Molecular Markers of Hemostatic Disorders: Implications in the Diagnosis and Therapeutic Management of Thrombotic and Bleeding Disorders

Jawed Fareed,¹ Rodger L. Bick,² Grace Squillaci,¹ Jeanine M. Walenga,¹ and Edward W. Bermes, Jr.¹

With current technological advances, it is now possible to measure in <50 µL of plasma picomolar amounts of circulating products of platelet activation, products of protease activation related to coagulation and fibrinolytic pathways, and prostaglandin metabolites formed during a physiologic or pathologic process. Most of these markers, which circulate in blood in nanogram or picogram amounts per milliliter during or after pathologic activation, provide pertinent information on the status of a patient in terms of specificity and early detection, and will be of crucial value in the diagnosis of hemostatic defects and the management of newer antithrombotic drugs that cannot be monitored by currently available assays. Currently, ¹²⁵I- and ³H-based simple radioimmunoassays are available for platelet factor 4, β-thromboglobulin, fibrinopeptide A, Bβ 15-42 related peptides, thromboxane B₂, and the prostaglandins 6-keto-PGF₁α and PGE₂. Nonisotopic methods such as enzyme-linked immunosorbent assays and fluorimnunoassays are being developed. Serotonin and ADP, products of platelet activation, are measurable by liquid-chromatographic, immunoenzymatic, and spectrophotofluorometric methods. Analytical methods for fibrin split products (fragments D and E) and serine protease inhibitor complexes such as thrombin–antithrombin-III, factor Xa–antithrombin-III, and kallikrein–Ca₂⁺-esterase are also being developed. We have evaluated all of these methods and found them to be very sensitive to those components of endogenous activation of the hemostatic system listed above.

Additional Keyphrases: coagulation abnormalities · coagulation assays · β-thromboglobulin · serine proteases · thromboxanes · prostacyclin · hemostasis · thrombosis · vascular endothelium · antithrombin-III · a₂-antiplasmin · fibrin split products · fibrinopeptide A · Bβ 15-42 related peptides · serotonin · hemophilia · prostaglandins · normal values

The maintenance of blood in a state of fluidity is often termed "hemostasis," whereas a transition into nonfluidity is designated as "thrombosis." A complex network of coagulation and fibrinolytic pathways, platelets, and endothelium plays a crucial role in the overall regulation of hemostasis. Many pathologic states, however, may alter the balance of these components and lead to thrombotic or hemorrhagic conditions. The individual components of this system are regulated by various biochemical pathways and their activation/inhibition results in the release or suppression of the release of certain enzymes, products of enzyme reactions, and other well-defined chemicals, which can be measured to assess specific aspects of the overall system (I–10).

Because most of our testing methods were developed to investigate only hypocoagulable states, thromboembolic conditions were recognized clinically only at the time of pathologic manifestation, reliable laboratory methods for early diagnosis being almost nonexistent. Our current knowledge on the generation of various molecular markers during hemostatic activation, however, has led to the development of new test panels to assess various bleeding and thrombotic problems. The clinical application of this concept of molecular markers and its diagnostic value in the study of various disorders is relatively new (J1–13); however, many groups of investigators have quantified various molecular markers to evaluate hemostatic disorders and to monitor drug-induced alterations in the hemostatic system.

Table 1 lists various components of the hemostatic system, their physiologic role, pathologic manifestation, drug modulation, and type of molecular marker released upon activation or pathophysiologic transformation of these pathways. Numerous chemically defined products are formed at the injured site in various amounts and circulate in blood in their stabilized form. Because the biologic half-life of each of these products differs considerably (from seconds to minutes), blood drawn at the time of activation or during a pathologic episode provides the most reliable sample for analysis. It is, however, imperative that the blood specimen be drawn properly in special anticoagulant, because the stability of these markers is very sensitive to blood drawing and handling procedures. Unlike the conventional methods, these tests are performed on native nonactivated plasma or whole blood and provide a true picture of the physiologic status of a given patient. Subsequently, laboratorians are now able to assess hyper- as well as hypocoagulable states.

In this manuscript we will briefly review various pathways of the hemostatic system, with particular reference to the molecular markers released or generated during pathologic or therapeutic modulation of this system. We will also discuss some of the preliminary analytical results we have obtained in our laboratory.

Hemostasis and Thrombosis: An Overview

Blood Vessels and Platelets

Figure 1 shows a cross section of a blood vessel. The vascular bed has the capacity to react to platelets as well as humoral substances released from the cellular elements of blood (erythrocytes, leukocytes) by constricting or dilating the vessel, thus modulating blood flow. Platelets and leukocytes respond rapidly when exposed to damaged or pathologically altered (fibrotic or atherosclerotic) endothelium or subendothelial tissues by adhering to these surfaces and aggregating to form a plug at the site of injury. Many biochemical processes are activated at this time, and the types of biochemical transformations are determined by the nature of pathophysiologic changes. The endothelial cell lining is metabolically very active in synthesizing coagula-

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Table 1. Various Components of the Hemostatic System and Their Role in the Pathophysiology and Drug-Induced Modulation of This System

<table>
<thead>
<tr>
<th>Component</th>
<th>Physiology</th>
<th>Pathology</th>
<th>Pharmacology</th>
<th>Molecular markers of activation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood coagulation</td>
<td>Hemostasis</td>
<td>Thrombosis</td>
<td>Anticoagulants</td>
<td>FPA, enzyme-inhibitor complexes</td>
</tr>
<tr>
<td>Fibrinolysis</td>
<td>Regulation of fibrinolysis</td>
<td>DIC, primary and secondary fibrinolysis</td>
<td>Antifibrinolytics</td>
<td>FDP, Bβ 15-42 related peptide, enzyme-inhibitor complexes</td>
</tr>
<tr>
<td>Kallikrein–kinin</td>
<td>Regulation of hemodynamics</td>
<td>Shock, Inflammation</td>
<td>Antishock drugs</td>
<td>Bradykinin, enzyme-inhibitor complexes</td>
</tr>
<tr>
<td>Complement pathways (C1-esterase)</td>
<td>Mediation of immune complex disease</td>
<td>Anticomplement action</td>
<td>Antiphlogistics</td>
<td></td>
</tr>
<tr>
<td>Platelets</td>
<td>Hemostasis</td>
<td>Thrombosis</td>
<td>Antiplatelet drugs</td>
<td>Prostaglandin derivatives, granular release material</td>
</tr>
<tr>
<td>Blood vessels</td>
<td>Hemostasis</td>
<td>Bleeding</td>
<td>Antiplatelet drugs</td>
<td>Prostaglandin products of platelet and protease system</td>
</tr>
<tr>
<td></td>
<td>Vascularity</td>
<td>Clotting</td>
<td>Anticoagulant drugs</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Vascular spasm</td>
<td>Vasoactive drugs</td>
<td></td>
</tr>
</tbody>
</table>

