There are many reports in the literature of blood test abnormalities occurring in patients with venous or arterial thrombosis. Most of these have not used acceptable criteria for establishing an association between thrombosis and blood tests and, therefore, their interpretation is questionable. Recently, sensitive and specific assays have been developed for the detection of products of intravascular thrombin formation, of plasmin digests of fibrin or fibrinogen and of platelet specific proteins that are released into the plasma when platelets react with stimuli. Blood abnormalities have been sought that can either predict or detect venous thrombosis. Many of the predictive tests evaluated are nonspecific acute phase reactant responses to inflammation; of these, only reduced fibrinolytic activity has been consistently reported to be associated with postoperative venous thrombosis. Hereditary antithrombin III deficiency has been consistently shown to predispose patients to venous thrombosis. Abnormalities of the plasminogen and fibrinogen molecule have also been described in patients with familial or recurrent venous thrombosis but these are rare and the association could be coincidental. 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 both are nonspecific. Elevated levels of beta thromboglobulin and platelet factor 4 have been reported in patients with arterial thromboembolism but the sensitivity and specificity of these findings is presently unknown.

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.

From the Departments of Pathology and Medicine, McMaster University Medical Centre, Hamilton, Ontario, Canada.

Address reprint requests to Dr. J. Hirsh, Department of Pathology, McMaster University, 1200 Main Street West, Hamilton, Ontario L8N 4J9, Canada.

© 1981 by Grune & Stratton, Inc.

For personal use only on October 22, 2017. From www.bloodjournal.org by guest.
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-

...
tion in plasminogen activator activity, an increase in white cell count and platelet count and fever.

The increase in factor VIII results in a shortening of coagulation tests such as the activated partial thromboplastin time (aPTT) 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. The increase in antithrombin 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.

Tissue injury is also associated with systemic activation of blood coagulation. 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. 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.

Thrombin cleaves fibrinopeptides A and B from fibrinogen, converting fibrinogen into fibrin monomer; through a feedback mechanism it changes factors V and VIII to a functional form; it stimulates prostaglandin synthesis by platelets; and it induces the platelet release reaction independently of prostaglandin synthesis.

The effects of a number of these interactions can be detected by sensitive blood assays. Fibrinopeptide A can be measured by radiimmunoassay. Fibrin monomer copolymerizes with other fibrin monomers or with fibrinogen or fibrin degradation products and can be detected either by paracoagulation tests or by affinity chromatography, or by gel filtration. The activation of platelet prostaglandin synthesis can be detected by measuring thromboxane B₂ in the circulation and the products of the platelet release reaction, β-thromboglobulin and platelet factor 4.

Thrombin and other activated coagulation factors form complexes with antithrombin III that can be detected by immunoassay. If thrombin is generated in sufficiently large quantity, it produces an increase in antithrombin III turnover and in fibrinogen turnover and, if generated in very large quantities, it produces a reduction of fibrinogen and other coagulation proteins.

If a critical concentration of fibrin monomer is reached, large soluble polymers are formed that are precipitated intravascularly as fibrin. This may occur in the microcirculation where it is usually asymptomatic, in venous valve pockets or venous sinuses where it is also usually asymptomatic but, under appropriate conditions, these venous thrombi grow larger and become occlusive. 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. One of these plasma digests, fragment E, can be assayed in nanogram concentrations by specific radiomimmunoassay. Thrombin is adsorbed onto fibrin in the thrombus 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 and this can also lead to local large vessel thrombosis, for example, following hip surgery.

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 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, the turnover of fibrinogen of other coagulation proteins or of antithrombin III or levels of antithrombin III and certain of the coagulation factors. The detection of circulating complexes is a promising approach but further refinements of the method are required before it can be evaluated clinically. The turnover of fibrinogen, other coagulation proteins and antithrombin II/III have been evaluated to a limited extent but appear to be considerably less sensitive than the fibrinopeptide A assay and the assay for fibrin/fibrinogen degradation product fragment E. Assay of antithrombin III has not proven to be a particularly sensitive marker for the presence of venous thromboembolism 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 βTG 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 nonthrombogenic 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 sensitivity 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 infarction that may complicate arterial thromboembolism. 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.
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. Polycythemia.
5. Low levels of functional plasminogen.
6. Reduced fibrinolytic activity.
7. Platelet survival and platelet turnover.
8. Other abnormalities 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 disease.

**BLOOD TESTS FOR THE DIAGNOSIS OF VENOUS AND ARTERIAL THROMBOSIS**

Tests for Prediction of Postoperative Venous Thrombosis

Reduced fibrinolytic activity is the only abnormality 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
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 arteral 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.

REFERENCES

26. Nossel HL, Yudelman I, Canfield RE, Butler VP Jr,
44. Fletcher AP, Alkjaersig NK, O'Brien JR, Tulevski V: Fibrinogen catabolism in the surgically treated patient and in those with postoperative venous thrombosis. Correlation of plasma fibrinogen chromatographic findings with 125I-labelled fibrinogen scan findings. J Lab Clin Med 89:1349, 1977
BLOOD TESTS AND THROMBOSIS

72. Handin RI, Tomkins B, Collins J: Plasma content of platelet factor 4 in patients with prosthetic or porcine cardiac valves. (submitted for publication)
88. Gurewich V, Hume M, Patrick M: The laboratory diagnosis of venous thromboembolic disease by measurement of fibrinogen/


149. Gaston LW: Studies on a family with an elevated level of factor V (proaccelerin) and a tendency to thrombosis. J Pediatr 36:367, 1966


Blood tests for the diagnosis of venous and arterial thrombosis

J Hirsh