.* CRC Press, Boca Raton, FL, 1982.
35. Summaria L, J Sandesara, G Yang, JP Vagher, JA Caprini: In vitro comparison of fibrinolytic activity of plasminogen activators using a thrombelastographic method; in vivo evaluation of the B-chain-streptokinase complex in the dog model using pre-titered doses. Thromb Haemostas 56:71–79, 1986.
36. Summaria L, L Arzadon, P Bernabe, KC Robbins, GH Barlow: Characterization of the amino terminal glutamic acid and the amino terminal lysine forms of human plasminogen isolated by affinity chromatography and isoelectric focusing methods. J Biol Chem 248:2984–2991, 1973.
37. Haverkate F, DW Traas: Dose-response curves in the fibrin plate assay. Fibrinolytic activity of proteases. Thromb Diathes Haemorrh 32:356–365, 1974.
38. Rothbard RL, PG Fitzpatrick, DM Caton, CW Francis, WB Hood Jr, VJ Marder: Relationship of systemic lytic state to acute thrombolysis with standard dose and low-dose intracoronary streptokinase. Circulation 67 (suppl. III):38, 1983 (Abst.).
39. Lew AS, T Neer, L Rodriquez, IL Geft, PK Shah, W Ganz: Clinical failure of streptokinase due to an unsuspected high titer of anti-streptokinsae antibody. J Am Coll Cardiol 1:183–185, 1984.
40. Chmielewska J, M Ranby, B Wiman: Evidence for a rapid inhibitor to tissue plasminogen activator in plasma. Thromb Res 32:427–436, 1983.
41. Collen D: Mechanisms of inhibition of tissue-type plasminogen activator in blood. Thromb Haemostas 50:678, 1983.
42. Korninger C, JM Stassen, D Collen: Turnover of human extrinsic (tissue-type) plasminogen activator in rabbits. Thromb Haemostas 46:658–661, 1981.
43. Matsuo O: Turnover of tissue plasminogen activator in man. (Letter.) Thromb Haemostas 48:242, 1982.

The Approach of Thromboelastography in the Monitoring of New Synthetic Antithrombotic Agents, GL-522 and GL-2021

OMER IQBAL, M.D., WALTER JESKE, B.S.,
DEBRA HOPPENSTEADT, M.S., MT (ASCP), JEANINE M. WALENGA, Ph.D.,
and JAWED FAREED, Ph.D.

There are several antithrombotic drugs available on the market, and an extensive array of newer ones are being evaluated in various laboratories for their possible clinical usage. Although many sophisticated laboratory tests and various animal models are available to determine the antithrombotic potential of an agent, thromboelastography can be a very useful research tool due to its simplicity and the comprehensive information obtained. The thromboelastograph measures the viscoelastic changes in a sample of whole blood as it undergoes clot formation. The analysis can be qualitatively and quantitatively interpreted in terms of a normal, hypo- or hypercoagulable state of the sample as well as the degree of clot lysis achieved.[1] Thromboelastographic (TEG) analysis alone cannot be used as a specific diagnostic test, but rather as an index of relative coagulability.[2] Thromboelastography has proven itself as a useful research tool in studies of clot lysis,[3–5] of red blood cell and platelet interactions,[6] detection of hypercoagulable states,[7–11] and determination of clot structure stiffness.[12–14] We have incorporated TEG analysis in our evaluations of novel antithrombotic agents. The results obtained with one class of synthetic agents will be described.

A number of synthetic polyanions have been synthesized by Genelabs (Redwood City, CA). One compound, designated as GL-522-Y-1, is a proprietary synthetic organic compound that manifests dose-dependent antithrombotic effects when administered orally or parenterally. Unlike heparin, GL-522-Y-1 shows little anticoagulant activity in conventional global clotting assays (prothrombin time and activated partial thromboplastin time) and does not demonstrate antithrombin III mediated inhibition of thrombin. Its unique mechanism of action may involve direct inhibition of protease generation.[15] GL-2021, the methyl ether derivative of GL-522-Y-1, displays a similar anticoagulant and antithrombotic profile. While these new compounds are being evaluated for development as orally active antithrombotic drugs for the prophylaxis and treatment of venous and arterial thrombosis, their apparent lack of anticoagulant effect on the global clotting tests led us to study their actions by thromboelastography.

MATERIALS

GL-522-Y-1, a synthetic polysulfonic acid salt, and GL-2021, a methyl ether derivative of GL-522-Y-1, were obtained from Genelabs. Hellige Thromboelastograph-D Model 0,4A Si M 1,25G, was obtained from (Hellige Germany). Hemochron, activated clotting time (ACT) machine and celite-activated ACT tubes, was manufactured by International Technidyne Corporation, (Edison, NJ); 3 and 5 mL plastic syringes were from Becton-Dickinson (Rutherford, NJ), and butterfly needles 21 gauge, 3/4 inch and 12 inch tubing were from Abbott Hospitals (North Chicago, IL).

METHODS

Stock solutions of the two agents, GL-522-Y-1 and GL-2021, were made at 500 mg/mL and 250 mg/mL.

From the Departments of Pathology and Pharmacology, Loyola University, Chicago, Illinois, and Stritch School of Medicine, Maywood, Illinois.

Reprint requests: Dr. Fareed, Department of Pathology, Loyola University Medical Center, 2160 South First Avenue, Maywood, IL 60153.

From each of these stock solutions 0.3 mL was taken and prefilled in three different 5 mL syringes. Utilizing a double-syringe technique, blood from three healthy human volunteers was drawn in the respective syringes up to the 3-mL mark, so as to obtain a final blood concentration of 50 and 25 mg/mL for each of the two agents. Saline supplementation to each volunteer's blood served as the control.

After uniformly mixing the contents of the syringes, a celite-ACT and TEG measurement were immediately performed. The ACT, a routinely used whole blood clotting assay, was performed in addition to the TEG to determine the anticoagulant potential of these two agents. The results of the ACT in seconds were recorded and the TEG tracing obtained. The thromboelastograph was analyzed and the parameters reaction (R) time, coagulation (K) time, maximum amplitude (MA), and the divergence angle were determined. The R time signifies the time taken for the initiation of clot formation. R time is the distance measured from the beginning of the tracing (event mark) to the point of 2 mm divergence. RK time is the time or the distance from the event mark to the 20 mm divergence of the graph. The K time is obtained by subtracting the R time from the RK time. This is the time to a standard clot firmness. The MA is the measurement in millimeters of the width of the tracing at the point of maximum divergence. This corresponds to the maximum shear modulus of the clot. The divergence angle denotes the kinetics of clot growth.

Statistical analysis was performed using analysis of variance (ANOVA) from the Primer of Biostatistics computer program (McGaw Hill, Evanston, IL).

RESULTS

A comparison of the anticoagulant effects of GL-522-Y-1 and GL-2021 in freshly drawn human blood in the celite-ACT test system showed a significant difference between the two agents at the final concentration of 50 and 25 mg/mL (Fig. 1). At a final concentration of 25 mg/mL, there was a 41-second elevation of the ACT with GL-522-Y-1 ($p = 0.014$) and 22-second elevation with GL-2021 ($p = 0.030$) when compared to their respective saline controls. At a final concentration of 50 mg/mL there was a 122-second elevation of ACT with GL-522-Y-1 ($p = 0.001$) and 24-second elevation with GL-2021 ($p = 0.003$) when compared to their respective saline controls. There was a marked concentration-dependent increase of the ACT with GL-522-Y-1 from a final concentration of 25 mg/mL to a final concentration of 50 mg/mL. However, there was not such a marked concentration dependent increase of ACT with GL-2021.

Analysis of the TEG parameters indicated that the R time was dose dependently increased for both agents

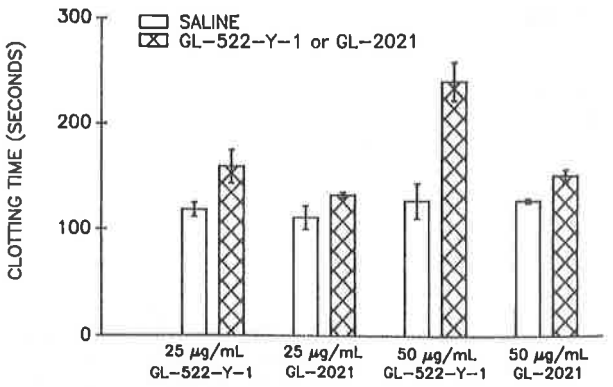

FIG. 1. A comparison of the anticoagulant effects of GL-522-Y-1 and GL-2021 in freshly drawn human blood as measured by the celite-ACT indicated a significant difference between the two agents at the concentrations tested at 25 mg/mL ($p = 0.046$) and at 50 mg/mL ($p = 0.001$).

(Fig. 2). There was a 7 mm increase of R time with GL-522-Y-1 ($p = 0.030$) at a final concentration of 25 mg/mL and a 3 mm increase with GL-2021 ($p = 0.090$) when compared with their respective saline controls. At the same final concentration of 25 mg/mL the RK time showed an 11 mm increase with GL-522-Y-1 ($p = 0.065$) and an 8 mm increase with GL-2021 ($p = 0.067$) (Fig. 3). The K time recorded a 4 mm increase with GL-522-Y-1 ($p = 0.222$) and a 4 mm increase with GL-2021 ($p = 0.234$) (Fig. 4). The MA showed an 8 mm decrease with GL-522-Y-1 ($p = 0.333$) and a 5 mm decrease with GL-2021 ($p = 0.425$) when compared with their respective saline controls (Fig. 5). The divergence angle showed a 7° decrease with GL-522-Y-1 ($p = 0.425$) and 9° decrease with GL-2021 ($p = 0.177$) when compared with their saline controls (Fig. 6).

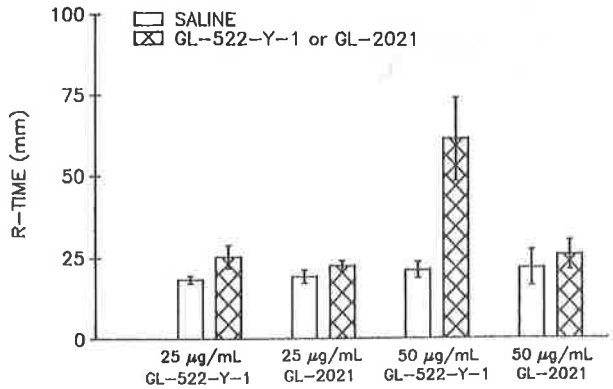

FIG. 2. The reaction time demonstrated a concentration-dependent increase for both agents at final concentrations of 25 and 50 mg/mL. The increase was more pronounced for GL-522-Y-1 than for GL-2021. All increases were statistically significant relative to their respective saline controls ($p = 0.002$).

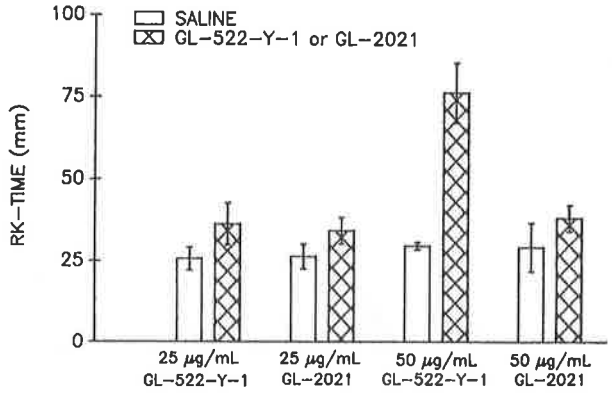

FIG. 3. The reaction plus coagulation time was concentration dependently increased by both agents relative to their respective saline controls. The increase induced by GL-522-Y-1 was statistically significant ($p = 0.002$).

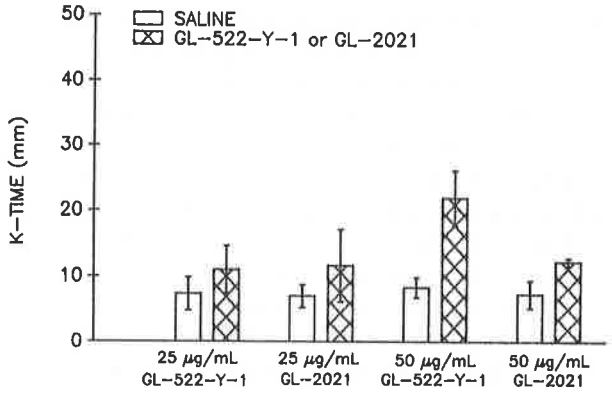

FIG. 4. The coagulation time was significantly increased by GL-522-Y-1 relative to control when the agents were supplemented at concentrations of 50 mg/mL. At a concentration of 25 mg/mL, no significant changes were noted.