DIC, disseminated intravascular coagulation; FPA, fibrinopeptide A; FDP, fibrin degradation products.

Fig. 1. Schematic representation of the cross section of a blood vessel
The endothelial cell lining plays a crucial role in the overall generation of such molecular markers of hemostatic activation as thromboxanes and prostacyclins.

The net result of the type of molecular markers generated depends on many endogenous and exogenous factors and fibrinolytic proteins and modulating the prosta-
glandin pathways (4–7).

Release of platelet-specific granular products, serotonin, platelet factor 4, β-thromboglobulin, clot-promoting agents such as platelet factor 3, factor VIII, and calcium trigger a complex process resulting in constriction of blood vessels and activation of coagulation and (or) fibrinolytic systems. Simultaneously, prostaglandin pathways are activated, leading to the formation of thromboxanes and prostacyclins. The net result is the generation of various functionally active and inactive substances, which are measurable in arterial or venous blood. A diagrammatic illustration of this process is shown in Figure 2. Within 3 to 10 s after adhesion to the vessel wall, platelets undergo release, followed by a slow phase of clot formation involving the plasma procoagu-
lants. Numerous pathways are involved in the complex interactions that occur during the activation of procoagu-
lants; however, this process can be best described only by analyzing the process of clot formation and reconstructing the pathways leading up to it. Under normal circumstances clot stabilization occurs within 5 min and the vessel subsequently recovers; in various pathologic states, however, the initial thrombus (clot) becomes larger and often is dislodged from its site of production into smaller emboli that are capable of producing various satellite clots in the heart, lung, brain, and other areas.

Platelets contribute to thrombosis directly and respond to various pathologic states. Concentrations of platelet granular products in plasma have been used extensively to predict pathologic events related to platelet activation (14–23). Increased production of platelets in myeloproliferative disorders is associated with thrombosis and can be reliably monitored by measuring platelet factor 4 and β-thromboglobulin (1). Concentrations of platelet factor 4 and β-throm-
boilubulin have also been used as diagnostic tools in thrombotic episodes in diabetes and chronic renal failure (24, 25). The therapeutic efficacy of antiplatelet agents can be measured by quantifying platelet release products in peripheral blood (26).

Coagulation and Fibrinolysis

Fibrin clots form during a thrombotic episode in the presence of small amounts of thrombin, by either the extrinsic or intrinsic pathways (Figure 3). In some instances, specific tissue proteases have been suspected of activating both the intrinsic and extrinsic pathways. For example, trypsin and kallikrein released from the pancreas could induce formation of procoagulants to initiate thrombin formation. Similarly, proteases released during inflammatory disorders can also accelerate clotting mechanisms. Once thrombin is generated, it cleaves the A and B peptides from the α and γ chains of fibrinogen, respectively (27). The initial cleavage generates monomers, which are eventually polymerized in the presence of a transamidase, factor XIII. Measurements of fibrinopeptide A have been reliable in the diagnosis and treatment of venous thrombosis and related disorders (28-37). Fibrinopeptide B, which forms at a much slower rate during a thrombotic process, may not truly reflect the action of thrombin or fibrinogen. Once formed, the clot (fibrin) is eventually digested by various proteolytic enzymes, particularly plasmin (EC 3.4.21.7). Both fibrin and fibrinogen are subjected to sequential proteolytic degradation. The initial attack on fibrinogen is at the carboxy terminal portion of the α chain, followed by cleavage of peptide bonds in the α and γ chains. The large molecular fragments produced first (fragment X) are subsequently degraded (fragments Y, D, and E) as shown in Figure 4.

Fibrinogen plays a key role in both the cellular and humoral mechanisms involved in hemostasis (38-50). Numerous chemically and immunologically identifiable products are formed during the activation of coagulation and fibrinolysis. In the evaluation of hemostatic disorders, a study of fibrinogen and its derivatives provides a molecular profile of the protease activities of various pathologic states. The fibrinolytic mechanism may be activated by both the

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**Fig. 3. Activation of the coagulation system via the extrinsic pathway (left) and intrinsic pathway (right)**

Serine proteases such as kallikrein and factors XIIa and XIa as well as tissue thromboplastin released upon trauma can activate the extrinsic pathway. The primary molecular marker generated in this process is fibrinopeptide A; however, prekallikrein also decreases. In the intrinsic pathway, factor XII is activated in certain pathologic states such as gout, sepsis, and vascular disease, and subsequently triggers the activation of the coagulation process via factor IX; fibrinopeptide A is the primary molecular marker of this process.

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**Fig. 4. Plasmin degradation of fibrinogen into various low-Mr fractions, which can be quantified immunocohemically**

Peptide-cleavage products of the Bβ chain, referred to as Bβ-related peptides, are a reliable index of activation of fibrinolysis.
intrinsic and extrinsic pathways, as shown in Figure 5. The type of degradation products depends on the nature of the activation. By measuring enzymatically modified fibrinogen or its proteolytic derivatives, we can reliably quantify the action of the specific enzymes in both in-vitro and in-vivo fibrinolytic conditions (51–54). With such methods as affinity chromatography, electrophoresis, immunoassay, and monoclonal antibody techniques, we can acquire data on the structure and function of fibrinogen digestion products in various pathophysiologic states (55–64).

