CLINICAL SIGNIFICANCE AND STANDARDIZATION OF THROMBOKINETICS

by

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Part One: UNDERSTANDING COAGULATION

Introduction

Coagulation is a rapidly growing field of interest in medicine. As knowledge of the coagulation process increases, methods used to monitor clot formation in the clinical laboratory become increasingly sophisticated. A particularly new concept in coagulation monitoring is the use of coagulation profilers linked to chart recorders which enable technologists to visualize clot formation graphically. The patterns formed by the chart recorder are called thrombokinetographs. The use of these thrombokinetographs as diagnostic tools when performed in conjunction with an activated partial thromboplastin time (APTT) is the topic of discussion of this paper. The usefulness of this measurement in diagnosis can not be realized without a basic understanding of the coagulation process.

Coagulation: Primary Hemostasis, Extrinsic System, and Intrinsic System

Clot formation is a dynamic process involving platelets which are anucleate cells representing portions of the cytoplasm of megakaryocytes and polypeptide chains which are termed collectively "clotting factors". There have been sixteen clotting factors described at this point in time, but the list is expected to grow in the future.

The coagulation process has been divided into two systems: the intrinsic system and the extrinsic system. Injury to an
endothelial cell results in the release of tissue thromboplastin which activates the extrinsic system while collagen serves as an activator for the intrinsic system. Both systems result in the formation of a fibrin clot. Refer to page 13 for a list of the clotting factors.

Injury to a capillary endothelial cell results in exposure of collagen to circulating platelets. There are receptor sites on the platelet surface that adhere to the collagen, and a shape change of the platelet is initiated. This shape change exposes Platelet Factor 3 on the membrane surface which is necessary to the intrinsic system of coagulation, and at the same time there is a release of substances from the platelets. The most important of these substances is ADP which stimulates the shape change and subsequent release mechanism in other platelets. As more and more platelets are "activated", aggregation of the platelets begins to occur and a primary plug of platelets is formed at the site of injury to the vessel.

As the platelets form the primary plug there is simultaneous activation of the intrinsic and extrinsic coagulation systems. Occurring most rapidly is the extrinsic coagulation reaction. The tissue thromboplastin released by the traumatized endothelial cell reacts readily with circulating Factor VII. A complex of calcium, tissue thromboplastin, and Factor VII causes the activation of Factor X. Activated Factor X, calcium, Factor V, and tissue thromboplastin activate Factor II (prothrombin) to Factor IIa (thrombin). Thrombin brings about the conversion of Factor I (fibrinogen) to Factor Ia (fibrin). Factor XIII then comes along to stabilize the fibrin clot.
The intrinsic system, on the other hand, appears to be activated by collagen. The series of reactions is more complex than the extrinsic reactions and is best demonstrated diagrammatically; the relationship of the intrinsic and extrinsic systems is also illustrated in this diagram:

\[\text{In vivo} \quad \text{Collagen} \quad \downarrow \]
\[\text{XII} \rightarrow \text{XIIa} \]
\[\text{XI} \rightarrow \text{XIa} \quad \uparrow \text{Ca}^{++} \]
\[\text{IX} \rightarrow \text{IXa} \quad + \quad \text{VIII, Ca}^{++}, \text{Platelet Factor 3} \]

Extrinsic system
\[\text{Factor VII + Ca}^{++} \quad \text{and} \quad \text{Tissue Thromboplastin} \quad \downarrow \]
\[\text{X} \rightarrow \text{Xa} \quad + \quad \text{V, Ca}^{++}, \text{Platelet Factor 3} \]
\[\text{Prothrombin} \rightarrow \text{Thrombin} \]
\[\text{Fibrinogen} \rightarrow \text{Fibrin} \quad \downarrow \text{Factor XIII} \]
\[\text{Stable fibrin clot} \]

As the complexed fibrin molecules polymerize there are fibrin threads forming a lattice work on and around the aggregating platelets. As the lattice work becomes more and more stabilized, the hemostatic plug is firmly anchored.