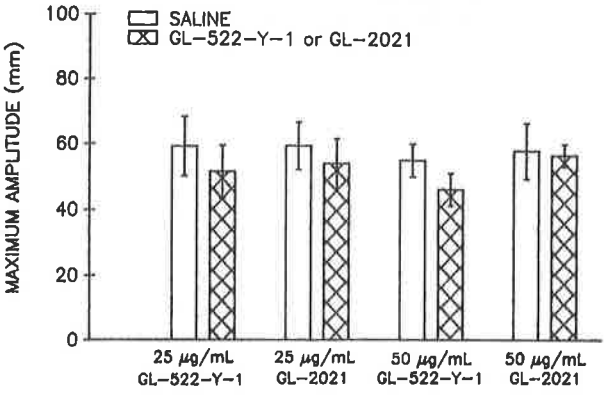

FIG. 5. Maximum amplitude was minimally effected by either of the agents. Although a trend toward decreasing values was noted with increasing GL-522-Y-1 concentration, no statistically significant differences were noted.

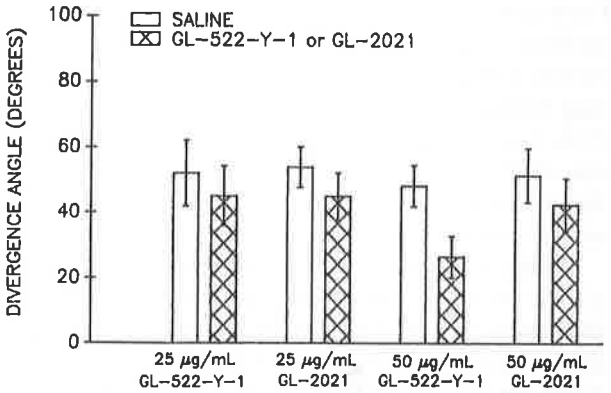

FIG. 6. A significant decrease in divergence angle ($p = 0.033$) was noted at a GL-522-Y-1 concentration of 50 mg/mL. GL-2021 had little effect on the divergence angle.

At a final concentration of 50 mg/mL, the R time recorded a 40 mm increase with GL-522-Y-1 ($p = 0.004$) and a 4 mm increase with GL-2021 ($p = 0.391$) (Fig. 2). The RK time showed a 47 mm increase with GL-522-Y-1 ($p = 0.002$) and a 9 mm increase with GL-2021 ($p = 0.142$) (Fig. 3). The K time showed a 14 mm increase with GL-522-Y-1 ($p = 0.012$) and a 5 mm increase with GL-2021 ($p = 0.016$) when compared with their respective saline controls (Fig. 4). The MA showed a 9 mm decrease with GL-522-Y-1 ($p = 0.149$) and a 1 mm decrease with GL-2021 ($p = 0.814$) (Fig. 5). The divergence angle showed a 22° decrease with GL-522-Y-1 ($p = 0.033$) and a 9° decrease with GL-2021 ($p = 0.252$) when compared with their respective saline controls (Fig. 6).

DISCUSSION

The ACT (celite) results indicated that GL-522-Y-1 is a more potent anticoagulant when compared with GL-2021 at final concentrations of 25 and 50 mg/mL. Both agents demonstrated a dose-dependent increase of the ACT when compared with their respective saline controls. The prolongation of the clotting time by GL-522-Y-1 was more marked than by GL-2021, and all observed increases were statistically significant.

Based on the TEG parameters GL-522-Y-1 also demonstrated an increased anticoagulant effect when compared with GL-2021. The R time, RK time, and K time were concentration dependently increased for both agents, although much more so with GL-522-Y-1. The time needed for the initiation of clot formation, R time, was only significantly prolonged by GL-522-Y-1. The time to standard clot firmness, the K time, was observed to be significantly prolonged relative to saline control by both GL-522-Y-1 and GL-2021 only at a concentration

of 50 mg/mL. Prolongations induced by the lower concentration of either agent were not statistically significant. The MA was concentration dependently decreased for both the agents, the decrease being more marked with GL-522-Y-1 when compared with GL-2021. No statistically significant differences were noted with this parameter. The MA which reflects the maximum shear modulus of the clot or the maximum strength of the clot was minimally affected by GL-22-Y-1 than GL-2021 when compared with their respective saline controls. The divergence angle, which reflects the kinetics of clot formation, showed a concentration dependent decrease for both the agents, although only the higher concentration of GL-522-Y-1 was seen to reduce this parameter significantly.

CONCLUSION

The GL-522-Y-1 showed a significant anticoagulant response as measured by the ACT (celite) and various TEG parameters when compared with GL-2021, the methyl ether derivative of GL-522-Y-1, following ex vivo supplementation of these two agents to freshly drawn human blood. The lower activity exhibited by GL-2021 in this study is consistent with the lower in vitro anticoagulant potency previously observed in our laboratory using global clotting assays.

Acknowledgment. We gratefully acknowledge the expert assistance of Sally Healey in typing the manuscript.

REFERENCES

1. Thromboelastograph® Operations Manual and CTEG User's Guide. Haemoscope Corporation, 1990, p 9.
2. Caprini JA, L Tuckermam, E Cohen, JP Vagher, V Lipp: The identification of accelerated coagulability. Thromb Res 9:167–180, 1976.
3. Von Kaulla KN: Continuous automatic recording of fibrin formation and fibrinolysis: A valuable tool for coagulation research. J Lab Clin Med 49:304–312, 1957.
4. De Nicola P, GM Mazzetti: Evaluation of thromboelastography. Am J Clin Pathol 25:447–452, 1955.
5. Astrup T, K Egerbald: Thromboelastographic patterns produced by fibrinolytic agents incorporated in fibrin. Am J Physiol 209:84–94, 1965.
6. Ramstack JM, L Zuckerman, LF Mockerds, JA Caprini, JP Vagher: Effect of platelet count and hematocrit on the coagulability of heparinized blood. Res Commun Chem Pathol Pharmacol 21:327–342, 1978.
7. Howland WS, EB Castro, JB Fortner, P Gould: Hypercoagulability, thromboelastographic monitoring during extensive hepatic surgery. Arch Surg 108:605–608, 1974.
8. Raby C: Control of heparin therapy by thromboelastography. *In:* Kakkar VV, DP Thomas (Eds): *Heparin, Chemistry and Clinical Usage.* Academic Press, London, 1976, pp 211–218.
9. Raby C: Heparinotherapic controllee. Presse Med 74:1526, 1976.
10. Hume M: The relation of "hypercoagulability" to thrombosis. Surg Sci 2:133–158, 1966.
11. Collins GJ, DJ Aher, NM Rich, CA Anderson: Detection and management of hypercoagulability. Am J Surg 132:767–770, 1976.
12. De Nicole P: *Thromboelastography.* Charles C Thomas, Springfield, IL, 1957.
13. Merskey C: Pathogenesis and treatment of altered blood coagulability in patients with malignant tumors. Ann NY Acad Sci 230:289–296, 1974.
14. Gollub S, C Schaefer: Structural alteration in canine fibrin produced by colloid plasma expanders. Surg Gynecol Obstet 127:783–793, 1968.
15. Jeske W, B Lojewski, J Fareed: Biochemical and pharmacologic studies on a novel polysulfonated oral antithrombotic agent (GL-522-Y-1). Blood 80(Suppl 1):322a, 1992.

Comparison of the Anticoagulant Activities of Thrombin Inhibitors as Assessed by Thromboelastographic Analysis

DEMETRA CALLAS, B.S., OMER IQBAL, M.D., and JAWED FAREED, Ph.D.

Anticoagulation is an important component in the management of cardiovascular diseases. The degree of anticoagulation must be closely monitored so that an optimal balance between thrombosis prevention and hemorrhagic risk is attained. The method for monitoring anticoagulation is chosen based on the type of agent used to induce an anticoagulant stage. There is a wide variety of functional methods available for monitoring anticoagulation, ranging from whole blood clotting tests to global clotting tests, and often more than one are used to ensure accurate estimation of the condition. The principles underlying these tests are based on the mechanism of action of the particular anticoagulant agent. Thus, for agents that produce anticoagulation via inhibition of the extrinsic pathway, the prothrombin time (PT) plasma clotting test is used.

Lately, there has been an increasing interest in the development of anticoagulants and antithrombotics other than heparin derivatives. The source of these new agents ranges from recombinant technology to synthetic methodology. The mechanisms of action of some of these products are complex and thus deciding on a method for the monitoring of the degree of anticoagulation can be problematic. This study was designed to compare the anticoagulant profile as assessed by thromboelastographic (TEG) measurements that some of these new agents exhibit.

Most coagulation tests have been developed for the detection of anticoagulant states. Because of this limitation they are unable to detect procoagulant (hypercoagulability) states. To examine the sensitivity of the TEG method toward procoagulant states, recombinant tissue factor (r-TF) was used as the positive control. TF is an extrinsic activator of factor VII, which leads to conversion of factor X to factor Xa directly or via activation of factor IX.[1] Activated factor X can then further activate factor VII, which results in an approximately 100-fold increase in coagulant activity. This ultimately leads to conversion of prothrombin to thrombin, the key enzyme in converting fibrinogen to solid fibrin clots. r-TF is thus a strong thrombotic agent, suitable for examining the sensitivity of coagulation tests toward procoagulant states.

MATERIALS AND METHODS

TEG measurements were made on freshly drawn whole blood samples from healthy volunteers, supplemented with various concentrations of the agents used in this study and preincubated at 37°C in a glass tube for 3 minutes prior to testing. The Hellige Thromboelastograph Coagulation Analyzer (Haemoscope, Skokie, IL) automatically records the viscoelastic changes in a sample of whole blood as the sample clots, retracts, and lyses. The resulting profile is a measure of the kinetics of clot formation, clot dissolution, and clot quality. The thromboelastograms generated in this manner are analyzed using the standard procedure as described by Fiedel and Ku[2]: the length from the beginning of the recording until the point where the graph reaches a 2 mm divergence is recorded as the reaction (R) value; the length from the beginning of the thromboelastogram until the point where the graph reaches a 2 mm divergence is recorded as the RK value; the difference between the R and RK values is the coagulation time (K) value. The anticoagulant agents examined in this study were recombinant hirudin (Knoll AG, Lud-

From the Departments of Pathology and Pharmacology, Loyola University, Chicago, Illinois, and the Stritch School of Medicine, Maywood, Illinois.

Reprint requests: Ms. Callas, Department of Pharmacology, Loyola University Medical Center, 2160 South First Avenue, Maywood, IL 60153.

wigshafen, Germany) and two tripeptide aldehydes from the Institute for Drug Research, Budapest, Hungary. The structures of the peptides are: D-MePhe-Pro-Arg-H and Boc-D-Phe-Pro-Arg-H. Each agent was prepared in saline and supplemented at different final concentrations to freshly drawn whole blood.

In addition to the above anticoagulant agents, the procoagulant agent r-TF was also examined. The effects of r-TF on blood parameters were examined both after in vitro supplementation and in vivo supplementation.

In the in vitro supplementation study, freshly drawn blood samples from male New Zealand White rabbits were supplemented with two concentrations of r-TF (2.5 and 5 ng/mL) and the TEG assays were carried out as just described. Saline was used as the control (n = 5 for each treatment group). For the in vivo effects of r-TF, two groups of rabbits (n = 5) were lightly anesthetized with ketamine/xylanine and r-TF (50 ng/mL) or saline (0.25 mL/kg) was then injected into each group. After a circulation time of 15 minutes, cardiac puncture was performed. TEG measurements were carried out on all blood samples drawn as already described.

RESULTS

Each of the anticoagulant agents was supplemented to freshly drawn whole blood from healthy volunteers at final concentrations of 10, 5, 2.5, and 1.25 mL. After a 3-minute (37°) incubation period, the blood samples were placed in the TEG cups, the pins were lowered and the TEG results recorded. The records were then processed and the R, RK, and K values were calculated. Figure 1 is a graphical representation of the R values. All agents produced a concentration-dependent prolongation of the R value. On an equigravimetric basis, hirudin appeared to be the more effective anticoagulant followed by the methylated tripeptide. The Boc-protected peptide was the weakest, although it also possessed concentration-dependent anticoagulant activities.