The biologic role of the fibrinolytic process is to remove fibrin deposits from blood vessels and tissues. Although both plasmin and nonplasmin-mediated fibrinolysis occur, many additional factors (activators, inhibitors) also regulate this process. When fragment X is generated, a peptide fragment is also released from the Bβ chain; it is known as Bβ 1-42 peptide (65–67). Because this peptide is released during the earliest stages of plasmin proteolysis, its measurement may be of value in the clinical investigation of early fibrino(ogen)lytic disorders.

Figure 6 shows the formation of various peptides from fibrinogen/fibrin upon the action of thrombin (EC 3.4.21.5) and plasmin and illustrates the structural sequence of Bβ 15-42 peptide. Various methods for the measurement of Bβ 15-42 related peptides have been developed (66–69). Trypsin (EC 3.4.21.4) is capable of releasing Bβ 1-118 peptide, whereas plasmin releases Bβ 15-42, Bβ 1-42, and Bβ 1-118 chains from fibrinogen or fibrin. The immunoidentity of each of these peptides to the antiserum raised against the Bβ 15-42 peptide makes it possible to quantitatively collect all peptide fragments of the Bβ chain having the 15-42 amino acid sequence.

The vascular surface and contact activation is capable of initiating the fibrinolytic and kallikrein systems. The role of factor XII in the regulation of various pathways is as follows:

1. Initiation of coagulation
   via activation of factor IX
   via activation of X
2. Activation of fibrinolysis
   via plasminogen activation
3. Activation of complement system
   via plasminogen activation
4. Activation of kallikrein system

Binding of factor XII and Fitzgerald factor (a kiningen of high relative molecular mass, \( M_r \)) to negatively charged surfaces changes the conformation of these proteins; in the presence of trace amounts of kallikrein, factor XII is transformed into factor XIIa (EC 3.4.21.38), which initiates various clotting processes. Factor XIIa in turn is capable of activating prekallikrein (Fletcher factor) into kallikrein, which is responsible for the generation of kinins. Activation of the kallikrein–kinin system, e.g., by septicemic shock, produces circulating concentrations of bradykinin (a nonapeptide), kallidin (a decapeptide), and Met-Lys-bradykinin, as well as kallikrein–inhibitor complexes. Glundal kallikrein (EC 3.4.21.35) produces kallidin and Met-Lys-bradykinin, whereas plasma kallikrein (EC 3.4.21.43) produces bradykinin. Although significant amounts of kinins are released upon kallikrein activation, they have a very short half-life and are difficult to measure; immunologic quantifi-
cation of peptides and high-M₉ kininogens provides a reliable measure of kinin release (70–74). Activation of the kallikrein–kinin system will also produce a consumption of Fletcher and Fitzgerald factors, which can be measured by both functional and immunologic methods.

Figure 7 shows the interaction of complement and hemostatic pathways. The generation of plasmin clearly can activate the complement system via the direct activation of C₁-esterase (EC 3.1.1.6). During the activation of the complement system, there is a marked consumption of C₁-esterase inhibitor. Certain other products generated, e.g., C₃a (anaphylatoxin), can also be quantified and thus considered as a marker of hemostatic activation.

During pathologic or therapeutic (defense) hemostatic activation, numerous proteases are generated; however, the relative amount of each of these proteases is primarily determined by the pathophysiologic state of a given patient. Figure 8 shows the formation of a complex between antithrombin-III and factor Xa, and the modulating effects of heparin on the formation of this complex. The role of serine protease inhibitors in regulating the function of coagulation enzymes such as thrombin, Xa, and plasmin is well understood, and many other serine proteases are now known (75–77) to form complexes with physiologic inhibitors (Table 2). With the introduction of monoclonal antibodies, we can now quantify these complexes in circulating blood, their presence being an indication that hemostatic activation has taken place. Simultaneous determination of these complexes, as a profile, may provide a useful procedure for assessing the relative activation of coagulation, fibrinolysis, kallikrein, and complement systems.

In hemophiliac patients with factor VIII procoagulant inhibitors, various prothrombin complex concentrates have been used therapeutically. These concentrates comprise a complex mixture of activated serine proteases and are capable of activating the coagulation, fibrinolytic, platelet,
and vascular pathways, as shown in Figure 9. Fibrinopeptide A and platelet-release proteins can be used to evaluate the therapeutic efficacy of these concentrates, and concentrations of Bβ 15-42 related peptides are useful in predicting the effects of the concentrates on fibrinolytic activation, which is undesirable in this situation. During this form of therapy the thrombotic potential of these products is also assessable by quantification of fibrinopeptide A. Some prothrombin complex concentrates can cause serious problems such as defibrination and disseminated intravascular coagulation (DIC). It is therefore imperative to evaluate the effects of these complexes through the molecular markers generated after the infusion. In the near future, many newer therapeutic products, containing active proteases, will become available. Because these therapeutic agents exert no effect on the routinely performed methods, quantification of molecular markers may prove very useful in monitoring the effects of these concentrates.

Prostaglandins and Their Derivatives

The vascular system, in particular the arterial system, is susceptible to the thrombogenic effects of platelets. The faster flow rate in the arterial sites renders platelets and coagulation factors much more capable of activation; moreover, the resistance produced by increased blood viscosity affects flow by creating local stasis, thus promoting activation of the coagulation system. This activation also leads to changes in the endothelial surface, causing platelets to adhere to the surface of the blood vessel. Platelet substances released during this process (e.g., β-thromboglobulins, serotonin, and ADP) are capable of activating acylhydrolases, which regulate the generation of prostacyclin and thromboxane. Endothelial damage or degenerative arteriosclerotic manifestations provide sites for constant platelet interaction—adhesion and release of substances that contribute to thrombosis (78–87). Platelet vessel walls regulate the formation of thromboxanes and prostacyclins, which are strong modulators of platelet aggregation and vascular spasm (88–93). In conditions such as strokes or transient ischemic attacks, coronary artery spasms, followed by ischemia and infarction, are known to develop with platelet activation and release of thromboxanes. Platelet activation can be assessed to determine if there is (a) increased platelet sensitivity to aggregating agents, (b) increased platelet generation and release of thromboxane B2, (c) increased circulating thromboxane A2, as measured by its stable metabolite thromboxane B2, or (d) increased platelet-release products such as platelet factor 4 or β-thromboglobulin. The current information on the role of platelets and these molecular markers is somewhat limited in the diagnosis of thrombosis; however, numerous reports on the diagnostic usefulness of thromboxane B2 and related substances in endotoxic shock, hypertension, cerebrovascular accidents, hyperlipidemia, renal transplant rejection, antiplatelet therapy, and many other disorders have been published (94–102).