Coagulation Disorders

A qualitative or quantitative defect in any of these factors (except Factor XII) is associated with a bleeding disorder. Classical hemophilia (Hemophilia A) is caused by a defect in the Factor VIII molecule. It has been shown that a large percentage
of patients exhibiting a decreased Factor VIII activity do actually produce a non-functional or partially functional Factor VIII molecule. This inability of the defective molecule to effectively participate in the intrinsic clotting system poses real hazards when the patient is traumatized. If the hemophilia is severe (<2% Factor VIII activity), the patient may have spontaneous bleeding into muscles and joints which can result in crippling. It should be noted here that a hemophiliac has a normal Ivy or Mielke bleeding time because his primary hemostatic system is entirely functional i.e. platelet aggregation results in the formation of a primary plug which stops the bleeding. However, a stable clot is not formed, so this patient may "rebleed" after dental extractions, minor surgery, etc. This is a characteristic common to clotting factor disorders. The genetic transmission of Hemophilia A is sex-linked recessive; females carry the trait on the X chromosome, and it is exhibited in statistically one-half of their male children.

Von Willebrand's disease involves an actual decrease in the amount of Factor VIII produced, and its genetic transmission is usually autosomal dominant. Unlike Hemophilia A patients, persons with von Willebrand's have prolonged Ivy and Mielke bleeding times because they lack von Willebrand's factor which is necessary to platelet aggregation. These patients are especially treacherous surgical subjects since they have a defective primary hemostatic system and a non-functional intrinsic clotting system.
Factor IX deficiency (Hemophilia B or Christmas Disease) can give the same clinical picture as classic hemophilia. It, like Hemophilia A, appears very rarely in females since the inheritance pattern is sex-linked recessive. About 15% of hemophiliacs have Factor IX deficiency. Factor IX deficiency can be distinguished from Factor VIII deficiencies by factor identification panels.

Other factor deficiencies causing coagulation problems may be the result of deficient synthesis of the factor, production of functionally inactive forms, or production of functionally abnormal forms. A listing of the factors giving information about frequency, genetic transmission and severity is on page 13.

Since the clotting factors are protein in nature, severe liver damage may result in a decreased amount of all the clotting factors (except Factor VIII which does not appear to be synthesized in the liver). Factors II, VII, IX, and X are dependent upon Vitamin K for their synthesis, therefore a lack of Vitamin K can result in decreased production of these factors.

Finally, one should realize that any qualitative or quantitative disorder of platelets can interfere with coagulation. Since platelet dysfunction is not measured by the automated partial thromboplastin time, this topic will not be discussed at this point. The interested reader may consult any hematology reference book for a detailed description of coagulation disorders involving platelets.
The automated partial thromboplastin time (APTT) is the single most useful procedure available for routine screening of coagulation disorders. The APTT screens the entire coagulation mechanism except Factor VII, XIII, and platelets. Blood is collected in tubes containing sodium citrate as an anticoagulant. The citrate binds the ionic calcium to prevent clotting in the tube. Platelets are removed by centrifugation. By adding calcium, a phospholipid substance (to replace Platelet Factor 3), and an activator of the intrinsic system, clotting is initiated in the tube and the time necessary for clot formation is the activated partial thromboplastin time. Deficiencies of any of the clotting factors in the intrinsic system except the two mentioned above can be detected. Factor VII of course participates only in the extrinsic system.

In the event that the APTT is prolonged, indicating a factor abnormality, this same test with some modifications can be used for specific factor identification and for assaying specific factor activity. For example, if a male patient has a prolonged APTT and other coagulation tests are normal, the probable factor deficiency is VIII or IX. Factor VIII is totally consumed in clot formation so it is not present in serum, but Factor IX is present in serum. On the other hand, Factor IX is absorbed by barium sulfate but Factor VIII is not; so plasma that has been absorbed with BaSO₄ contains Factor VIII
but no Factor IX. These characteristics of the factors can be used to the advantage of the technologist in the identification of missing factors. If the patient's plasma is mixed with serum reagent containing no Factor VIII, and the APTT returns to normal limits, the deficient factor must be Factor IX because the patient's plasma supplies Factor VIII and the serum reagent supplies Factor IX. If the time is corrected with BaSO₄ absorbed plasma, but not with serum reagent, the missing factor is Factor VIII.

After identification of the deficient factor has been made, the actual activity can be measured by performing dilutions using plasma containing a known activity, plotting a curve of time for clot formation versus concentration of the factor, and comparing the patient's time to the graph.

Thrombokinetics and it's Role in Diagnosis of Coagulation Disorders

The formation of thrombokinetographs is a new concept in coagulation testing. The purpose of the formation of the graph is to visualize the actual clot formation as it occurs. Since a factor deficiency can be as low as twenty-five percent of normal activity and still have an APTT within normal limits, use of the TKG in conjunction with the APTT gives visual information about the clot formation.