The comparative effects of these agents on the TEG RK value are illustrated in Figure 2. The order of potency was the same as in Figure 1, that is, hirudin > D-MePhe-Pro-Arg-H > Boc-D-Phe-Pro-Arg-H. All agents produced a concentration-dependent anticoagulation.

Figure 3 depicts the effects of these agents on the TEG K parameter. Interestingly, hirudin and Boc-D-Phe-Pro-Arg-H had no concentration-dependent effect on the K value, but the D-MePhe-Pro-Arg-H compound had an effect. Furthermore, although hirudin was still more potent than Boc-D-Phe-Pro-Arg-H in prolonging the K value, when compared to D-MePhe-Pro-Arg-H at the highest concentration (5 mg/mL), the methylated tripeptide surpassed the effect of hirudin.

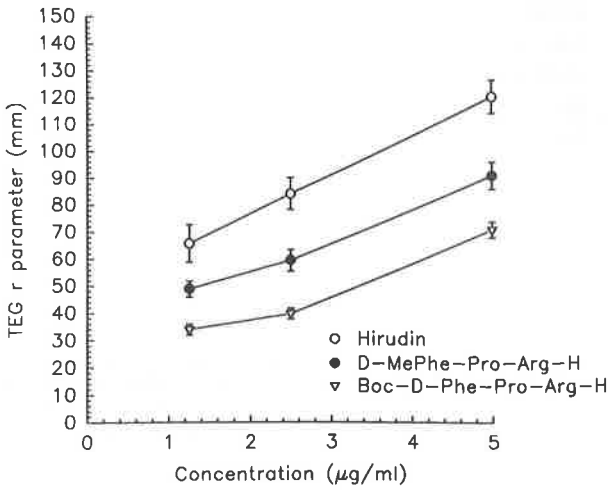

FIG. 1. In vitro effect of human whole blood supplemented with hirudin, D-MePhe-Pro-Arg-H or Boc-D-Phe-Pro-Arg-H on the thromboelastographic reaction time parameter. All agents were supplemented to human whole blood at final concentrations of 5, 2.5, and 1.25 mg/mL. All results represent the mean ± 1 SEM, n = 5.

The in vitro supplementation of r-TF to freshly drawn rabbit whole blood resulted in clot formation before the end of the 3 minute (37°) incubation period preceding the initiation of TEG recording, thus hampering analysis of these graphs (results not shown). On the other hand, in vivo administration of r-TF resulted in opposite results, namely, prolongation of time to clot formation.

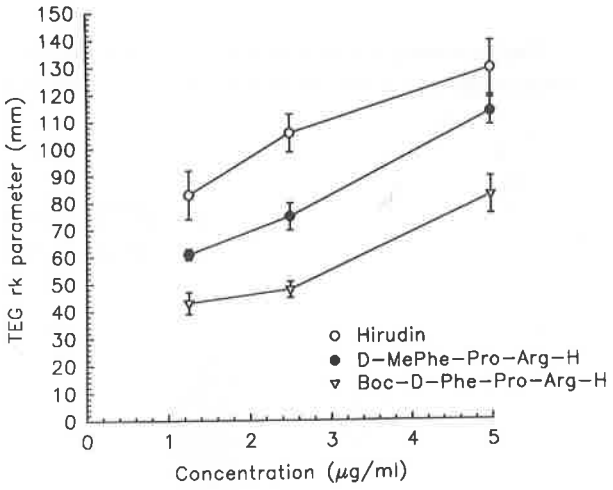

FIG. 2. In vitro effect of human whole blood supplemented with hirudin, D-MePhe-Pro-Arg-H, and Boc-D-Phe-Pro-Arg-H on the thromboelastographic reaction - coagulation time parameter. All agents were supplemented to human whole blood at final concentrations of 5, 2.5, and 1.25 mg/mL. All results represent the mean ± SEM, n = 5.

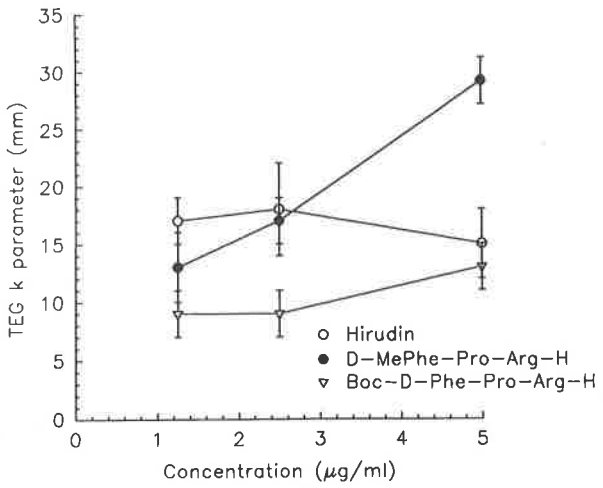

FIG. 3. In vitro effect of human whole blood supplemented with hirudin, D-MePhe-Pro-Arg-H, and Boc-D-Phe-Pro-Arg-H on the thromboelastographic coagulation time parameter. All agents were supplemented to human whole blood at final concentrations of 5, 2.5, and 1.25 mg/mL. All results represent the mean ± SEM, n = 5.

The results of the analyses of the TEG tracings of this study are illustrated in Figure 4. Injection of r-TF at 50 μg/kg prolonged the R, RK and K value of the whole blood thromboelastographs significantly when compared with saline. In addition, the TEG α angle of the whole blood from the r-TF–treated animals was reduced when compared with the control.

DISCUSSION

The increasing number of potential antithrombotics and anticoagulants under development pose a multitude of questions, ranging from standardization of these new agents to assessment of their activities in vitro and in vivo. These agents are designed as specific targets for various blood components. Conventional plasma clotting tests may not be the appropriate methods for monitoring the anticoagulant activities of these agents, since only the plasma fraction is examined in these tests. Whole blood clotting tests would be more advantageous, since all of the components of the hemostatic mechanisms, including platelets, are incorporated in the clot formation.

The TEG method for monitoring the progress of the clot formation may have the advantage that in addition to providing a relative time scale to clot formation (R parameter), certain characteristics of the clot itself may be enlightening as well, such as the RK value, which corresponds to the time to a fixed clot firmness. The K parameter (K = RK − R) is then an indication of the rate of growth of the clot. In the comparison of the anticoagulant agents with this method, as expected, the R and RK values increase in a concentration-dependent manner. However, when considering the K parameter, hirudin and Boc-D-Phe-Pro-Arg-H do not have a concentration-dependent effect on the prolongation of the rate of growth of the clot, but the D-MePhe-Pro-Arg-H agent does exhibit this unique characteristic. This would not be detectable in an ordinary whole blood clotting method in which the endpoint is the initiation of clot formation (corresponding to the R parameter).

The results from the r-TF studies are unexpected. Although supplementation of r-TF to whole blood results in strong procoagulant effects (clots are formed before the TEG recording is initiated), in vivo administration produces anticoagulant effects reflected in all of the TEG parameters. It is of interest to note that the plasma fractions of the whole blood after in vivo injection of r-TF did not show anticoagulation with the global clotting tests

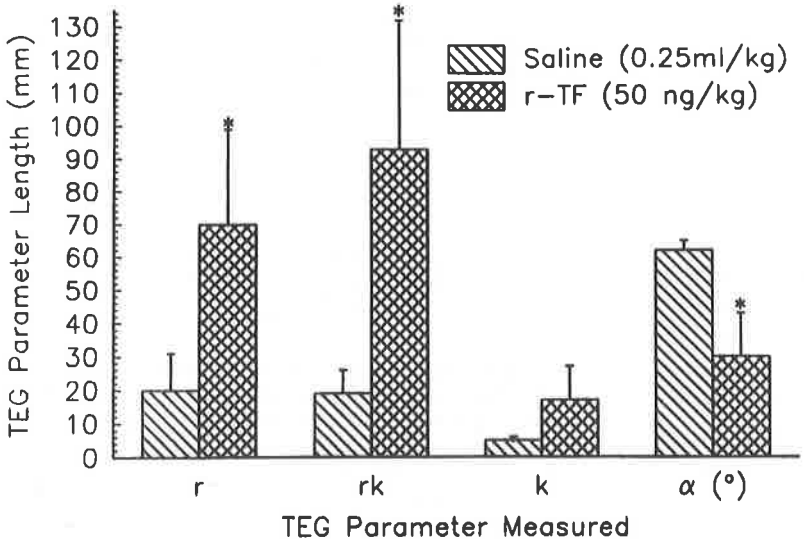

FIG. 4. Thromboelastographic parameters of blood samples from rabbits treated with r-TF or saline in vivo. Each point represents the mean ± SD of five samples from five animals. *The TEG parameter after injection of r-TF is significantly different from the TEG after injection of saline, at $p < 0.05$.

(activated partial thromboplastin time, prothrombin time, Heptest, thrombin time). This may be explained by the fact that various tissues such as the brain, adventitia, organ capsules, and the epidermis are rich in TF,[3] which is necessary to activate the hemostatic mechanisms when vessel injuries occur and blood is exposed to these tissues. In addition, TF can be expressed on surfaces of monocytes or endothelial cells in response to lymphokines or monokines.[3] In a recent study by Sueishi et al,[4] TF was localized in human atherosclerotic intima and was expressed mainly by macrophages at the site along with platelet growth factor B chain. Therefore, since the plasmatic fractions lack platelets, red blood cells, and other cellular components to which TF may bind, only the whole blood tests, such as the TEG, are capable of reflecting the true coagulation state.

The TEG results of our study clearly demonstrate the type of information that this method is capable of providing. This information is more global and in-depth than that obtained with the conventional plasma clotting assays. Furthermore, it allows for qualitative differentiation of the various anticoagulants utilized in this study. The TEG method thus may be more clinically useful than the other methods, especially with the new anticoagulants and antithrombotics, because it is physiologically more relevant to the whole blood effects.

REFERENCES

1. Nemerson Y: Tissue factor and hemostasis. Blood 71:1–8, 1988.
2. Fiedel BA, CSL Ku: Further studies on the modulation of blood coagulation by human serum amyloid P component and its acute phase homologue C-reactive protein. Thromb Haemostas 55:406–409, 1986.
3. Drake TA, JH Morrissey, TS Edgington: Selective cellular expression of tissue factor in human tissues. Am J Pathol 134:1087–1097, 1989.
4. Sueishi K, C Yasunaga, T Murata, M Kumamoto, K Nakagawa, S Kono: Endothelial function in thrombosis and thrombolysis. Jpn Circ J 56:192–198, 1992.

Thromboelastographic Analysis of Patients Receiving Aprotinin with Comparisons to Platelet Aggregation and Other Assays

MICHAEL J. KOZA, B.S., JEANINE M. WALENGA, Ph.D., YELENA N. KHENKINA, M.S., HENRY J. SULLIVAN, M.D., ALVARO MONTOYA, M.D., and ROQUE PIFARRE, M.D.

Impaired platelet function is considered the main cause of bleeding after patients undergo cardiopulmonary bypass surgery (CPB). The requirement for blood products and other postoperative drugs has not been eliminated even with improvements in technology.[1] Various studies have shown the activation of platelets when in contact with the extracorporeal unit.[2,3] Yet, there are data that demonstrate that this response may be reversible.[4]

Aprotinin, a protease inhibitor, has recently been used in the United States on a compassionate-use basis in cardiovascular surgery patients determined to be at high risk for bleeding. Previous studies in Europe have concluded that the use of aprotinin decreases postoperative blood loss by about one-half.[5,6] In addition to inhibiting trypsin, kallikrein, and plasmin, aprotinin is also thought to preserve the adhesive abilities of platelets via the glycoprotein Ib (GP Ib) receptor.[7,8] Although questionable, some studies have demonstrated a correction of the diminished interaction between platelets and the subendothelial layer in the presence of aprotinin.[9,10]

Due to the consistent decrease in postoperative blood loss, there has been a growing interest in the monitoring of aprotinin and in more precisely determining its mechanism of action. More importantly, a limited number of postoperative cases of thrombosis have been reported with the clinical use of aprotinin.[11] Current studies are thus centering on the effect of aprotinin on heparin monitoring and the effect of aprotinin on platelet function.[12] Among other methods, the thromboelastograph (TEG) can be utilized for assessing hemostasis in the presence of aprotinin and for identifying postoperative bleeding.