Figure 10 shows the cyclooxygenase pathway of arachidonic acid metabolism. The enzyme cyclooxygenase is the rate-limiting enzyme in these pathways. Malonaldehyde, a stable metabolite of labile endoperoxide prostaglandin A1, is produced during the formation of thromboxane A2 (103), and can be easily quantified by a thiobarbituric acid condensation method. Thromboxane A2, a potent aggregating and spasmogenic agent, is primarily synthesized in the platelets. Because it is produced via a platelet-stored enzyme, thromboxane A2 has been used to study platelet activation and the effectiveness of antiplatelet therapy (104). Prostacyclin, on the other hand, a potent anti-aggregating agent, is formed by the action of a vascular endothelial enzyme. Normal endothelial cell lining is capable of this transformation, but pathological endothelium is not. Therefore simultaneous measurement of the stable metabolites of prostacyclin and thromboxane provides a useful index of vascular function to differentiate between the patients at risk and not at risk for clot formation. These determinations are especially useful after stress testing.

Substantial direct and indirect evidence from clinical and epidemiological studies on the relationship between dietary fat and occlusive vascular disease indicate that atherosclerosis and thrombosis are chief determinants of these occlusive lesions. Conversely, prostacyclins have been implicated in the increased bleeding tendency that accompanies uremia (105). Therefore, a balance between the production of thromboxane and prostacyclin is crucial in the overall expression of the pathophysiology of atherosclerosis and arterial thrombosis. Cyclooxygenase in platelets is more sensitive to inactivation by aspirin than the cyclooxygenase in the arterial wall. Aspirin and other nonsteroidal anti-inflammatory agents in low doses are effective nontoxic antithrombotic agents for various pathological states.

Arachidonic acid can be transformed by an alternate route, the lipoxygenase (EC 1.13.11.12) pathway (Figure 11), which plays an important role in the mediation of inflammatory responses. In contrast to the cyclooxygenase system, the lipoxygenase pathway consists of heterogeneous enzymes at various sites. Generally, the lipoxygenase reactions are less complicated than those of cyclooxygenase. Numerous products formed throughout this pathway play a key role in the mediation of allergy and inflammation, and have been extensively reviewed by various workers (106–128). Lipoxygenase converts arachidonic acid into

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![Figure 9](image-url) - Proposed mechanisms by which the activated prothrombin complex concentrates produce their procoagulant effects. Currently, measurements of prothrombin time and partial thromboplastin time are not capable of detecting the effects of these concentrates; however, their therapeutic effects can be easily investigated by measuring fibrinopeptide A and B on 15-42 related peptides. PK, prothrombinase; PL, phospholipid.

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5-hydroperoxyeicosatetraenoic acid. This hydroperoxide is then converted into a 5(6)-hydroperoxyeicosatetraenoic acid (leukotriene A). Leukotriene A is subsequently converted into leukotrienes B, C, D, and E after various chemical modifications. Leukotriene D₄ has been established as identical to the slow-reacting substance of anaphylaxis, which plays a key role in the pathophysiology of asthma and other pulmonary disorders.

Another functionally potent derivative of arachidonic acid that plays a key role in the mediation of the inflammatory...
response and activation of platelets is platelet-activating factor (Figure 12). Although structurally similar to arachidonic acid, it is not derived from either the lipoxygenase or cyclooxygenase pathways. Furthermore, its release from platelets and pathophysiological roles are independent of ADP release. At concentrations between 10 and 100 pmol/L, platelet-activating factor induces shape change, aggregation, and release reaction in platelets (129–133). Its structure has now been shown to be O-alkyl-2-acetyl-SN-glyceryl-3-phosphorylcholine. Several structurally similar substances have also been reported to be secreted during the activation of immunologic systems.

Table 3 lists various metabolites of arachidonic acid and their sites of production. Leukotrienes, platelet-activating factor, and thromboxanes are released during bronchoconstriction, changes in blood pressure, cardiac dysfunction, increased capillary permeability, and intravascular con-

**Table 3. Production of Arachidonic Metabolites**

<table>
<thead>
<tr>
<th>Product</th>
<th>Site of production</th>
<th>Pathway</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thromboxanes (TXb2)</td>
<td>Platelets (TXb2)</td>
<td>Cyclooxygenase (TXb2 synthetase)</td>
</tr>
<tr>
<td>6-Keto-PGF1β</td>
<td>Platelets (TXb2)</td>
<td>Cyclooxygenase (TXb2 synthetase)</td>
</tr>
<tr>
<td>Prostaglandins (PGF1α, PGF2α)</td>
<td>Endothelium</td>
<td>Cyclooxygenase (prostacyclin synthetase)</td>
</tr>
<tr>
<td>Leukotrienes</td>
<td>Leukocytes</td>
<td>Lipooxygenase</td>
</tr>
<tr>
<td>Hydroperoxyicosatetraenoic acid (HPETE)</td>
<td>Leukocytes</td>
<td>Lipooxygenase</td>
</tr>
<tr>
<td>Hydroxyicosatetraenoic acid (HETE)</td>
<td>Leukocytes</td>
<td>Lipooxygenase</td>
</tr>
<tr>
<td>Platelet-activating factor</td>
<td>Platelets</td>
<td>Other</td>
</tr>
</tbody>
</table>

RIAs for TXb2, 6-keto-PGF1β, PGF2α, and PGF3α are now commercially available. An assay for cyclooxygenase is also available. Only research-based methods are available for the measurement of leukotrienes, HPETE, HETE, and platelet-activating factor.