In this particular study, use is made of the Bio/Data CP-8 Coagulation Profiler. Bio/Data integrated a chart recorder to its coagulation analyzer. The line drawn on the chart is a visualization of the dynamics of clot formation as observed by a photocell receiving light through the actual tube in which the
APTT is being performed. As the clot forms, the absorbance of light increases. A continuous series of electrical signals from the photocell to the recorder demonstrates the coagulation process as a pattern. Displayed on the chart is a graph of the rate of change of the absorbance (optical density).

There have been reports of detection of mildly factor deficient patients that were detected by the TKG although the APTT was normal. The graphs formed in these cases showed decreased slope and decreased amplitude indicating that the rate of clot formation was abnormal; thus a factor deficiency was suggested. This is important information to physicians, particularly when used as part of a pre-surgical panel. Patients with mild factor deficiencies are potential hazards in surgery, but if the problem is known before surgery, steps can be taken to minimize the risk to the patient.
Part Two: CLINICAL SIGNIFICANCE AND STANDARDIZATION OF THROMBOKINETICS

Objectives

At Ball Memorial Hospital I am currently involved in a study to correlate the thrombokinetic patterns of the activated partial thromboplastin times with respect to various clinical states. Hopefully certain thrombokinetic patterns will emerge which can be directly correlated with clinical diagnoses and treatment of patients.

The objectives of this study are: 1) to establish a normal range of thrombokinetic patterns for male and female subjects using two different reagents. 2) to evaluate TKG patterns from 750 consecutive APTT's from the hospital population. The data collected includes the activated partial thromboplastin times, the thrombokinetic patterns, the patients' diagnoses, medications, age, and sex.

Experimental Procedure

Whole blood specimens were collected in 3.8% sodium citrate (one part citrate to nine parts blood) taking care to cause as little trauma as possible during venipuncture. The specimens were drawn with a syringe and iced immediately. If a slightly abnormal APTT was obtained, the specimen was redrawn with a two-syringe technique to ensure that there was no activation of the clotting systems.

Specimens were centrifuged at 2000 rpm for five minutes. The supernatant plasma was removed and stored in ice until performance of the APTT. All plasma was tested within two hours.
after collection, and the tests were performed in duplicate. For the procedure used at Ball Memorial Hospital, refer to page 14.

For the patients on whom APTT's were performed, information was obtained either from the patients' records at the nursing stations or from their files in medical records. Of particular interest were the patients' ages, diagnoses, and medications.

It was also necessary that we establish normal values for the thrombokinetic patterns. Subjects used as normals were male and female volunteers from the laboratory and from Ball State University. Volunteers ranged in age from nineteen to forty-five years old.

The data was tabulated for each patient using the number value for the activated partial thromboplastin time, slope and amplitude of thrombokinetograph, and age. Additional variables that were selected were those that appeared to be recurrent throughout the study; for these parameters the patient was marked either applicable or non-applicable. These additional parameters include the following:

A. Disease - deep vein thrombosis, arteriosclerotic heart disease, tonsillectomy and adenoidectomy, gastrointestinal bleeding, respiratory disease, liver disease

B. Medications - heparin, Dalmane, Keflin, Darvon, Lanoxin, Valium, Tylenol, aspirin

Unfortunately, at this writing our data has not been processed by the computer center at Ball State. The finished paper will be presented Wednesday, October 27, 1976 at the fall meeting of the
Although no definite conclusions can be made at this point, there are a few statements that can be made concerning what has evolved at this point.

Comparison of the curves obtained on the same person using the two different reagents (Dade and General Diagnostics Automated APTT) shows that although the slopes and amplitudes are comparable, they do not match. For instance, a slope of 2.5 and amplitude of 3.0 using Dade reagent may produce a slope of 3.0 and amplitude 3.9 using General Diagnostic reagent when tested on the same plasma sample; but the slopes and amplitudes obtained using General Diagnostics are not consistently higher than those obtained with Dade reagents. It is encouraging to note that the general shape of the curves formed using the two reagents on the same sample are very similar. See page 14.