The TEG provides data based on the viscoelastic properties of clot formation as determined from alterations in shear elasticity. Different parameters, including the reaction time (R), coagulation time (K), maximum amplitude (MA), and angle (A), are used to describe whole blood coagulation as measured by the TEG. R represents the initiation of clot formation, which is prolonged by anticoagulant therapy or factor deficiency and shortened by hypercoagulable conditions. K depicts the time to the formation of a clot at a fixed level of firmness. Shortening of this value is an indication of increased platelet function, whereas elevated values represent anticoagulant or physical impairment of platelet function. MA defines the maximum shear modulus or strength of a developed clot. The strength of a clot is composed of two elements, the strength of the fibrin strands and the contribution of platelets. The angle is the measurement of the rate of fibrin polymerization and is closely related to K. A coagulation index (CI) can be calculated to provide a descriptive product of the overall coagulation function of the patient based on all of the above parameters of the TEG. Normal CI values fall within a range of −2.0 to +2.0.

$$\text{CI (native)} = -(0.1227)\text{R} + (0.0092)\text{K} + (0.1655)\text{MA} - (0.0241)\text{A} - 5.0220$$

$$\text{CI (celite)} = -(0.3258)\text{Rc} - (0.1886)\text{Kc} + (0.1224)\text{MAc} + (0.0759)\text{Ac} - 7.7922$$

From the Department of Thoracic and Cardiovascular Surgery, Loyola University Medical Center, Maywood, Illinois.

Reprint requests: Dr. Walenga, Department of Thoracic and Cardiovascular Surgery, Loyola University Medical Center, 2160 South First Avenue, Maywood, IL 60153.

$$\begin{aligned}\text{CI (native-celite)} = {} & (0.386)R - (0.508)RK \\ & - (0.03816)MA - (0.1639)A \\ & - (2.088)Rc + (1.966)RKc \\ & + (0.1732)MAc + (0.877)A(c) \\ & - 55.55\end{aligned}$$

Various modifications of the TEG have been established to provide versatility for clinical and experimental observations. Two of the most commonly used methods are the original native (nonactivated) and celite-activated whole blood analyses.[13] With the use of 1% celite, residual anticoagulant activity is overcome to allow for a quicker clotting response. This response is compared to the native whole blood response to provide a more relevant discrimination between different hemostatic states (hypo-, hyper-, or normal coagulation).

To determine what influence aprotinin has on whole blood clot formation, as measured by the TEG and other platelet function assays, we analyzed samples taken from those patients who received aprotinin during CPB and compared the results to other patients who had not been given aprotinin during surgery.

MATERIALS AND METHODS

Blood samples were taken prior to sternotomy from seven patients undergoing redo coronary bypass grafting who did not receive aprotinin. In 11 individuals undergoing the same procedure receiving aprotinin, baseline samples were taken just prior to aprotinin administration. Aprotinin administration was terminated when the patient left the operating room. Postoperative samples were taken from each patient after 30 minutes in the intensive care unit. Samples were drawn from a catheter placed in the internal jugular vein. The initial 10 mL of blood were drawn and discarded to remove any contaminants. Blood was placed into clean empty glass tubes or tubes containing either sodium citrate, ethylene diaminetetraacetic acid (EDTA), or EDTA plus indomethacin (500 μg/mL).

Blood collected in clean tubes was immediately analyzed using both the native and 1% celite activation methods on the Haemoscope Thromboelastograph (Skokie, IL) by a procedure previously reported.[13] Blood from a group of 13 normal volunteers was also evaluated to validate further the method used.

Citrated samples were processed for platelet-rich plasma (PRP) for platelet aggregation and adhesion studies. Platelet-poor plasma (PPP) was obtained and frozen at −70°C for future analysis. Platelet aggregations were performed on a Biodata PAP4 aggregometer (Hatboro, PA) with 450 μL PRP and 50 μL of aggregating agent. Platelet agonists were used at various concentrations to determine if the threshold of platelet aggregation response had increased after CPB. Adenosine diphosphate (ADP) at 2.5, 1.25, and 0.625×10^{-5} M, epinephrine at 500, 250, and 125 μg/mL, and ristocetin at 100, 50, and 25 μg/mL were used. All are final concentrations used during analysis.

Platelet adhesion studies were performed with PRP by utilizing a silicon-treated Bürker counting chamber as previously described.[14]

Samples collected in EDTA were later evaluated for blood cell count, in particular the platelet count, on a Coulter counter system.

The EDTA plus indomethacin sample was processed for PPP and frozen at −70°C. Platelet factor 4 (PF4) levels were determined on these plasmas by a radioimmunoassay method (Abbott, North Chicago, IL).

Postoperative blood loss was assessed by measuring the amount of drainage from the patients' chest into the Pleur-Evac unit after 24 hours.

Statistical analysis was performed using descriptive analysis, analysis of variance (ANOVA), and linear regression from the program Primer of Biostatistics from McGraw Hill (Evanston, IL).

RESULTS

The use of aprotinin showed lower postoperative blood loss than the nontreated group (Fig. 1); however, due to the low number of patients the difference was not significant. The amount of time on CPB varied (1.5 to 3.5 hours) among both groups, which could have contributed to the varying degree of blood loss between each group. However, in the control group 71% of the patients had excessive bleeding (more than 1000 cc over 24

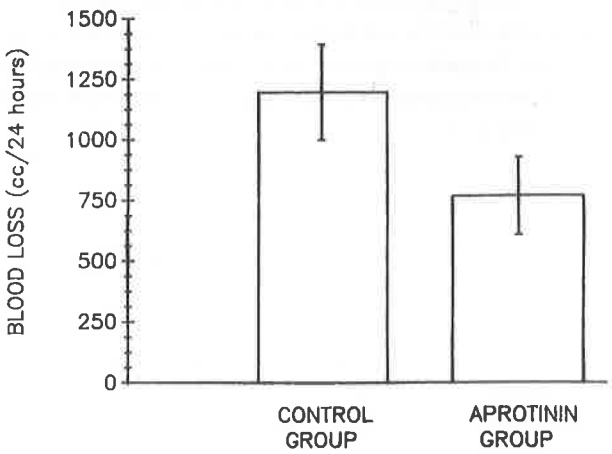

FIG. 1. Postoperative blood loss of the control and aprotinin groups showing lower blood loss for the aprotinin-treated group. Data are represented as mean ± SEM.

TABLE 1. Comparison of Normal, Preoperative, and Postoperative Thromboelastographic Parameters and Indexes*

			Postoperative	
Parameter	*Normal*	*Preoperative*	*Control*	*Aprotinin*
Native				
R (mm)	13 ± 2	32 ± 8†	19 ± 5	11 ± 4
K (mm)	13 ± 2	14 ± 5	14 ± 4	15 ± 5
Angle (°)	46 ± 3	24 ± 7	41 ± 5	38 ± 4
MA (mm)	38 ± 3	42 ± 7	36 ± 8	38 ± 7
Index	0.2 ± 0.6	−2.6 ± 0.5	−1.1 ± 0.7	−0.9 ± 0.4‡
Celite				
R (mm)	4 ± 1	5 ± 1	4 ± 1	13 ± 4†‡
K (mm)	5 ± 1	8 ± 1	6 ± 1	14 ± 2†‡
Angle (°)	59 ± 4	49 ± 4	45 ± 10	34 ± 2†‡
MA (mm)	57 ± 6	53 ± 3	41 ± 8	39 ± 2†‡
Index	1.1 ± 3.8	−0.7 ± 0.9	−1.8 ± 0.8	0.7 ± 0.2
Combined index	−1.9 ± 1.1	−4.8 ± 2.7	−15.2 ± 3.3†‡	−9.7 ± 2.3‡
Number	13	18	7	11

* R: reaction time; K: coagulation time; MA: maximum amplitude. Preoperative represents the combined preoperative responses from both the aprotinin and nontreated groups. Data are represented as mean ± SEM.
† $p < 0.05$ versus normal.
‡ $p < 0.05$ versus preoperative value.

hours), whereas only 18% of the patients in the aprotinin-treated group experienced excessive bleeding.

The TEG results from normal volunteers (Table 1) were comparable to those previously described with the mean indexes falling within the normal value ranges (−2.0 to +2.0).[13] The preoperative data for the patients are also shown in Table 1. The baseline data for the control (nontreated) and aprotinin groups were combined, since there were no differences in patient characteristics. Hypocoagulable TEG results were seen in several preoperative patient samples with prolonged R ($p < 0.05$), as well as diminished angle values, in the native system in comparison to the normals. The preoperative celite assay results showed relatively normal responses in the patients, indicating that the inhibiting factors of coagulation and platelet function detected in the native system analysis could be overcome with the addition of a contact activator (celite).

The postoperative aprotinin group showed less hypocoagulability (shorter R) but no difference in the index of the native blood analysis compared with the nontreated group. The celite-activated blood of the aprotinin patients, however, showed more hypocoagulability than the nontreated patients (prolonged R and K and smaller angle), and the index revealed an overall difference between the two groups. This hypocoagulable state of the aprotinin group could be an assay artifact, since aprotinin itself will interfere in contact activation (celite), causing a prolongation of the time to clot. Because the native blood analysis did not show this effect, the celite data may not represent the actual clinical situation. Although the data revealed differences between the aprotinin- and nonaprotinin-treated groups in both the native and celite systems, there was no statistical significance.

The postoperative results of the aprotinin group revealed a better clotting response than the patients' preoperative TEG in the native blood analysis (shorter R, wider angle). The higher index value of the postoperative TEG (native) was significantly different from baseline ($p < 0.05$). In the celite-activated analysis there remained the (false?) hypocoagulable response (longer R, longer K, smaller angle A, smaller MA) in comparison to the baseline values ($p < 0.05$).

The postoperative results of the aprotinin group, in comparison to the normal values, showed no difference between the two data sets in the native blood analysis. In other words, a normal postoperative coagulation response was obtained with aprotinin treatment.

The celite-activated system again showed significantly prolonged (falsely?) time to clot (R, K, MA, angle) when the aprotinin and normal groups were compared ($p < 0.05$).

The postoperative control group (nontreated) revealed no significant differences from the preoperative patient results in the native blood analysis, although there was some indication of a more normal coagulable response (R and angle). The celite-activated blood analysis showed no significant differences between preoperative (baseline) and postoperative responses in the nontreated patients, except for an indication of reduced platelet function postoperatively (angle, MA). In comparison to the normal volunteer TEG data, no significant differences

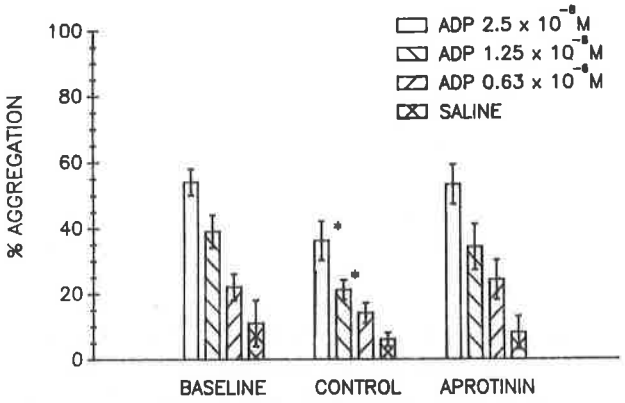

FIG. 2. Platelet aggregation induced by various concentrations of adenosine diphosphate (ADP) in patients with or without aprotinin (control, nontreated group). Baseline represents the combined preoperative response from both the treated and nontreated groups. Data are represented as mean ± SEM (*$p < 0.05$).

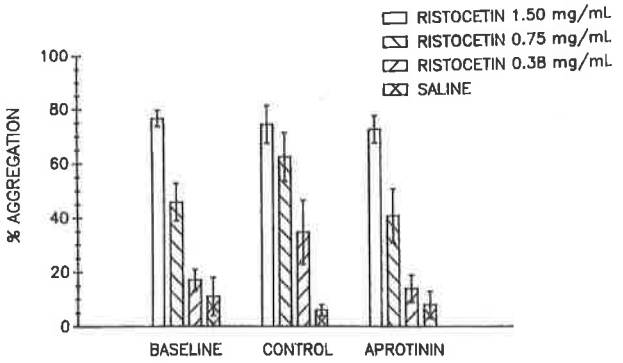

FIG. 4. Platelet aggregation induced by various concentrations of ristocetin in patients with or without aprotinin (control, nontreated group). Baseline represents the combined preoperative response from both the treated and nontreated groups. Data are represented as mean ± SEM.

were observed in the nontreated patients. However, overall a hypocoagulable state was detected postoperatively in the nonaprotinin group ($p < 0.05$).