Alm ast all the body serotonin, under normal conditions, is present in the dense granules of platelets. This biogenic amine, released by the dense granules when they are activated, plays an important role in the regulation of vascular tone, and can be measured by radioenzymatic and chromatographic methods. Serotonin is capable of producing strong contractions of smooth muscles in the vessel walls, activating platelets, and initiating the synthesis of prostaglandins via activation of the acylhydrodase.

Upon activation of the hemostatic system or its pathologic and therapeutic modulation, various well-defined molecular markers are generated that are absent in blood during normal physiologic states. Because these markers are generated in small amounts during the initial phases of hemostatic modulation, sensitive methods are needed for their quantification. Until recently, it was relatively difficult to quantify chemical substances in the nanogram or picogram range. With the introduction of isotopic and nonisotopic immunoassays and other analytic methods—radioimmunoassays, enzyme-linked immunosorbent assays, "high performance" liquid-chromatographic (HPLC), gas-chromatographic, and
mass-spectrophotometric methods—such measurements are now possible (135–150). Table 4 lists some of the commercially available kits for the measurement of molecular markers in biologic fluid. Many newer kits are under development and will become available for routine use in the near future. Most of the available methods are based on immunoassay principles.

Table 5 is a compilation of the reported normal concentrations in plasma of various molecular markers of hemostatic activation. Only trace amounts of these markers are detectable in anticoagulated plasma from normal, healthy persons; however, in various pathophysiologic states, and during procoagulant and thrombolytic therapy, significant increases occur. A systematic study, including the use of proper measures for the collection of blood, will establish the normal range for each of these markers. Unlike other coagulation methods, assays for these molecular markers cannot be done with citrated plasma or whole blood. The mode of blood collection also significantly affects the concentrations of the markers, which are very sensitive to blood-drawing techniques. Moreover, because blood drawn into a glass tube is prone to activation, plastic tubes must be used (33, 151). Most of the commercially available kits contain special instructions and anticoagulant mixtures for the proper collection of blood sample and processing.

Results of Diagnostic Evaluation

During the last few years we have conducted a comprehensive study on the diagnostic and prognostic efficacy of

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**Table 4. Molecular Markers of Hemostatic Disorders, Their Diagnostic Significance, and Commercial Kits for Their Measurement**

<table>
<thead>
<tr>
<th>Molecular markers (and kit manufacturer)</th>
<th>Source</th>
<th>Physiologic role</th>
<th>Diagnostic value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrinopeptide A, (Mallinckrodt, IMCO, Diagnostica Stago)</td>
<td>Activation of clotting enzymes</td>
<td>Unknown</td>
<td>Thrombosis</td>
</tr>
<tr>
<td>B 715-42 peptide (IMCO)</td>
<td>Activation of fibrinolytic enzymes</td>
<td>Unknown</td>
<td>Fibrinolysis</td>
</tr>
<tr>
<td>Platelet factor 4 (Abbott)</td>
<td>Activation of platelets</td>
<td>Antithrombin</td>
<td>Thrombosis</td>
</tr>
<tr>
<td>β-Thromboglobulin (Amersham)</td>
<td>Activation of platelets</td>
<td>Regulation of vascular tone</td>
<td>Thrombosis</td>
</tr>
<tr>
<td>Thromboxane B 2 (New England Nuclear Corp.)</td>
<td>Activation of platelets, vascular deficiency</td>
<td>Platelet activation, vascular spasm</td>
<td>Thrombosis</td>
</tr>
<tr>
<td>6-Keto-PGF1α (New England Nuclear Corp.)</td>
<td>Activation of platelets, vascular disorders</td>
<td>Platelet inhibition, vascular dilation</td>
<td>Vascular disease, shock</td>
</tr>
<tr>
<td>Leukotrienes (New England Nuclear Corp.)</td>
<td>Activation of platelets and neutrophils</td>
<td>Mediation of inflammation</td>
<td>Thrombosis</td>
</tr>
<tr>
<td>Serotonin (numerous methods)</td>
<td>Platelet release</td>
<td>Vasodilation</td>
<td>Thrombosis</td>
</tr>
<tr>
<td>Serine protease-inhibitor complexes</td>
<td>Activation of protease pathways</td>
<td>Coagulation, fibrinolysis, production of kinins</td>
<td>DIC, shock</td>
</tr>
</tbody>
</table>

Serotonin can be measured with chromatographic and radioenzymatic methods, the other markers with isotopic and nonisotopic methods capable of detecting subnanogram amounts.
some of these molecular markers, modifying some currently available assays and proposing methods for quantifying various substances in biologic fluids (152). Most of these markers were evaluated in anticoagulated plasma by established methods, and were measured in terms of nanograms or picograms per milliliter, to be easily transformable into other units. "Reference" values were obtained from normal healthy volunteers of both sexes, ages 20–40 years, for comparison with concentrations measured in patients having various diseases.

Figure 14 shows scattergram data on serotonin concentrations in platelet-rich plasma, platelet-poor plasma, and serum of normal individuals and patients with carcinoid syndrome, as measured by HPLC (153). Note the significantly increased amounts of serotonin in platelet-poor plasma and serum from patients with carcinoid syndrome.

Fibrinopeptide A is a sensitive marker of the activation of the coagulation processes. Upon the generation of trace amounts of thrombin, transformation of fibrinogen to fibrin results in the release of fibrinopeptides A and B (30, 33, 35). Pathological conditions in which concentrations of fibrinopeptide A are increased include: hypercoagulable state, neoplastic conditions, myocardial infarctions, burns, bone fractures, lupus erythematosus, and hemolytic anemias—sickle-cell crisis and thalassemia major (31–34). Fibrinopeptide A is also useful in the monitoring of anticoagulant and procoagulant therapy, and in the differential diagnosis of DIC, and primary fibrinolysis. In DIC, simultaneous activation of coagulation and fibrinolysis results in the formation of both fibrinopeptide A and Bβ 15-42 peptides, whereas in primary fibrinolysis only Bβ 15-42 related peptides are formed.

