REVIEW ARTICLE

Blood Tests for the Diagnosis of Venous and Arterial Thrombosis

By J. Hirsh

For years, both the clinician and the investigator have been seeking blood tests that can be used to predict thrombosis in high risk patients or to confirm or exclude the diagnosis of thrombosis when this is suspected clinically. Attempts have also been made to use blood tests to investigate the role of thromboembolism in the complications of ischemic heart disease, cerebrovascular disease, renal failure, hyperlipoproteinemia, and in the vascular complication of diabetes. The interpretation of most of the published studies is difficult because many of the tests used have lacked sensitivity and/or specificity and because the experimental design of the studies has been inadequate. At best, these studies have provided a stimulus to investigators to perform more rigorously designed studies seeking an association, either causal or otherwise, between blood tests and thrombosis.

Over the last few years, sensitive and specific biochemical and immunochemical tests have been developed that are able to detect small concentrations of products of intravascular thrombin generation, of intravascular fibrin formation and of platelet-specific proteins that are released into the plasma as a consequence of activation of the platelet release reaction. Direct tests have also been developed for detecting circulating activated clotting factors and their inhibitors but these have not been applied clinically. Investigators have also become much more aware of the need to use objective and clinically relevant endpoints to identify thrombotic events and to ensure that the design and evaluation of these studies are appropriate.

The execution of these studies is difficult. Care must be taken to ensure that the test under evaluation is properly standardized, that the effect of technical factors such as blood drawing, speed and temperature of centrifugation, and time of storage are standardized and that the endpoints used for the diagnosis of the thromboembolic event are objective and both sensitive and specific for the thromboembolic event. Bias in patient selection should be avoided and the control groups should be both appropriate and realistic.

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Sensitive and specific tests are available for the diagnosis of venous thrombosis, pulmonary embolism, and peripheral arterial thrombosis, and considerable progress has been made in recent years in quantifying the severity of atherosclerosis in arteries accessible to angiography.

The control group used for comparison is important. It is potentially misleading to compare the results from the patients under study with those obtained in normal controls. Rather, all patients with the suspected clinical disorder (e.g., venous thrombosis, pulmonary embolism, arterial thromboembolism) should be investigated and the results of blood tests under study analyzed and compared in patients with and without objective evidence of the thromboembolic event. It is also valuable to screen a large number of hospital controls to determine the effect of a variety of comorbid conditions on the specificity of the test.

Potential relationships between blood tests and thrombosis could be causal or incidental. In practice, it may be difficult to differentiate between these two and, more particularly, to determine whether an abnormal blood test precedes or occurs as a consequence of the thromboembolic event. In either case, the demonstration of a relationship is potentially of practical importance since it may be used as a marker or predictor of a thromboembolic event (either alone or in combination with other blood tests or clinical risk factors) and, if it is causal, it could provide valuable information about the pathogenesis of a thromboembolic event that in turn could be applied to prophylaxis and treatment.

A number of criteria should be fulfilled to establish an association between blood tests and thrombosis. These include: (1) Adequate study design. A statistically significant relationship should be demonstrated between the test(s) used and thrombosis in a prospective study on a representative sample of the population using objective, specific and clinically relevant endpoints for the diagnosis of thromboembolism. (2) Consistency. The likelihood that the association is real is increased if consistent results are found in a number of studies using similar or preferably identical tests. (3) Biologic gradient. The probability that the association is real is increased if a correlation exists between the degree of abnormality of the test result and the risk of thrombosis. (4) Biologic plausibility. The likelihood that the association is real is increased if there is a plausible explanation for the mechanism of an association between the abnormal blood test and thrombosis.

Proof of causality is much more difficult to obtain and probably impossible to prove unequivocally in man. A causal relationship is likely if a therapeutic maneuver that normalizes the result of a blood test also reduces the frequency of thrombosis. This is particularly so if a biologic gradient can be shown to exist between the effect of a therapeutic maneuver on both the blood test and on reduction of thrombosis. In addition, the temporal relationship between blood testing and identifying the thrombosis must be appropriate. Causality is supported but not proved by demonstrating in animal experiments that the induction of the specific blood test abnormality is followed by an increased frequency of thrombosis.