Platelet aggregation analysis (Figs. 2, 3, and 4) demonstrated a concentration dependency to each platelet agonist (ADP, epinephrine, and ristocetin) in the baseline samples (all patients preoperative). A decrease in aggregation was seen in the postoperative samples with no significant difference observed between the aprotinin and nontreated groups. The postoperative control group, however, showed a significantly lower response with the two highest concentrations of ADP than the responses obtained at baseline ($p = 0.032$). The postoperative aprotinin group demonstrated a significantly lower response to the highest concentration of epinephrine than the baseline results ($p = 0.001$). No statistical difference was seen in the ristocetin-induced aggregation assays.

Other platelet function assays, including the platelet count, platelet adhesion, and platelet factor 4 values as a measure of platelet activation are shown in Figures 5, 6, and 7. Platelet count was decreased in both aprotinin and nontreated groups postoperatively, but significantly decreased in the aprotinin group ($p = 0.008$). Platelet adhesion did not change significantly between baseline and postoperative results. A higher degree of variation was noted in the nontreated group. In the aprotinin group, there was a tendency for less postoperative platelet activation as depicted by more normal (less hyperactive) adhesion and a more consistent response. PF4 levels were equally increased in both groups postoperatively in comparison to the baseline; however, neither group showed a significant increase over baseline.

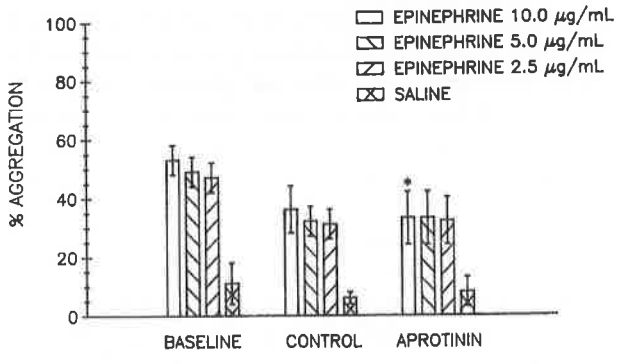

FIG. 3. Platelet aggregation induced by various concentrations of epinephrine in patients with or without aprotinin (control, nontreated group). Baseline represents the combined preoperative response from both the treated and nontreated groups. Data are represented as mean ± SEM (*$p < 0.05$).

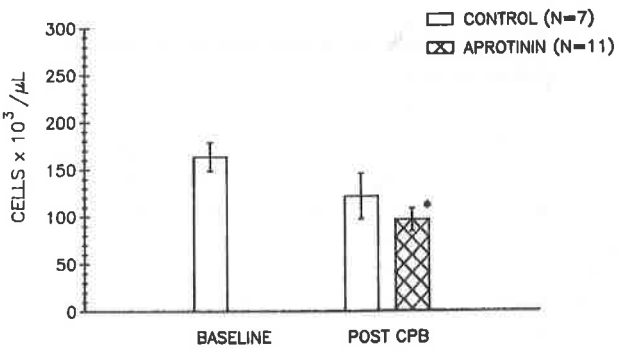

FIG. 5. Comparison of postoperative platelet count in patients with and without aprotinin (control, nontreated group). Baseline represents the combined preoperative response from both the treated and nontreated groups. Data are represented as mean ± SEM.

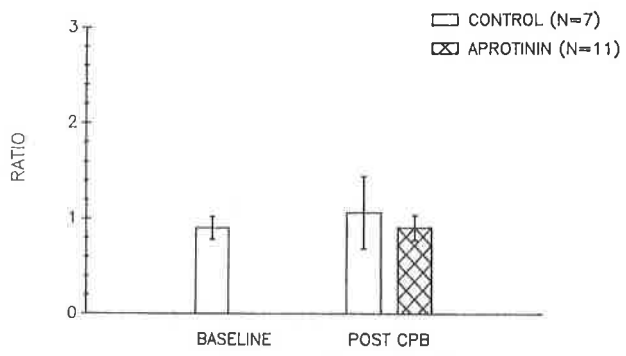

FIG. 6. Comparison of platelet adhesion postoperatively in patients with and without aprotinin (control, nontreated group). Baseline represents the combined preoperative response from both the treated and nontreated groups. Data are represented as mean ± SEM.

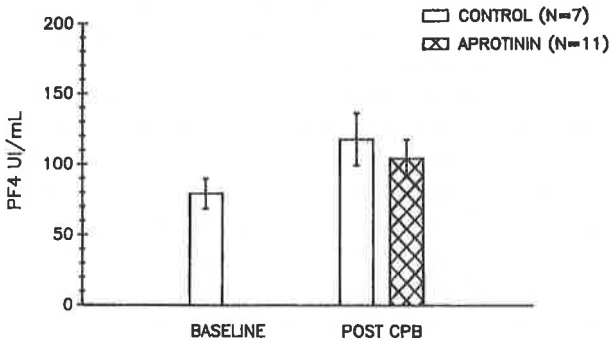

FIG. 7. Comparison of postoperative platelet factor 4 release in patients with and without aprotinin (control, nontreated group). Baseline represents the combined preoperative response from both the treated and nontreated groups. Data are represented as mean ± SEM.

DISCUSSION

The data of this study demonstrate the effectiveness of aprotinin to decrease postoperative blood loss correlating with previously published studies.[5,6] Severe bleeding, observed after CPB, can be due to drug therapy and coagulation irregularities; however, partial loss of platelet function is regarded as the principal cause.[15,16] Platelet function can be easily evaluated with the TEG to determine the abnormalities due to activation by contact with foreign surfaces, release of endogenous substances such as ADP or thrombin, hypothermia, or other means of activation.

In this study preoperative TEG results from the cardiac patients, specifically the native sample, generally expressed hypocoagulable conditions, probably as a result of preoperative administration of various pharmaceutic agents, including heparin. The calculated indexes of the postoperative samples were not significantly different between the aprotinin and nontreated groups. However, a trend was observed with the postoperative aprotinin group having more normal coagulation and platelet function in comparison to those patients who did not receive aprotinin.

An interesting finding was that the clotting parameters in the celite assay system were prolonged in those patients who received aprotinin in comparison to the nontreated group. This is consistent with observations noted by other investigators that aprotinin interferes with the clotting of celite-based activation systems.[12,17,18] Taking this into account and looking only at the native blood analysis, the aprotinin group still showed near normal TEG results postoperatively (not hypo- or hypercoagulable), whereas the nontreated group was somewhat hypocoagulable.

With respect to a linear correlation between blood loss and the TEG findings, the postoperative TEG celite index and combined index correlated well with blood loss in the nontreated group ($r^2 = 0.72$, 0.69, respectively). The native TEG index demonstrated a weaker correlation ($r^2 = 0.31$) and in some cases demonstrated normal coagulation even though blood loss was elevated. This is consistent with previous studies that used only the native whole blood TEG method to predict blood loss.[19] None of the postoperative TEG individual parameters (celite or native) or the indexes from the aprotinin group could be correlated with blood loss. This is not surprising for the celite TEG data because of the interference of aprotinin in the celite assay. Perhaps the native TEG assay values did not reveal a correlation due to the lower amount of blood loss in the aprotinin patients.

A strong trend in the platelet aggregation response indicated that platelets could better aggregate postoperatively in the patients who received aprotinin. This was independent of platelet count, which was not significantly different between the treated and nontreated groups. The ADP, epinephrine, and ristocetin platelet aggregations, however, did not correlate with blood loss in the nontreated group, and the enhanced platelet aggregation response from the aprotinin group could not be correlated with decreased blood loss.

The platelet adhesion studies measured the functional capability of the platelet to respond to stimuli. It is through the GP Ib receptor that adhesion is mediated. A trend was seen in the nontreated group to have stronger adhesion properties (more hyperactive) than the aprotinin group postoperatively. These data suggest that platelets with aprotinin were less stimulated by surgery and thus showed a more normal adhesion response postoperatively. Platelet adhesion did not, however, correlate with blood loss in either group. Previous studies have suggested that

aprotinin preserves the platelet by protecting the GP Ib receptor from activation when in contact with the foreign surfaces of the extracorporeal unit.[8] Our data maintain this concept; however, further investigation is needed to establish this firmly and to seek other mechanisms of action of platelet protection.

The PF4 data show that the platelet secretion response to stimulation during surgery was the same for both the aprotinin-treated and the nontreated groups. We hypothesize that the total platelet activation response in the presence of aprotinin can be differentiated so that the release response is not diminished by aprotinin, yet the aggregation capacity of the platelets is better retained and adhesion is less hyperactive postoperatively. The postoperative aprotinin-treated platelet appears to be more "normal," less hyperactive, and less spent than a nontreated platelet. This could be observed in the TEG data as well. Furthermore, the nontreated patients showed more unpredictable variation (less consistency) in response than the aprotinin-treated platelets.

In conclusion, the TEG could provide reliable data to assess a patient's hemostatic condition. Our study has shown that the native TEG should not be used alone when the TEG is utilized for hemostatic monitoring. Both native and celite-activated analyses should be used to provide a more detailed understanding of the hemostatic function. However, when aprotinin is used, prolonged clotting times for the TEG are obtained and the analysis of clot formation becomes limited. Other surface activators, such as kaolin, which has shown less effect by aprotinin than celite, should be tried.[12,18]

Following surgery with aprotinin, consistent trends were observed, indicating more normal coagulation and platelet function as judged by TEG, platelet aggregation, and platelet adhesion. Even though no difference in platelet count was observed between the treated and nontreated groups, aprotinin-treated platelets were less activated during surgery (normal, not increased postoperative adhesion), and they were better able to aggregate to multiple agonists. In this study, however, no strong correlation could be made between the TEG, functional platelet activity, and blood loss. Our study will continue so that a larger database can be formed and to determine if a replacement to the celite-activated TEG (that is, kaolin) should be used with aprotinin-treated patients.