Figure 15 shows the distribution pattern of fibrinopeptide A in normal human plasma and serum samples as determined by a simplified RIA method (141). Only trace amounts of fibrinopeptide A (2.0 µg/L) are found in anticoagulated plasma and no significant sex-related differences are noted. Serum, on the other hand, contains considerable

\[ \text{SEROTONIN} \]
fibrinopeptide A (thrombin being generated during the formation of serum), and is therefore not a good test sample for this molecular marker.

We used a Mallinkrodt radioimmunoassay kit to study the effect of leukapheresis on concentrations of fibrinopeptide A in 12 patients. Although this procedure exerts no significant alterations of the routine coagulation parameters, fibrinopeptide A increased from 3.38 (SD 0.93) μg/L to 12.1 (SD 7.84) μg/L for a mean increase of 398% (range 132–620%). Thus the coagulation system may be activated by minute alterations of the hemostatic system, and more sensitive monitoring techniques are needed.

We also compared fibrinopeptide A concentrations in malignancy (n = 10), pregnancy–associated primary fibrinolysis (n = 10), and in DIC, and found significantly higher values than in normals. The pronounced increase in the fibrinopeptide A in DIC is from intravascular coagulation. Similarly, we measured fibrinopeptide A (μg/L, mean ± SD, n = 10) in patients with burns (17.6 ± 6.1), hypercoagulable state (21.8 ± 8.4), lupus erythematosus (13.6 ± 2.8), and neoplastic conditions (12.6 ± 3.1). In most of these conditions, activation of coagulation evidently increases the circulating concentrations of fibrinopeptide A. There are three commercially available methods for the quantification of fibrinopeptide A in biologic fluids. The kits from Mallinkrodt (St. Louis, MO) and IMCO (Stockholm, Sweden) are based on radioimmunoassays; the Asserachrom kit from Diagnostica Stago (Asnieres, France) is based on enzyme-linked immunoassays (I41, I42). For normal samples all kits performed identically; however, for pathological conditions the IMCO kits reported significantly higher concentrations than the other two kits: 23 (SD 15) μg/L vs 31 (SD 19) μg/L. Figure 16 illustrates the day-to-day performance characteristics of the Mallinkrodt RIA kit for fibrinopeptide A in high- and low-concentration controls.

Table 6 shows concentrations of Bβ 15-42 related peptide in 25 normal individuals and in various groups of patients with activated fibrinolytic conditions resulting from neoplastic disorders. Many of these patients showed pronounced hypofibrinogenemia with proportionately increased Bβ 15-42 related peptides. Several other reports have shown similar data in other states of fibrinolysis. Although the Bβ 15-42 related peptides increase with pronounced fibrinolysis, the increases of these peptides in plasma are detectable in patients with mild fibrinolysis, which cannot be noted in routine coagulation laboratory tests. Therefore, drug-induced fibrinolysis and mild fibrinolytic disorders, which are often undetectable, can be routinely assessed by quantifying Bβ 15-42 related peptides (154–157).

Fibrinopeptide A and Bβ 15-42 related peptides in patients with various hematologic diseases are compared in Table 7. As stated previously, a differential diagnosis between the primary and secondary fibrinolytic disorders can be made by measuring these two peptides. Perhaps a ratio between the two determinations will provide a reliable index of the activation of fibrinolysis and coagulation. This would be extremely useful for the therapeutic modulation of these diseases.

Table 8 shows concentrations of platelet factor 4 and β-thromboglobulin in normal volunteers and in patients with hypercoagulable states, post-cardiac surgery, disseminated intravascular coagulation, and after stress testing, as measured by RIA methods. These two proteins are primarily associated with the light granules of platelets; upon platelet

---

**Table 6. Concentrations of Bβ 15-42 Related Peptide in Patients with Activated Fibrinolytic Conditions Resulting from Neoplasia**

<table>
<thead>
<tr>
<th>Category</th>
<th>n</th>
<th>Fibrinogen, g/L</th>
<th>Bβ 15-42 peptide, ng/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>25</td>
<td>2.60 ± 0.40</td>
<td>24 ± 7</td>
</tr>
<tr>
<td>Group I</td>
<td>10</td>
<td>2.48 ± 0.30</td>
<td>160 ± 41</td>
</tr>
<tr>
<td>Group II</td>
<td>10</td>
<td>1.76 ± 0.19</td>
<td>140 ± 68</td>
</tr>
<tr>
<td>Group III</td>
<td>10</td>
<td>1.30 ± 0.14</td>
<td>&gt;500</td>
</tr>
<tr>
<td>Group IV</td>
<td>5</td>
<td>0.70 ± 0.11</td>
<td>&gt;500</td>
</tr>
</tbody>
</table>

Bβ 15-42 related peptides were measured in plasma with an IMCO RIA kit.