CLINICAL EVIDENCE OF AN ASSOCIATION BETWEEN BLOOD TESTS AND THROMBOSIS

Blood tests which have been evaluated fall into two categories; transient abnormalities and longstanding abnormalities.

(A) Transient abnormalities occur (1) as an acute phase reactant response to trauma or inflammation or (2) as a specific reflection of activation of blood coagulation or the platelet release reaction. These tests have been evaluated as possible predictors of venous or arterial thrombosis or as markers for the presence of venous or arterial thromboembolism. (B) Longstanding abnormalities that may be inherited or occur in association with a chronic disease or disorder. These abnormalities have been reported to be associated with recurrent or idiopathic thrombosis.

PATHOPHYSIOLOGIC BASIS FOR TRANSIENT ABNORMALITIES

Venous Thrombosis

Tissue injury, whether produced by surgical or nonsurgical trauma, by infection, inflammation or infarction, is associated with a variety of local and systemic manifestations, some of which are reflected in blood changes that can be readily detected by testing a peripheral venous sample. Tissue injury also predisposes to thrombosis both locally and at remote sites. In general, there is a correlation between the severity of tissue injury and both the magnitude of blood changes and the risk of thrombosis. It is inevitable, therefore, that a variety of changes will occur in patients with tissue injury and that some of these blood abnormalities will be associated with thrombosis. It is possible also that some blood changes may be of predictive value and that some will reflect the presence of thrombosis.

The systemic response to injury includes changes that represent both an acute phase reaction and a response to activation of blood coagulation. The acute phase reaction response includes an elevation of a number of plasma proteins including fibrinogen, factor VIII, alpha 1 antitrypsin (an antiplasmin which accounts for approximately 10% of the total antiplasmin activity), other alpha globulins, a reduc-
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The increase in factor VIII results in a shortening of coagulation tests such as the activated partial thromboplastin time\(^{12,15,19,23,24}\) and the combination of this change plus an increase in platelet count and plasma fibrinogen level has often been referred to as a hypercoagulable state. This term implies that the observed changes are prethrombotic although there is no evidence that these changes per se actually predispose to thrombosis.\(^{15}\) The increase in antiplasmin activity and decrease in plasminogen activator activity results in a decrease in blood or plasma fibrinolytic activity, a blood change that could be causally related to the development of postoperative or post-traumatic thrombosis.\(^{19,20-22}\)

Tissue injury is also associated with systemic activation of blood coagulation.\(^{12,15,18}\) This may be caused either by tissue thromboplastin that is released from damaged tissue or from interaction of plasma coagulation factors and platelets with the injured vessel wall.\(^{12}\) If activation of blood coagulation is sufficiently marked, thrombin is generated and this can produce a number of changes in the peripheral blood that can be detected by laboratory tests.\(^{25}\)

Thrombin cleaves fibrinopeptides A and B from fibrinogen, converting fibrinogen into fibrin monomer\(^{25,27}\); through a feedback mechanism it changes factors V and VIII to a functional form\(^{22}\); it stimulates prostaglandin synthesis by platelets; and it induces the platelet release reaction independently of prostaglandin synthesis.\(^{33,36}\)

The effects of a number of these interactions can be detected by sensitive blood assays. Fibrinopeptide A can be measured by radioimmunoassay.\(^{25-27}\) Fibrin monomer copolymerizes with other fibrin monomers or with fibrinogen or fibrin degradation products\(^{37}\) and can be detected either by paracoagulation tests\(^{38,42}\) by affinity chromatography,\(^{43}\) or by gel filtration.\(^{44}\) The activation of platelet prostaglandin synthesis can be detected by measuring thromboxane B\(_2\) in the circulation\(^{44}\) and the products of the platelet release reaction, \(\beta\) thromboglobulin and platelet factor 4.\(^{36,45-49}\)

Thrombin and other activated coagulation factors form complexes with antithrombin III that can be detected by immunoassay.\(^{2}\) If thrombin is generated in sufficiently large quantity, it produces an increase in antithrombin III turnover\(^{50}\) and in fibrinogen turnover\(^{51}\) and, if generated in very large quantities, it produces a reduction of fibrinogen and other coagulation proteins.\(^{52,53}\)