REFERENCES

1. van Oeveren W, MD Kazatchkine, B Descamps-Latscha: Deleterious effects of cardiopulmonary bypass: A prospective study of bubble versus membrane oxygenation. J Thorac Cardiovasc Surg 97:235–239, 1989.
2. Musial J, S Niewiarowski, D Hershock, TA Morinelli, RW Coleman, LH Edmunds Jr: Loss of fibrinogen receptors from platelet surface during simulated extracorporeal circulation. J Lab Clin Med 105:514–522, 1985.
3. Wenger RK, H Lukasiewicz, BS Mikuta, S Niewiarowski, LH Edmunds: Loss of platelet fibrinogen receptors during clinical cardiopulmonary bypass. J Thorac Cardiovasc Surg 97:235–239, 1989.
4. Zilla P, R Fasol, P Groscurth, W Klepetko, H Reichenspurner, E Wolner: Blood platelets in cardiopulmonary bypass operations: Recovery occurs after initial stimulation, rather than continual activation. J Thorac Cardiovasc Surg 97:379–388, 1989.
5. Royston D, BP Bidstrup, KM Taylor, RN Sapsford: Effect of aprotinin on need for blood transfusion after repeat open heart surgery. Lancet 2:1289–1291, 1987.
6. Bidstrup BP, D Royston, RN Sapsford, KM Taylor: Reduction in blood loss and blood use after cardiopulmonary bypass with high-dose aprotinin (Trasylol). J Thorac Cardiovasc Surg 97:364–372, 1989.
7. Dietrich W, M Spannagl, M Jochum, P Wendt, W Schramm, A Barankay, F Sebening, JA Richter: Influence of high-dose aprotinin treatment on blood loss and coagulation patterns in patients undergoing myocardial revascularization. Anesthesiology 73:1119–1126, 1990.
8. van Oeveren W, MP Harder, KJ Roozendaal, L Eijsman, CRH Wildevuur: Aprotinin protects platelets against the initial effect of cardiopulmonary bypass. J Thorac Cardiovasc Surg 99:788–797, 1990.
9. Lavee J, N Savion, A Smolinsky, DA Goor, R Mohr: Platelet protection by aprotinin in cardiopulmonary bypass: Electron microscopic study. Ann Thorac Surg 53:477–481, 1992.
10. Lavee J, Z Raviv, A Smolinsly, N Savion, D Varon, DA Goor, R Mohr: Platelet protection by low-dose aprotinin in cardiopulmonary bypass: Electron microscopic study. Ann Thorac Surg 55:114–119, 1993.
11. Cosgrove DM III, B Heric, BW Lytle, PC Taylor, R Novoa, LAR Golding, RW Stewart, PM McCarthy, FD Loop: Aprotinin therapy for reoperative myocardial revascularization: A placebo-controlled study. Ann Thorac Surg 54:1031–1038, 1992.
12. Najman DM, JM Walenga, J Fareed, R Pifarre: Effects of aprotinin on anticoagulant monitoring: Implications in cardiovascular surgery. Ann Thorac Surg 55:662–666, 1993.
13. Zuckerman L, E Cohen, JP Vagher, E Woodward, JA Caprini: Comparison of thromboelastography with common coagulation tests. Thromb Haemostas 46:752–756, 1981.
14. Breddin K: Zur Messung der Thrombozytenadhäsivität. Thromb Diath Haemorh 12:269–280, 1964.
15. Mohr R, M Golan, U Martinowitz, F Rosner, DA Goor, B Ramot: Effect of cardiac operation on platelets. J Thorac Cardiovasc Surg 92:434–441, 1986.
16. Mohr R, U Martinowitz, M Golan, A Luski, DA Goor, B Ramot: Platelet size and mass as an indicator for platelet transfusion after cardiopulmonary bypass. Circulation 74 (Suppl 3):153–158, 1986.
17. Hunt BJ, JM Murkin: Heparin resistance after aprotinin. Lancet 341:126, 1993.
18. Wendel HP, W Heller, MJ Gallimore, H Bantel, H Müller-Beissenhirtz, HE Hoffmeister: The prolonged activated clotting time (ACT) with aprotinin depends on the type of activator used for measurement. Blood Coagul Fibrinolysis 4:41–45, 1993.
19. Wang JS, CY Lin, WT Hung, MF O'Conner, RA Thisted, BK Lee, RB Karp, MW Yang: Thromboelastograph fails to predict postoperative hemorrhage in cardiac patients. Ann Thorac Surg 53:435–439, 1992.

Procoagulant and Anticoagulant Effects of Intravascular Contrast Media as Assessed by Thromboelastography

OMER IQBAL, M.D., WALTER JESKE, B.S.,
DEBRA HOPPENSTEADT, M.S., MT(ASCP), MICHAEL KOZA, B.S., MT(ASCP),
JEANINE M. WALENGA, Ph.D., JAWED FAREED, Ph.D.,
and ROGELIO MONCADA, M.D.

Contrast agents were first used by Osborne and colleagues in 1923 at the Mayo Clinic, as sodium iodide solution for urography.[1] The ionic contrast media are basically tri-iodinated derivatives of benzoic acid and are formulated as salts with cations of sodium or meglumine. Ionic contrast media have been associated with side effects, such as adverse hemodynamic changes, cardiac electrical, and rhythm alterations, and allergic reactions. Transformation of the ionizing carboxyl group at the number one position of the benzene ring to a nondissociating amide group results in a nonionic contrast media (NICM). Following interesting milestones of development, Torsten Almén[2] in 1969 introduced metrizamide, the first commercial NICM classified as a low osmolality contrast media (LOCM). Since then, newer LOCM such as iohexol (Omnipaque, Sterling Winthrop, New York, NY), iopamidol (Isovue, Bristol-Myers Squibb, New Brunswick, NJ), ioversol (Optiray, Mallinckrodt, St. Louis, MO), and ioxaglate meglumine-ioxaglate sodium (Hexabrix, Mallinckrodt) have been introduced.

The NICM have proven to cause fewer of the side effects associated with the ionic contrast media.[3] However, several clinical and experimental investigators have reported on potential thrombogenic risks with the use of NICM. Robertson[4] reported blood clot formation in angiographic syringes containing NICM. Kopko and coworkers indicated that thrombin can be generated in mixtures of blood and NICM. Grollman et al[6] reported thrombotic complications during coronary angiography. Gasparetti et al[7] reported the association of NICM with thrombus formation in percutaneous transluminal coronary angioplasty. Grines et al[8] reported on the different effects of ionic and NICM in a canine model of thrombosis caused by acute arterial injury. Hwang and coworkers[9] demonstrated the risk of thromboembolism during diagnostic and interventional cardiac procedures with NICM.

Although typical hemodynamic clinical side effects are reduced with NICM-LOCM, the tremendous cost of these agents causes confusion about their appropriate use.[10–16] Furthermore, the clot formation that may occur when blood is mixed directly with NICM during angiographic procedures[4,17–20] is not acknowledged by all clinicians. Jacobson and Rosenquist[21] raised an important issue of the medical, economic, and legal issues pertaining to the appropriate use of contrast media and stated that the use of the NICM-LOCM agents should be reserved for high-risk patients.

Thus, there exists a concern among some clinicians regarding the safety of nonionic contrast media in interventional radiologic diagnosis. A pragmatic approach to understand these agents better would serve to elicit some basic findings as to the anticoagulant or procoagulant nature of these contrast agents. Thromboelastography, a clinically proven diagnostic tool, is a sensitive technique to evaluate patients undergoing interventional radiologic procedures in which contrast media are used, to help make a rational selection of the contrast media tailored to the physiologic suitability of the patient. In this study

From the Departments of Pathology and Pharmacology, Loyola University, Chicago, Illinois, and the Stritch School of Medicine, Maywood, Illinois.

Reprint requests: Dr. Fareed, Department of Pathology, Loyola University Medical Center, 2160 South First Avenue, Maywood, IL 60153.

TABLE 1. Anticoagulant Effect of Ioxaglate Meglumine-Ioxaglate Sodium, Iohexol, and Iopamidol as Measured by Celite Activated Clotting Time

Saline control (n = 5)	109.80 ± 1.92	Ioxaglat meglumine-ioxaglate sodium	124.00 ± 3.19
Saline control (n = 5)	112.20 ± 4.82	Iohexol	113.60 ± 3.91
Saline control (n = 5)	118.20 ± 13.75	Iopamidol	119.20 ± 16.80

we have used the thromboelastograph to evaluate the potential procoagulant effect of NICM.

MATERIALS AND METHODS

The following materials were used: Iohexol; iopamidol; Ioxaglate meglumine-ioxaglate sodium; 0.9% sodium chloride (Baxter Healthcare, Deerfield, IL); activated clotting time (ACT) instrument (Hemochron, International Technidyne Corporation, Edison, NJ); ACT assay tubes (celite), (Hemochron, International Technidyne Corporation); and Thromboelastograph-D Model 0,4A Si M 1,25G (Hellige, Freiburg, Germany).

Ex vivo supplementation of the contrast agents was performed in freshly drawn human blood (n = 5) in a fashion to simulate the clinical situation where a radiologist injects the contrast agent during angiography. Separate 5 mL syringes were prefilled with 62.5 μL of the contrast agent or saline and blood was drawn up to 5 mL. After uniformly mixing the contents of the syringes by inverting them several times, the syringes were kept at room temperature for 5 minutes. During these 5 minutes, the contents of the syringes were periodically mixed. After 5 minutes, thromboelastography and the celite-ACT assays were performed. ACT results were recorded in seconds and the thromboelastograph analyzed to determine the parameters such as reaction (R) time, reaction-coagulation (RK) time, coagulation (K) time, maximum amplitude (MA), and divergence angle. The ionic contrast media ioxaglate meglumine and nonionic contrast agents iohexol and iopamidol were studied. A saline control was simultaneously performed for the same whole blood each time a contrast agent was tested. Statistical analysis was done using ANOVA from the Primer of Biostatistics Computer Program (McGraw Hill, Evanston, IL).

RESULTS

The celite-ACT results indicated a significant elevation (15 seconds) of the ACT for the ionic contrast agent when compared with the saline control ($p < 0.05$). The ACT values for iohexol and iopamidol supplemented blood samples were not significantly changed when compared with their respective saline controls (Table 1; Figure 1).

Analysis of the thromboelastograph and calculation of different thromboelastographic parameters such as reaction time, reaction-coagulation time, coagulation time, MA, and angle are shown in Table 2. The reaction time is significantly increased for ioxaglate meglumine-aglate sodium ($p < 0.05$) when compared with the corresponding saline control, suggesting an anticoagulant effect or prolongation of the time to clot (Fig. 2). A slight but not significant increase of R time was seen for iohexol when compared against its respective control. Iopamidol showed a slight decrease of R time when compared with its saline control, although this was not statistically significant.

The other thromboelastographic parameters K time (Fig. 3), MA (Fig. 4), and the angle (Fig. 5) did not show statistically significant changes following ioxaglate meglumine ioxaglate sodium, iohexol, or iopamidol supplementation when compared with their respective saline controls. The RK and K times were variable for ioxaglate meglumine-ioxaglate sodium and iohexol, showing both increased and decreased means with no clear trend. However, the maximum amplitude of clot formation suggests an anticoagulant effect by ioxaglate meglumine-ioxaglate sodium (slightly decreased MA), a procoagulant effect

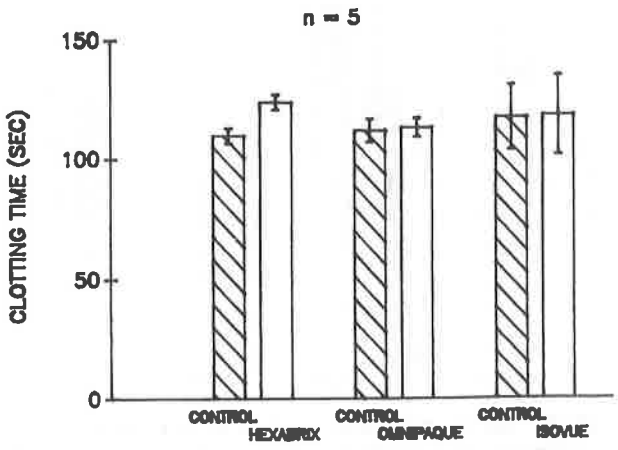

FIG. 1. The celite-activated clotting time results indicate a significant elevation of the activated clotting time (ACT) for the ionic contrast agent (ioxaglate meglumine-ioxaglate sodium) when compared to the saline control ($p < 0.05$). The ACT values for iohexol- and iopamidol-supplemented blood samples were not changed when compared to their respective saline controls.

TABLE 2. Thromboelastographic Parameters Following Supplementation of Ioxaglate Meglumine-Ioxaglate Sodium, or Iohexol, or Iopamidol to Freshly Drawn Human Blood (n = 5)*

	R	*RK*	*K*	*MA*	*Angle*	*R*	*RK*	*K*	*MA*	*Angle*
Saline						Ioxaglate meglumine-ioxaglate sodium				
Mean	28.20	39.40	11.00	53.60	39.60	33.20	42.40	9.20	47.80	40.80
±	±	±	±	±	±	±	±	±	±	±
SD	8.73	11.82	3.32	5.46	9.02	3.97	7.57	3.70	6.83	6.91
Saline						Iohexol				
Mean	25.60	36.40	9.60	48.20	37.00	28.40	41.00	12.60	52.00	36.80
±	±	±	±	±	±	±	±	±	±	±
SD	4.34	3.05	2.41	6.98	5.34	2.07	6.28	4.83	5.87	6.46
Saline						Iopamidol				
Mean	26.20	35.20	10.00	54.60	39.00	23.20	34.00	10.80	54.80	40.60
±	±	±	±	±	±	±	±	±	±	±
SD	2.59	3.96	3.81	2.70	3.67	5.12	7.68	3.56	7.89	9.34

* R: reaction time; RK: reaction-coagulation time; K: coagulation time; MA: maximum amplitude.

by iohexol (slightly increased MA), and no effect by iopamidol (Fig. 4). The angle or rate of clot formation was not affected by any of the three agents (Fig. 5).

DISCUSSION

The ACT (celite) values of ioxaglate meglumine-ioxaglate sodium, iohexol, and iopamidol supplemented whole blood samples when compared with their respective saline controls indicate that there is a statistically significant increase between saline control and ioxaglate meglumine-ioxaglate sodium values (signifying the anti-coagulant nature of this agent), and there is no statistically significant difference when iohexol and iopamidol are compared with their respective saline controls.