**Table 7. Concentrations of Fibrinopeptide A (FPA) and Bβ 15-42 Related Peptides in Patients with Various Hematologic Diseases**

<table>
<thead>
<tr>
<th>Disease</th>
<th>Hemostatic manifestation</th>
<th>FPA Conc., ng/mL*</th>
<th>Bβ 15-42 Conc., ng/mL*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>None</td>
<td>2.6 ± 0.4</td>
<td>31.0 ± 4.2</td>
</tr>
<tr>
<td>Polycythemia vera</td>
<td>Hypercoagulable state</td>
<td>2.4</td>
<td>88.0</td>
</tr>
<tr>
<td>Poorly differentiated lymphoma</td>
<td>DIC</td>
<td>1.9</td>
<td>12.4</td>
</tr>
<tr>
<td>Severe anemia (hyperplastic)</td>
<td>DIC</td>
<td>26.0</td>
<td>144.0</td>
</tr>
<tr>
<td>Hemangiomia</td>
<td>DIC</td>
<td>26.4</td>
<td>128.0</td>
</tr>
<tr>
<td>Erythoblast leukemia and multiple myeloma with treatment with PCC</td>
<td>Hypercoagulable state DIC</td>
<td>24.0</td>
<td>128.0</td>
</tr>
</tbody>
</table>

*Mean of three determinations. We measured fibrinopeptide A with a Mallinkrodt RIA kit and Bβ 15-42 related peptides with an IMCO RIA kit.
activation and (or) consumption, they are released into the systemic circulation. All of the pathologic states investigated show significant increases of these platelet-specific proteins, thus providing a reliable means for detecting the activation of platelets (15–23). Furthermore, these measurements are useful in monitoring antiplatelet drugs.

Table 9 illustrates thromboxane B2 concentrations in patients with Prinzmetal's angina, as measured by RIA. Although various other techniques have been used to quantify thromboxane B2, RIAs are by far the most current and useful methods for this evaluation. Lewy et al. (168) have clearly shown an increase in the circulating concentrations of thromboxane B2 in patients who have experienced anginal pain. Numerous other reports have recently become available for the quantification of thromboxane B2 in various vascular disorders (82–85).

A comparison of fibrinopeptide A, Bβ 15-42 related peptides, and thromboxane B2 in women volunteers taking oral contraceptives is shown in Table 10. The high incidence of thrombotic problems in this group is well documented (1). Several investigators have also reported a high frequency of thrombotic and vascular problems in smokers taking oral contraceptives (84–86). The concentrations of fibrinopeptide A are significantly greater for these volunteers than for normal men and women controls. The Bβ 15-42 related peptides are also increased, suggesting a slight activation of the fibrinolytic process. The increases in thromboxane B2 indicate activation of platelets. There was a large scatter in the data, and we list only a range for these analytes.

Because hemostatic activation in different pathological states is triggered by various pathways, simultaneous measurement of various molecular markers provides a reliable profile of the components involved in the overall pathophysiological process. This profiling may be useful when various therapeutic agents are used to control the pathological process.

Table 11 illustrates composite molecular markers profiling in various pathological states and during therapy. By measuring absolute amounts of specific markers, the exact locus of the pathological trigger can be investigated. In presurgical patients and patients with disseminated intravascular coagulation, concentrations of one or more of the molecular markers may be increased. In a hypercoagulable state increased concentrations of fibrinopeptide A and platelet-release products, as well as thromboxane B2, are found. More information is needed on the Bβ 15-42 related peptides.

### Table 8. Platelet Factor 4 and β-Thromboglobulin in a Normal Group and in Various Groups of Patients

<table>
<thead>
<tr>
<th>Group (and no.)</th>
<th>Platelet factor 4 (mean ± 1 SD)</th>
<th>β-Thromboglobulin (mean ± 1 SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (50)</td>
<td>11.4 ± 4.9</td>
<td>26.0 ± 8.1</td>
</tr>
<tr>
<td>Hypercoagulable state (20)</td>
<td>19.8 ± 6.9</td>
<td>42.0 ± 11.1</td>
</tr>
<tr>
<td>Post-cardiac surgery (10)</td>
<td>31.0 ± 19.8</td>
<td>97.0 ± 13.8</td>
</tr>
<tr>
<td>Disseminated intravascular coagulation (10)</td>
<td>86.7 ± 30.4</td>
<td>118.0 ± 40.6</td>
</tr>
<tr>
<td>Post-stress testing (10)</td>
<td>32.6 ± 12.0</td>
<td>63.0 ± 23.8</td>
</tr>
</tbody>
</table>

Platelet factor 4 was measured with an RIA kit from Abbott Laboratories, and β-thromboglobulin with an RIA kit from Amersham Nuclear Corp.

### Table 9. Thromboxane B2 (by RIA) in Patients with Prinzmetal's Angina

<table>
<thead>
<tr>
<th>Condition</th>
<th>Thromboxane B2 (mean ± 1 SD), pmol/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angina-free</td>
<td>20</td>
</tr>
<tr>
<td>Spontaneous angina</td>
<td>14</td>
</tr>
<tr>
<td>5–10 min post-angina</td>
<td>11</td>
</tr>
<tr>
<td>ST-segment shift or ventricular arrhythmia</td>
<td>7</td>
</tr>
<tr>
<td>Source: Lewy et al. (168).</td>
<td></td>
</tr>
</tbody>
</table>

### Table 10. Molecular Markers in Volunteers Taking Oral Contraceptives

<table>
<thead>
<tr>
<th>Group</th>
<th>FPA (mean ± SD, ng/mL)</th>
<th>Bβ 15-42 (mean ± SD, pg/mL)</th>
<th>TxB2 (range), pg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (M &amp; F)</td>
<td>2.6 ± 0.8</td>
<td>21 ± 11</td>
<td>0–50</td>
</tr>
<tr>
<td>Control (F only)</td>
<td>2.3 ± 0.7</td>
<td>46 ± 18</td>
<td>0–50</td>
</tr>
<tr>
<td>Volunteers (F) taking contraceptives</td>
<td>6.8 ± 3.4</td>
<td>81 ± 26</td>
<td>0–130</td>
</tr>
</tbody>
</table>

The control group consisted of 50 normal men and women. Members of the control female group, ages 18–40 years, were not taking any oral contraceptives. The test group comprised age-matched women taking oral contraceptives. Fibrinopeptide A (FPA) was measured with RIA kit from Mallinkrodt, Bβ 15-42 related peptides with a kit from IMCO, thromboxane B2 (TxB2) with a kit from New England Nuclear Corp.

