Detection of Prethrombotic and Thrombotic States

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ABSTRACT

Newer methods for detecting subclinical thrombosis are discussed. Uses of radionuclides for thrombus localization are reviewed. Laboratory tests suitable for nonspecialized laboratories are highlighted. The serial use of functional antithrombin III and ethanol gelation assays are recommended to follow the effect of anticoagulant therapy. The use of heparin therapy in low dosage is discussed.

Thromboembolism is a significant medical problem. Embolic complications are sudden and catastrophic. Survival is associated with significant chronic sequelae. Pulmonary embolism alone accounts for 200,000 deaths annually in the United States. Over 90 percent of these deaths occur in hospitalized patients. The vast majority seen at autopsy are not diagnosed prior to death. The routine diagnostic approaches available in past decades as well as in recent years have had little impact in the diagnosis of thromboembolic disease. Prevention of embolization begins with the early detection of venous thrombosis. This has been difficult to achieve. Clinical diagnosis is not reliable and is often late when definite.

Newer measures for detecting subclinical thrombosis are being evaluated. They involve both radionuclide uptake and imaging. Great attention has been given to the deep venous systems of the legs. Examination of pelvic veins is somewhat more difficult and has been ignored as a consequence. The approaches, however, do not possess the potential morbidity of radiologic contrast venography. As with conventional radiographic techniques, serial examination is difficult and correlation with the results of anticoagulant therapy is limited.

Complementary approaches involve evaluation of the coagulation process itself and thrombin kinetics in particular. Correlation with the results of anticoagulant therapy is striking. Serial examination is not difficult. The techniques are not limited to disturbances of the deep venous systems of the legs. Localization of the clotting site cannot be reached, however.

The advent of low dose subcutaneous heparin therapy for the prophylaxis of pulmonary embolism intensified the need for development of imaging and laboratory techniques to identify patients at risk for thromboembolism and to follow objectively patient response to anticoagulant therapy.
Physiology

Thrombosis is a dynamic biochemical process which results in mechanical obstruction of blood flow in a vessel. Thrombi may propagate directly or with skip areas. Thrombus formation may be initiated by the action of collagen on factor XII or by the action of factor VII on tissue thromboplastin. Through either mechanism factor X is activated and bound. Thrombin is rapidly evolved.

Thrombin has a profound impact on the entire clotting mechanism. Thrombin cleaves fibrinogen directly, accelerates the activity of factors V, VIII and XIII, as well as potentiates the aggregation of platelets. The effect, then, of freely circulating thrombin is potentially catastrophic. Thrombin accelerates thrombin generation and clot stabilization. Of critical importance in antagonizing thrombin generation and maintaining the integrity of the clotting scheme is antithrombin III. Antithrombin III exerts its effect directly on thrombin1 and inhibits thrombin formation as well by inhibiting activated factors, X, IX, XI and XII.

Radionuclide Approaches

In the early 1960's efforts began to localize clots with radioiodinated fibrinogen and plasmin. The use of fibrinogen is predicated upon its incorporation into an actively forming clot. Other approaches use labelled red and white cells, platelets and other clotting components. Antibody to fibrinogen has also been employed. Plasminogen use is predicated upon penetration of or adsorption onto the formed thrombus to be activated for fibrin degradation. Streptokinase and urokinase have also been used to detect formed clots.

Recent reports of the use of 125-iodine-labelled homologous fibrinogen in clot detection show good results when compared with contrast venography. Radiolabelled fibrinogen is injected and uptake is determined over the course of the leg veins. False positive or high uptake areas are seen in patients with arthritis, edema, cellulitis or superficial thrombophlebitis. Anticoagulant therapy, at least in short term, does not affect uptake. While this is an advantage in detection, it limits the use of uptake studies in following the results of anticoagulant therapy. There is a hepatitis risk to use of homologous fibrinogen. Autologous fibrinogen is readily available. Isotopes other than 125-iodine may be used to label autologous fibrinogen. This may facilitate imaging.

Limitations of plasminogen use also include difficulty in obtaining reasonably pure fractions for radiolabelling and use. Radiolabelled plasminogen is more effective in detecting clots two days old or older than is radiolabelled fibrinogen.

Radionuclide venography relies upon conventional scanning agents such as labelled macroaggregated albumin, per-technitrate, or Technetium-albumin or Indium-transferrin. Criteria recently proposed for clot detection include diminished radioactivity at the site of the thrombus, the presence of collateral channels and pooling of radioactivity below the site of the thrombus. Comparison with contrast venography is quite good. False positive results may be seen in chronic venous disease. Small clots may escape detection.