Results of the thromboelastographic analysis indicate that when Hexabrix is compared to the corresponding saline control, the R time is significantly increased, suggesting that there is an anticoagulant effect consistent with the ACT. MA, which is the strength of the final clot (strength of fibrin strands and platelet contribution), is lower following ioxaglate meglumine-ioxaglate sodium supplementation when compared with saline control. However, there is little change in the angle or rate of fibrin polymerization. These changes in clot formation following ioxaglate meglumine-ioxaglate sodium supplementation suggest that there is an anticoagulant effect with this agent.

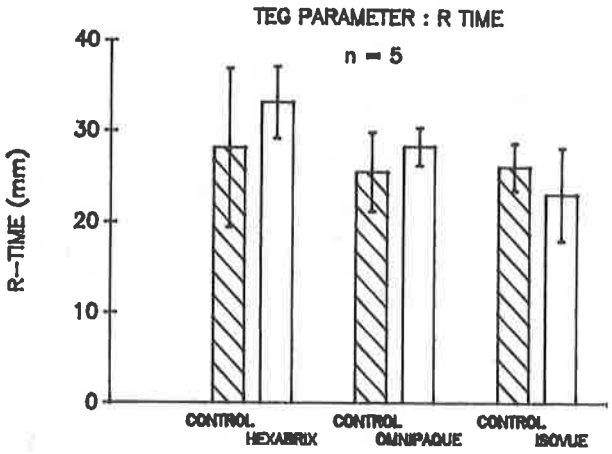

FIG. 2. The reaction time, or time to clot initiation, was significantly increased for ioxaglate meglumine-ioxaglate sodium ($p < 0.05$) when compared to the corresponding saline control, suggesting an anticoagulant effect. Such a significant increase of reaction time was not seen for iohexol when compared to its respective control. Although iopamidol showed a slight decrease of reaction time (procoagulant response) when compared to its saline control, this was not a statistically significant decrease.

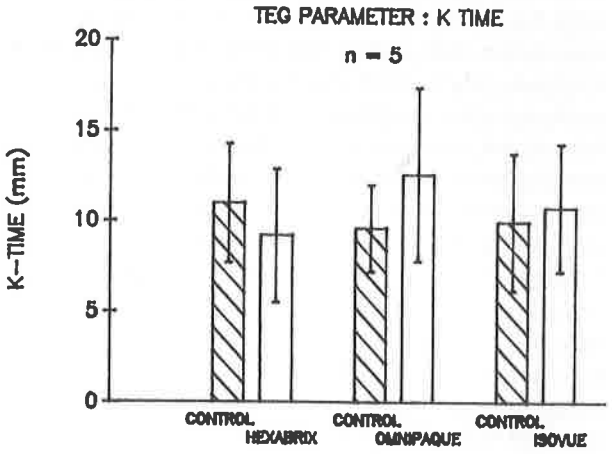

FIG. 3. There was a slight but not significant increase of coagulation time (time to a fixed level of clot firmness) when compared to its respective control for both iopamidol and iohexol. Ioxaglate meglumine-ioxaglate sodium showed a slight but not significant decrease in coagulation time when compared to its saline control.

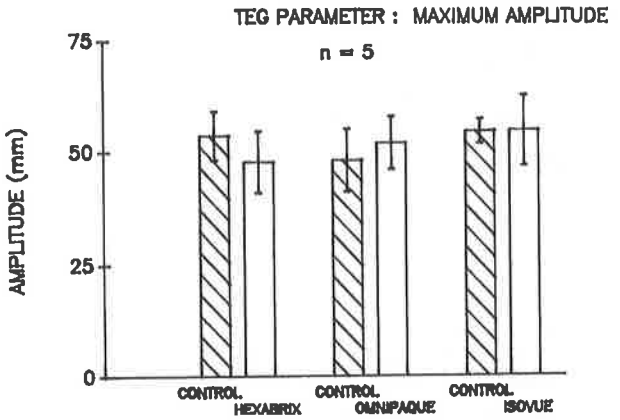

FIG. 4. The maximum amplitude (MA), or maximum strength of the final formed clot, was lower for ioxaglate meglumine-ioxaglate sodium when compared to the corresponding saline control, indicating an anticoagulant effect. The MA with iohexol supplementation was higher when compared to its corresponding saline control indicating that a stronger clot was formed. The MA for iopamidol was unchanged when compared to its corresponding saline control.

When the results of iohexol are compared to the saline control, there is no significant change in the R, RK, and K times, although all tend to increase slightly. This is inconsistent with the ACT data. However, with iohexol, several individual blood samples gave shorter time to clot values than the saline control. This was never observed with ioxaglate meglumine-ioxaglate sodium. The MA seen with iohexol supplementation was somewhat higher when compared to its corresponding saline control, indicating that a stronger clot was formed. The MA was also higher for iohexol than ioxaglate meglumine-ioxaglate sodium, indicating that the developed clot produced in iohexol-supplemented blood was firmer than the ioxaglate meglumine-ioxaglate sodium–supplemented clot. No change in the rate of clot formation (angle) was observed with iohexol.

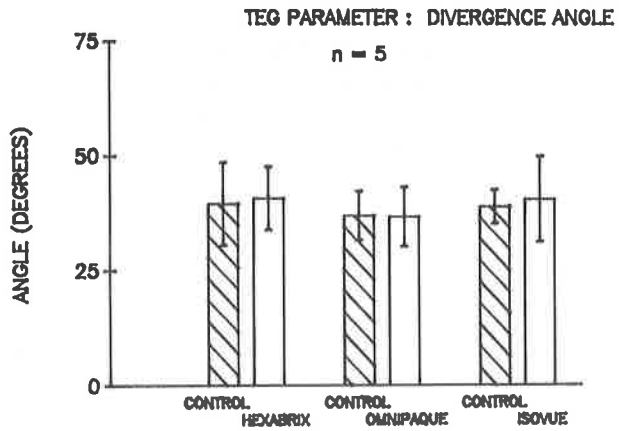

FIG. 5. There was no change in the divergence angle (rate of clot formation) for ioxaglate meglumine-ioxaglate sodium, iohexol, or iopamidol when compared to their corresponding saline controls.

When the results of iopamidol supplementation were compared to the saline control, it was observed that the thromboelastographic parameters were not significantly affected. R time, the time taken for the initiation of clot formation, was the single thromboelastographic parameter that showed a difference between the contrast agent when compared to its respective saline control. The shorter R time was indicative of a procoagulant effect.

Overall, a statistically significant difference was found between ioxaglate meglumine-ioxaglate sodium, iohexol, and iopamidol, with ioxaglate meglumine-ioxaglate sodium having the highest ACT value and R time or most prolonged initiation of time to clot ($p < 0.05$). Another distinction was the strength of the final clot, which was weakest for ioxaglate meglumine-ioxaglate sodium. The time to a fixed clot firmness for the three agents was approximately the same and the rate of clot formation did not change for any contrast media–supplemented blood compared to its control.

CONCLUSION

These studies suggest that an anticoagulant effect as indicated by a prolongation of the initiation of clot formation and final clot strength is produced by the ionic contrast media (ioxaglate meglumine-ioxaglate sodium), whereas the NICM (iohexol and iopamidol) tend to show a diminution of this anticoagulant effect. In some individual cases a procoagulant effect mediated by the NICM was suggested.

A pragmatic approach to the suitability of the use of NICM by the radiologist would be to assess the patients on an individual basis by performing simple techniques such as ACT and TEG on their whole blood supplemented with contrast media to determine any procoagulant effect. The use of NICM would be eliminated in patients who show an in vitro procoagulant response.

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REFERENCES

1. Osborne ED, CG Sutherland, AJ Scroll Jr, LG Rountree: Roentgenography of urinary tract during excretion of sodium iodide. JAMA 80:368–373, 1923.
2. Almén T: Contrast agent design: Some aspects on the synthesis

of water soluble contrast agents of low osmolality. J Theor Biol 24:216–226, 1969.
3. King BF, GW Hartman, B Williamson Jr, AJ LeRoy, RR Hattery: Low-osmolality contrast media: a current perspective. Mayo Clin Proc 64:976–985, 1989.
4. Robertson HJF: Blood clot formation in angiographic syringes containing non-ionic contrast media. Radiology 162:621–622, 1987.
5. Kopko P, DG Smith, BS Bull: Thrombin generation in nonclottable mixtures of blood and nonionic contrast agents. Radiology 174:459–461, 1990.
6. Grollman JH, C Chi, RA Astone: Thromboembolic complications in coronary angiography associated with the use of non-ionic contrast media. Cathet Cardiovasc Diagn 14:159–164, 1988.
7. Gasparetti CM, Feldman, LR Burwell, DA Angello, KH Haugh, RM Own, ER Powers: Influence of contrast media on thrombus formation during coronary angioplasty. J Am Coll Cardiol 18:433–450, 1991.
8. Grines CL, C Diaz, J Mickelson: Acute thrombosis in a canine model of arterial injury: Effect of ionic versus non-ionic contrast media. Circulation 80 (Suppl 2):411, 1989.
9. Hwang MH, ZE Piao, DK Murdock, J Giardina, I Pacold: The potential risk of thrombosis during angiography using non-ionic contrast media. (Abstr.) J Am Coll Cardiol 11:55, 1988.
10. McClennan BL: Low-osmolality contrast media, premises and promises. Radiology 162:1–8, 1987.
11. Wolf GL: Safer, more expensive iodinated contrast agents: How do we decide? Radiology 154:557–558, 1986.
12. Committee on Drug and Contrast Media: The current efficacious use of water soluble contrast agents for intravascular injections. ACR Bull 44:9–10, 1988.
13. Bettman MA: Radiographic contrast agents—a perspective. (Editorial.) N Engl J Med 317:891–893, 1987.
14. Bagg MNJ, TA Horwitz, L Bester: Comparison of patient responses to high and low osmolality contrast agents injected intravenously. AJR 147:185–187, 1986.
15. Granger RG: Low osmolar contrast media. (Editorial.) Br Med J 289:144–145, 1984.
16. Roberts CJ, SC Farron, MC Charney: How much can the NHS afford to spend to save a life or avoid a severe disability? Lancet 1:89–91, 1985.
17. Fareed J, JM Walenga, GE Saravia, RM Moncada: Thrombogenic potential of non-ionic contrast media? Radiology 174:321–325, 1990.
18. Parvez Z, R Moncada, J Fareed, HL Messmore: Effect of non-ionic contrast media (CM) on the components of coagulation and complement systems. Invest Radiol 18:279–284, 1983.
19. Rasuli P: Blood clot formation in angiographic syringes containing non-ionic contrast media. (Letter.) Radiology 165:582, 1987.
20. Skalpe IO, IM Anke: Complications in cerebral angiography: A comparison between the non-ionic contrast medium iohexol and meglumine metrizoate. Neuroradiology 25:157–160, 1983.
21. Jacobson PD, CJ Rosenquist: The introduction of low osmolar contrast agents in radiology: Medical, economic, legal, and public policy issues. JAMA 260:1586–1592, 1988.

Perspectives on Thromboelastography

JOSEPH A. CAPRINI, M.D., F.A.C.S., CLARA I. TRAVERSO, M.D., Ph.D., and JUAN I. ARCELUS, M.D., Ph.D.

This issue of *Seminars* and the literature cited in it arrive at two main conclusions. First, that thromboelastography (TEG) has been considered an interesting test of hemostasis since it was proposed by Hartert in 1948.[1] Studies evaluating its role in several medical fields continue to this day. Second, to interpret TEG properly, a comprehensive knowledge of the test is necessary, as well as experience with its use. The authors of this issue hope that it provides a better understanding of the rationale for the use of TEG, as well as providing, in some way, a consensus that places thromboelastography in its deserved position, based on the extensive experience gained throughout the years.

TECHNICAL ASPECTS

Before ordering a TEG or to interpret preexisting data, the kind of information that TEG can provide in each case needs to be understood, and the specimen needs to be selected according to the information required. The first major consideration is the type of specimen that is to be tested. Obviously, information is different from a TEG performed on plasma than from a TEG performed on whole blood; further differences are obtained if a thromboelastogram is performed on platelet-poor or on platelet-rich plasma, in native or decalcified whole blood. Similarly, the specific assay to be performed (technical modifications to the original test such as celite-activated TEG or different proportions of blood/anticoagulant) need to be specified. Applications of the test will differ depending on the specimen and the technique used. Therefore, before ordering the TEG test, the kind of information that TEG can provide in each case needs to be understood, and the specimen needs to be selected according to the information required.