It was also observed that many patients who had been receiving heparin (an anticoagulant) had curves that differed from normal even hours after the anticoagulant effect of the heparin had diminished. The normal shape of the graphs is approximately "bell-shaped"; but many of the patient's post-heparin therapy had curves that increased in slope to a peak but remained at that peak without a descent toward the baseline. This is an observation for which there is no explanation at this time.

I can not make any statements about correlation of the curves to the diagnoses of the patients without the results of the computer analysis. The completed text will be published in the American Journal of Clinical Pathology.
TABLE I

<table>
<thead>
<tr>
<th>Factor</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Fibrinogen</td>
</tr>
<tr>
<td>II</td>
<td>Prothrombin</td>
</tr>
<tr>
<td>III</td>
<td>Tissue Thromboplastin</td>
</tr>
<tr>
<td>IV</td>
<td>Calcium</td>
</tr>
<tr>
<td>V</td>
<td>Proaccelerin, labile factor</td>
</tr>
<tr>
<td>VII</td>
<td>Proconvertin, stable factor</td>
</tr>
<tr>
<td>VIII</td>
<td>Antihemophilic A factor (AHF) or antihemophilic globulin (AHG)</td>
</tr>
<tr>
<td>IX</td>
<td>Antihemophilic B factor (AHB) or Christmas factor</td>
</tr>
<tr>
<td>X</td>
<td>Stuart-Prower factor</td>
</tr>
<tr>
<td>XI</td>
<td>Plasma thromboplastin antecedent</td>
</tr>
<tr>
<td>XII</td>
<td>Hageman factor, contact factor</td>
</tr>
<tr>
<td>XIII</td>
<td>Fibrin stabilizing factor, fibrinase</td>
</tr>
</tbody>
</table>

Fitzgerald Factor
Fletcher Factor
Passovoy Factor

TABLE II

<table>
<thead>
<tr>
<th>Factor</th>
<th>Genetic Transmission</th>
<th>Frequency per million</th>
<th>Severity</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Autosomal recessive</td>
<td>&lt; 0.5</td>
<td>moderate</td>
</tr>
<tr>
<td>II</td>
<td>Autosomal recessive</td>
<td>&lt; 0.5</td>
<td>mild to severe</td>
</tr>
<tr>
<td>V</td>
<td>Autosomal recessive</td>
<td>&lt; 0.5</td>
<td>mild to severe</td>
</tr>
<tr>
<td>VII</td>
<td>Autosomal recessive</td>
<td>&lt; 0.5</td>
<td>mild to moderate</td>
</tr>
<tr>
<td>VIII</td>
<td>Sex-linked recessive</td>
<td>60-80</td>
<td>very severe to mild</td>
</tr>
<tr>
<td>IX</td>
<td>Sex-linked recessive</td>
<td>15-20</td>
<td>very severe to mild</td>
</tr>
<tr>
<td>X</td>
<td>Autosomal recessive</td>
<td>&lt; 0.5</td>
<td>mild to severe</td>
</tr>
<tr>
<td>XI</td>
<td>Autosomal recessive</td>
<td>~ 1.0</td>
<td>mild to moderately severe</td>
</tr>
<tr>
<td>XII</td>
<td>Autosomal recessive</td>
<td>~ 1.0</td>
<td>no disease</td>
</tr>
<tr>
<td>XIII</td>
<td>Autosomal recessive</td>
<td>&lt; 0.5</td>
<td>mild to moderately severe</td>
</tr>
<tr>
<td></td>
<td>von Willebrand's disease</td>
<td>Autosomal dominant</td>
<td>5-10</td>
</tr>
</tbody>
</table>
PROCEDURE FOR THE APTT

1. Pipette 0.1 ml Automated APTT into a cuvette.

2. Add 0.1 ml patient's plasma to the above, mix, incubate at 37°C for five minutes.

3. Pipette 0.1 ml 0.025 M calcium chloride into the plasma-automated APTT mixture. The Bio/Data profiler begins automatically with the addition of the calcium chloride.

4. A thrombokinnetogram is run with each sample. Controls run with each sample are Verify Normal, a normal and an abnormal control.
The graph on the left is that of a patient whose curve was made hours after heparin injection. Note that there is no decrease in amplitude after the peak. The curve on the right shows a curve with a peculiar "dip" at the peak. This is seen occasionally with no explanation.

Here are shown two normal curves on the same person showing the difference in slope and amplitude obtained when using General Diagnostic (left) and Lade (right) reagents.