Laboratory Approaches

Von Kaula and von Kaula have described a panel of laboratory tests useful in the laboratory detection of hyper-coagulable states. The panel includes factor VIII and fibrinogen levels. Both have been described as elevated in a number of clinical states associated with thrombus formation. Platelet counts and platelet aggregation studies are also performed. Others have added platelet coagulation studies. The kinetics of thrombin formation are evaluated by the
thrombin generation test. The activity of antithrombin is examined by a functional assay of antithrombin III. The single most useful test, however, is the thrombin generation test.35 The thrombin generation test quantitates thrombin generation and is independent of the patient's own fibrinogen. It permits detection of prethrombotic states in the presence of low fibrinogen levels. Plasma is incubated for varying periods and the time to clot formation assayed. The thrombin generation test, however, requires intensive training in its performance. This generally places the test beyond the capability of smaller nonspecialized laboratories.

The thrombin generation test can be replaced by the modified ethanol gelation test of von Kaulla.35 If evaluated in tandem with a serum antithrombin III level, a good approximation of the dynamics of thrombin formation and inactivation is obtained. The entire panel is not employed. Small amounts of thrombin in plasma produce a soluble form of fibrin12 (bearing an N-terminal glycine as fibrinogen20) which aggregates in the presence of ethanol to form a translucent gel. Soluble fibrin polymer has been described, without the addition of thrombin, in patients suffering venous thrombosis.10,11 Heparin does not interfere with the reaction.14 Fibrinolytic fibrin degradation products influence the reaction.17,21 As modified by von Kaulla, the trend of the gel test follows the thrombin generation test. A positive gel test indicates soluble fibrin is being generated and, by extension, thrombin. If in the presence of anticoagulant therapy the gel test is positive, the need for further therapy is indicated.

Antithrombin III activity reflects the presence or loss of a protective mechanism against thrombin traces in the blood. If antithrombin III levels are low, the presumption is that once clotting is initiated, it proceeds rapidly. Low levels of antithrombin III do not imply thromboembolism will follow. Thromboembolic complications have not been reported in affected persons below the age of ten years. The existence of people with low levels of antithrombin III, many of whom develop deep vein thrombosis and thromboembolism, supports the biological significance of antithrombin III.22,24 Acquired deficiency of antithrombin III with attendant risk of thromboembolism has been described in women on oral contraceptives,41 in persons with cirrhosis2 and in people of advancing age.2 Elevated levels may be induced with coumadin.

Antithrombin III levels may be evaluated by radial immunodiffusion or through a functional assay.3 Radial immunodiffusion requires 48 hours to perform and is of little use in acute clinical situations. The functional assay employed by us is adaptable to the fibrometer. Serum is examined at two, four and six minutes of incubation. A semi-log plot of clotting times to incubation times is linear. The six minute value is used to follow patients.

As von Kaulla demonstrated,35 in our experience and also in the experience of others, adequate anticoagulation is associated with a reversion of the gel test to negative and with a rising of antithrombin III levels.6 Walsh et al have demonstrated the strong predictive value of measurements of platelet coagulant activity.37 The tests, however, require intensive training in their performance. This generally places them beyond the capability of smaller nonspecialized laboratories.

Therapeutic Approaches

Low dose subcutaneous heparin prophylaxis of deep venous thrombosis has been extensively studied in Europe. The basic and most widely quoted study is an International Multicenter Trial.19 Patients evaluated had undergone major thoracic or abdominal surgery. Several major points have arisen from the study and have been reinforced in several American publications.11,39 The effectiveness of 5,000 units of heparin given twice daily in
preventing both deep venous thrombosis and pulmonary thromboembolism is established. Hemorrhagic complications are minimal, particularly in well screened patients. \(^1\) Heparin therapy is contraindicated in bleeding disorders. Low dose heparin therapy is not effective in operations involving much tissue destruction or release of thromboplastic substances. It is contraindicated in cranial surgery.

The role of low dose heparin therapy in preventing thrombosis of the deep veins of the leg in patients with myocardial infarction has been established.\(^1\) Its use in preventing mural thrombosis is not known. The role of low dose heparin therapy in other clinical states in which thromboembolic events are frequently observed has not been evaluated critically. Patients with a previous history of thrombosis or embolism, oral contraceptive use or with a history of congestive heart failure or respiratory failure must be examined.

The effective mechanism of action of heparin is thought to be the potentiation of antithrombin III activity.

**Summary**

New means of diagnosis and therapy of deep venous thrombosis and the prevention of embolism are now available. Laboratory tests to evaluate thrombin kinetics, antithrombin activity and platelet coagulation activity are available to identify prethrombotic states. Patient evaluation at admission and serially during hospitalization may identify those at risk for thrombus formation. Radionuclide techniques to identify formed clots are being explored. Laboratory tests to evaluate the adequacy of anticoagulation therapy are within the reach of non-specialized laboratories.

**References**

19. **INTERNATIONAL MULTICENTER TRIAL:** Pre-