If a critical concentration of fibrin monomer is reached, large soluble polymers are formed that are precipitated intravascularly as fibrin.\(^{28,34}\) This may occur in the microcirculation where it is usually asymptomatic,\(^{12}\) in venous valve pockets or venous sinuses where it is also usually asymptomatic\(^{55}\) but, under appropriate conditions, these venous thrombi grow larger and become occlusive.\(^{56}\) Intravascular fibrin is usually digested by the fibrinolytic enzyme system or by leukocytes to form soluble fibrin degradation products that can be detected in the serum.\(^{15}\)

One of these plasma digests, fragment E, can be assayed in nanogram concentrations by specific radioimmunoassay. Thrombin is adsorbed onto fibrin in the thrombus\(^{37}\) where it may interact with platelets, plasma coagulation factors or with fibrinogen to produce biochemical changes that can be detected by specific blood assays. The thrombus may also produce tissue damage and inflammation and thereby contribute to the original nonspecific acute phase response.

Vessel damage also produces activation of blood coagulation and platelet adhesion and aggregation at the site of injury\(^{58}\) and this can also lead to local large vessel thrombosis, for example, following hip surgery.\(^{59}\)

Tests that reflect intravascular thrombin generation or intravascular fibrin formation are likely to be sensitive to venous thromboembolism but are unlikely to be able to differentiate between large vessel thrombosis or pulmonary embolism and episodes of microvascular thrombosis that frequently complicate the course of sick, hospitalized patients and, therefore, they are unlikely to be specific for venous thromboembolism. These tests may also not be able to differentiate between intravascular and extravascular fibrin formation\(^{60}\) and so may not be able to distinguish between venous thrombosis and hematomas or inflammatory exudates containing fibrin. It is possible in future that improved specificity may result from combining tests reflecting fibrin formation with other tests and by monitoring the changes that occur in them following heparin therapy.

Other laboratory tests that could reflect activation of blood coagulation in vivo include the measurement of circulating complexes of activated clotting factors and inhibitors,\(^{2}\) the turnover of fibrinogen\(^{51,65}\) of other coagulation proteins or of antithrombin III or levels of antithrombin III and certain of the coagulation factors.\(^{62}\) The detection of circulating complexes\(^{57}\) is a promising approach but further refinements of the method are required before it can be evaluated clinically. The turnover of fibrinogen,\(^{21,61}\) other coagulation proteins\(^{62}\) and antithrombin III\(^{63}\) have been evaluated to a limited extent but appear to be considerably less sensitive than the fibrinopeptide A assay\(^{64}\) and the assay for fibrin/fibrinogen degradation product fragment E.\(^{65-67}\) Assay of antithrombin III has not proven to be a particularly sensitive marker for the presence of venous thromboembolism\(^{62,68,69}\) since a detectable...
decrease in antithrombin III levels occurs only when
the thrombotic state is relatively extensive. In addition,
low antithrombin III levels are not specific for
thrombosis since they occur in patients with liver
disease. Measurement of circulating fibrinogen,
factor VIII, factor V, and other coagulation factors
are both insensitive and nonspecific tests for venous
thromboembolism and are only significantly reduced
in very extensive venous thrombosis.

Arterial Thrombosis

Arterial thrombi usually complicate atherosclerosis
or occur when platelets come in contact with exposed
subendothelium or with a prosthetic surface. The
platelets adhere, undergo the release reaction, and
aggregate. The platelet aggregates embolize and are
then replaced by fresh platelets. If the aggregates
are sufficiently large or the atherosclerotic stenosis
marked, an occlusive thrombus is produced, however,
mORE frequently the aggregates embolize to obstruct
the arterial system distally. Evidence that platelets
have undergone the release reaction can be obtained
by measuring plasma levels of receptor and platelet factor
4 and evidence that the platelet prostaglandin pathway
has been activated can be obtained by measuring
thromboxane B2 in the circulation. The continuous
process of adhesion, aggregation and embolization
results in an increased platelet turnover that can be
detected by measuring the survival of isotopically
labeled platelets. It is likely, however, that many
thrombogenic stimuli can interact with platelets
and both induce the release reaction and result in a
reduction in platelet survival so that these changes
may not be specific for arterial thromboembolism.
Moreover, it has been difficult to establish the sensitiv-
ity of these tests for arterial thromboembolism
because of a lack of reliable and objective endpoints
for atherosclerosis and arterial thromboembolism.