By the same token, abnormal range for each thromboelastographic parameter needs to be established by every laboratory for each type of specimen. An alternative to this is to verify that normal values given by other laboratories are appropriate to a particular laboratory. These considerations have to be applied to research as well as to clinical usage. Additional considerations also come into play in research. For instance, there is a trend to hypercoagulability among normal rodents.

SOURCES OF ERROR

For several reasons, which will be discussed later, incorrect thromboelastograms are sometimes recorded. These equivocal tracings bias the information interpreted from the reading, and false hypercoagulable and hypocoagulable states may be diagnosed. The most frequent source of error is that a rigorous and standardized methodology has not been used. For practical reasons, this section will be divided into three separate areas: False hypercoagulability, false hypocoagulability, and other sources of error.

False Hypercoagulabilities

Any laboratory examination has a crucial step, which is proper, standardized blood sample collection.[2,3] A poorly withdrawn blood sample, for example due to difficult veins, results in a contamination of the specimen by tissue thromboplastin, which influences both longitudinal thromboelastographic parameters (the reaction time [R] and the clot formation time [K]). Values of both constants are diminished, and the shortening of both parameters

From the Department of Surgery, The Glenbrook Hospital and Northwestern University Medical School, Glenview, Illinois (J.A.C.), Department of Surgery and Related Specialties, University of Granada Hospital, Granada, Spain (C.I.T.), and Department of Surgery, Hospital of the Axarquía, Vélez-Málaga (Málaga), Spain (J.I.A.).

Reprint requests: Dr. Caprini, Department of Surgery, 2100 Pfingten Road, Glenview, IL 60025.

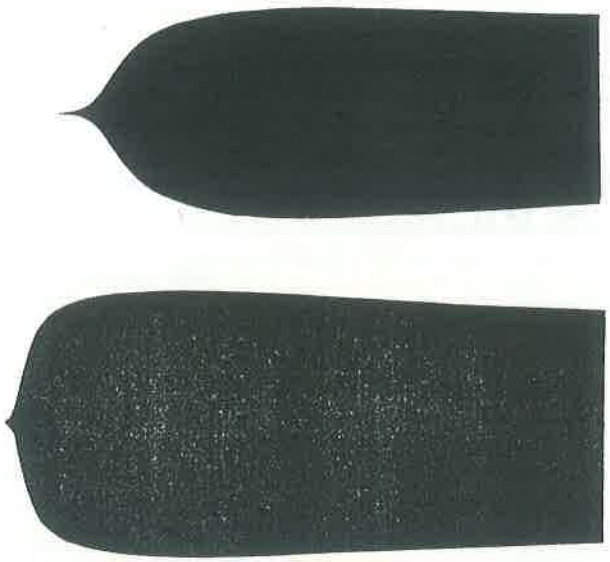

FIG. 1. Thromboelastography as a tool to display a contaminated blood specimen. (Authors' tracing.)

indicates a hypercoagulability that does not truly exist.[4,5] The two-syringe blood collection technique is used to assure reliable test results; however, sometimes, even when using this technique, blood collection is unsatisfactory.

A trace of thrombin in a cuvette due to a defective cleaning process may also generate a false hypercoagulability.[6] To assure the proper withdrawal of blood, Caprini and associates proposed using the celite-activated TEG technique.[7] When there is no suspicion of clotting or hemolysis in the tubes, or when poor blood collection has gone undetected, performing the combined analysis of native whole blood TEG and celite-activated whole blood TEG can display the false hypercoagulability derived from the improper blood collection. This is shown in Figure 1. The use of this combined technique in clinical practice has provided excellent results, and it is proposed as part of a standardized blood sample collection.

As with the withdrawal of blood, the standardization of the thromboelastographic technique is crucial. Accordingly, when one selects a thromboelastographic methodology, it is necessary to meticulously reproduce it, step by step, every time, to obtain reliable results.

False Hypocoagulabilities

When decalcified whole blood TEG is used false hypocoagulabilities may be recorded. Defective reagents, those in which calcium is lacking, generate a slowed coagulability.[5] An improper blood/anticoagulant ratio causes the same kind of error as does a lack of calcium. This is also noted when the sample in the cuvette is not properly covered with two to three drops of paraffin oil.[8] Inadequate manipulation of the decalcification/recalcification process strongly affects R (Fig. 2).[4] When plasma is used for the thromboelastographic analysis, an excessively high speed of centrifugation may remove part of the platelets, which imparts a false decrease on the maximal amplitude (MA), that is, a false hypocoagulability would be recorded.[9]

False changes in the thromboelastographic parameters may also be observed due to several other factors. If hemolysis of the blood sample takes place, R values are diminished due to the presence of microclots. Because of an accelerated clotting, platelets and fibrinogen are partially consumed and, subsequently, MA values are decreased. A similar error is incorporated when there is an excessively prolonged delay between the withdrawal of the blood and performing the test. Such a delay provokes an early lysis of platelets with a release of platelet

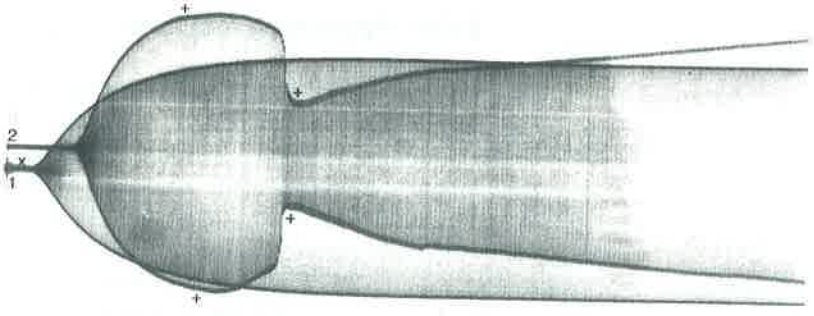

FIG. 2. Defective thromboelastogram due to technical errors. Tracing 1 shows inadequate manipulation (x). Tracing 2 shows an inadequate cleaning process (+), followed by the rupture of the clot (narrow portion labeled with +) as a consequence of a strong impact given to the table where the machine was located. (Authors' tracings.)

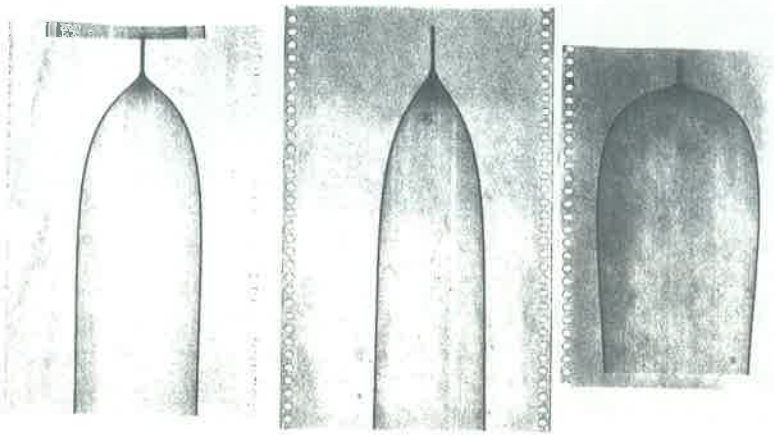

FIG. 3. Examples of tracings indicative of hypercoagulability (right), normocoagulability (center), and hypocoagulability (left). (Authors' tracings.)

factors and, subsequently, an accelerated thrombin generation. TEG shows this by a decrease in the R value, which resembles a hypercoagulability that does not truly exist. Likewise, lysed platelets do not take part in clot formation and, therefore, MA values diminish, which is indicative of a hypocoagulability that does not exist either.[5]

Other Sources of Error

Other errors of interpretation may be caused by vibrations of the table on which the TEG machine is located,[10] to which the older thromboelastographs are especially sensitive. A strong impact may abruptly rupture the clot (Fig. 2). Artifacts affecting the curved part of a thromboelastogram may be caused by inadequate cleaning of the cups or pins.

SUMMARY

The knowledge of these sources of error together with strict methodology assure reliable results and avoid what some authors consider limitations of the test. These so-called limitations are actually due to incomplete information or insufficient experience. It is our opinion that the key for success when using TEG is to use the test in those cases for which it has been indicated. Also, the fact that TEG is a global test of coagulation should be kept in mind and therefore the need for additional hemostatic tests should be evaluated when applicable (Fig. 3).

According to this review, the established applications of TEG are as follows: (1) detection of hypercoagulable states, particularly in the postoperative period and for patients with malignancies; (2) management of patients who are administered intravenous heparin; (3) monitoring the coagulation state during liver transplantation; (4) monitoring the coagulation state and management of patients during cardiopulmonary bypass; and (5) diagnosis and treatment of hematologic dysfunctions, particularly hemophilia. Management of patients under warfarin administration, as well as monitoring of the adjustment of subcutaneous heparin prophylaxis in several surgical procedures, should probably belong to this list, but further studies are needed to confirm these roles.

Used by experienced hands, TEG is a valuable hemostatic test, the future of which is already present.

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REFERENCES

1. Hartert HH: Blutgerinnungsstudien mit der Thromboelastographie, einem neuen Untersuchungsverfahren. Klin Wochenschr 26:577–583, 1948.
2. Leppanen EA, R Grasbeck: Experimental basis of standardized specimen collection: Effect of posture on blood picture. Eur J Haematol 40:222–226, 1988.
3. Owen J: The utility of plasma fibrinopeptide assays. Thromb Haemostas 62:807–810, 1989.
4. Gormsen J: Thromboelastographic findings in surgery. Acta Chir Scand 122:466–470, 1961.
5. Duraffourd P: Les conditions de la fiabilite et les advantages du thromboelastogramme. Coeur 12:447–455, 1981.
6. Audier M, A Serradimigni: *Savoir interpreter un thrombo-elastogramme ou l'exploration precise de la coagulation sanguine.* Maloine, Paris, 1962, pp 135.
7. Caprini JA, JP Vagher, S Yeager, G Harvey, J Mitchell: Hemostatic activation during two-syringe technique. (Abst.) Thromb Haemostas 46:393, 1981.
8. Neef H, M Ritcher, U Fisher: Einfluß der Wasserstoffionenaktivität auf die Blutgerinnung. Folia Haematol 3:303–311, 1973.
9. Raby C: Exploración del tiempo trombodinámico. *In: Hemorragias y trombosis.* Toray-Masson, Barcelona, 1968, pp 190–211.
10. Marchal G, ME Leroux, M Samama: La Thrombodynamographie (Thromboelastographie). Sa place actuelle en clinique. Presse Med 51:1919–1922, 1960.

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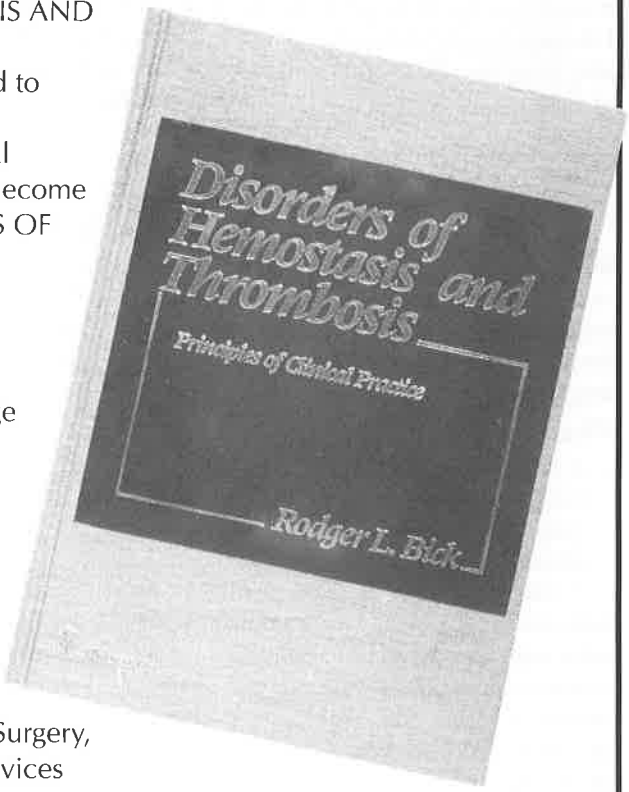

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