Blood changes also occur as a consequence of tissue
inflammation that may complicate arterial thrombo-
embolism. If tissue injury is marked, a typical acute
phase reactive response occurs and the process of
blood coagulation may be activated both locally and
systemically, leading to changes similar to those
described in the preceding section. If a large occlusive
thrombus forms, some of the changes described for
venous thrombosis might occur.

Specific Blood Tests Which
Have Been Evaluated

Transient Abnormalities

The following blood changes have been reported
following surgery and trauma and have been related to
the presence of postoperative venous thrombosis or
have been evaluated in patients with symptomatic
venous thromboembolism.

Transient Responses to Trauma or Inflammation

(1) Altered tests of blood coagulation. (2)
Reduced fibrinolytic activity. (3) Presence
of circulating tissue thromboplastin. (4)
Reduced levels of antithrombin III. (5)
Thrombocytosis. (6) Changes in tests of platelet function.

Tests That Reflect Activation of Blood Coagulation
or the Platelet Release Reaction

(1) Thrombin/antithrombin complexes. (2) Fibrinopeptide A assay. (3) Assay of fibrin
(5) Fibrinogen turnover. (6) Platelet factor 4
and β thromboglobulin assay.

Longstanding Abnormalities Associated With an
Increased Risk of Thrombosis

(1) Decreased antithrombin III activity. (2)
Dysfibrinogenemia. (3) Thrombocytosis with or
without spontaneous aggregation. (4) Polycy-
temia. (5) Low levels of functional plasminogen. (6)
Reduced fibrinolytic activity. (7) Platelet
survival and platelet turnover. (8) Other abnor-
malities of platelet function. (9) Increased
levels of coagulation factors.

Clinical Value of Blood Tests Evaluated

The development and application of specific tests
for thrombin generation, fibrin formation and its
dissolution and for the platelet release reaction have
provided valuable information about the pathogenesis
of intravascular thrombosis. At the present time,
however, they only have a very limited role in the
management of patients with thromboembolic dis-
ease.

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Tests for Prediction of
Postoperative Venous Thrombosis

Reduced fibrinolytic activity is the only abnormal-
ity that has been consistently reported to be associated
with postoperative venous thrombosis. Although the
measurement of the fibrinolytic activity may improve
the predictive power of clinical risk factors, this
improvement is marginal and the expense of these
tests does not justify their routine use in high risk
surgical patients. Many other blood abnormalities
have been reported in postoperative patients or in
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patients exposed to trauma but they have all been nonspecific and have lacked predictive power.

Tests for the Diagnosis of Clinically Suspected Venous Thrombosis

Two blood tests, the fibrinopeptide A assay and the assay for fibrin/fibrinogen fragment E, are highly sensitive to acute venous thromboembolism in symptomatic patients but are both nonspecific. At present, they have to be performed by radioimmunoassay, a procedure that is too complicated for routine clinical use. If, however, they are simplified either one of these two tests could be used to exclude the presence of venous thrombosis or pulmonary embolism in symptomatic patients.

Tests to Investigate Patients with Proven Venous Thrombosis

At present, a low antithrombin III level, less than 60% of normal, is the only longstanding or inherited abnormality that has been shown to be associated with a risk of venous thromboembolism. Abnormalities of the plasminogen molecule and of fibrinogen have been described in patients with thrombosis but these are rare and the reported association could be coincidental. There are reports that patients with idiopathic or recurrent venous thrombosis have impaired fibrinolytic activity but the significance of this association has never been demonstrated in rigorously designed studies.

Tests for Arterial Thromboembolism

There is an association between increased platelet turnover and arterial thromboembolism. However, considerable overlap exists between patients and controls and there is insufficient data available on hospital controls to make this a clinically useful test. Elevated levels of plasma βTG and PF₄ have also been reported in patients with thrombosis and arterial disease but the sensitivity and specificity of these findings is presently unknown.

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