### Table 11. Molecular Marker Profiling in Various Pathological States and during Therapy

<table>
<thead>
<tr>
<th>Fibropeptide A</th>
<th>Platelet factor</th>
<th>Thromboglobulin</th>
<th>Thromboxane B2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-surgical</td>
<td>↑ ↑ ↑</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>DIC</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Hypercoagulable state</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Heparin therapy</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Oral anticoagulant therapy</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Antiplalet therapy</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>Thrombolytic therapy</td>
<td>?</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>Hemophilia A and B</td>
<td>?</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>Platelet function profiling</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Drug-induced alteration of hemostatic function</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
</tr>
</tbody>
</table>

↑ increased, ↓ decreased, ↑↑ increase or decrease, ? no change reported.

Heparin is conventionally used in the prophylaxis and treatment of thrombosis. Patients suffering from a thrombotic state generally show increased concentrations of fibrinopeptide A. Treatment significantly decreases the initial increase in fibrinopeptide A, so that this marker can be used as a means of monitoring antithrombotic therapy (158, 159). During antiplalet therapy the activation of platelets is suppressed, and there is a pronounced decrease in platelet-release products. Simultaneous measurement of platelet-release products and thromboxane B2 is a reliable means of monitoring antiplalet therapy. Similarly, thrombolytic therapy can be monitored in terms of titers of the Bβ 15-42 related peptides. In hemophilia A and B various molecular markers can be used to investigate the hemostatic restoration with plasma concentrates. These markers can also be used in profiling platelet function and in studying the drug-induced alteration of hemostatic function (160–162). Disseminated intravascular coagulation (DIC) is the result of a multicomponent activation of various hemostatic pathways, and can be assessed as an uncontrolled activation.
of hemoatasis. Proteases of the coagulation, fibrinolysis, and kallikrein–kinin pathways are capable of generating fibrinopeptide A, Bβ 15-42 related peptides, and kinins, whereas the activation of platelets generates platelet-release proteins and serotonin (Figure 17). The platelet endothelial interactions are capable of generating products of prosta
glandin pathways. DIC may manifest in various forms in association with septicemia, cancer, gut, post-surgical trauma, complicated pregnancy, and many other diseases. Currently used laboratory methods for evaluation of DIC do not provide reliable information; molecular markers, on the other hand, are useful for diagnosis and monitoring treatment for this syndrome.

In Figure 18 various molecular markers of hemoatatic activation are compared for patients with defined DIC. Significant increases from the baseline values for the fibrinopeptide A, Bβ 15-42 related peptides, 5-hydroxytryptamine, and platelet factor 4 occur in these patients. Bradykinin concentrations are also increased in this syndrome, but not as markedly, perhaps because kinins have a very short biological half-life in circulating blood; however, during a sustained activated state, these peptides provide a reliable means of monitoring the activation of the kallikrein system.

**Discussion**

Current assays used to assess hemoatasis and monitor anticoagulant and procoagulant therapy not only are ill-defined and nonstandardized, but also are only capable of measuring bleeding problems. Furthermore, these methods do not necessarily provide a true hemoatatic picture, because the parameters measured, such as activation of factors or consumption of inhibitors, vary widely among individuals (163, 164).

With clearer biochemical understanding and technological advancements, it is now possible to quantify picograms or nanograms of specific substances generated during the various phases of hemoatatic activation (Table 12). We are now able to pinpoint pathophysiological effects of disease and drug therapy in hemoatatic, fibrinolytic, kallikrein–kinin, and platelet systems as well as related prosta
glandin, leukotriene, and complement systems. These pathways are closely interrelated and it is not surprising that one disorder is often associated with many others.

The introduction of molecular markers as diagnostic tools to study the alterations of hemoatatic pathways has introduced a new dimension to the laboratory evaluation of bleeding and clotting disorders. A comparison of molecular markers of hemoatatic activation with the conventional methods is given in Table 13. Unlike the general screening tests for hemoatatic system evaluation, these markers provide highly specific information on the biological pathways involved. They are extremely sensitive to pathophysiological changes, and minute aberrations of this system can be detected by quantifying them. Many of these markers can only be assessed by newer technologies such as the immunoassays and chromatographic, mass spectrometric, and isotope dilution methods (165–167). Although both immunoassay and gas-chromatography/mass spectrometry can be used to profile molecular markers, immunoassay offers definite advantages for the clinical laboratory.
Table 12. Molecular Markers of Hemostatic Activation

<table>
<thead>
<tr>
<th>Marker</th>
<th>Chemical nature</th>
<th>Site of production release</th>
</tr>
</thead>
<tbody>
<tr>
<td>*Fibrinopeptide A</td>
<td>Peptide</td>
<td>Blood (coagulation)</td>
</tr>
<tr>
<td>*Bb1 15-42 peptides</td>
<td>Polypeptides (heterogenous)</td>
<td>Blood (clot lysis)</td>
</tr>
<tr>
<td>*Platelet factor 4</td>
<td>Polypeptide</td>
<td>Platelets (light granules)</td>
</tr>
<tr>
<td>Serotonin</td>
<td>Indocyanine</td>
<td>Platelets (dense granules)</td>
</tr>
<tr>
<td>*Thromboxanes</td>
<td>Arachidonic acid derivative</td>
<td>Platelets/endothelial cells</td>
</tr>
<tr>
<td>*6-Keto-PGF1a</td>
<td>Arachidonic acid derivative</td>
<td>Endothelium</td>
</tr>
<tr>
<td>Leukotrienes</td>
<td>Arachidonic acid derivative</td>
<td>Leukocytes, platelets</td>
</tr>
<tr>
<td>Platelet-activating factor</td>
<td>Arachidonic acid derivative</td>
<td>Various sites, platelets</td>
</tr>
<tr>
<td>*Kinnis</td>
<td>Peptide</td>
<td>Blood (proteins)</td>
</tr>
</tbody>
</table>

* Analyses for which there are commercially available kits.

Table 13. Comparison of Molecular Markers of Hemostatic Activation and Conventional Coagulation Tests

<table>
<thead>
<tr>
<th>Molecular markers</th>
<th>Conventional tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrinopeptide A, Bb 15-42 related peptides, platelet factor 4, thromboxane B2, serotonin, kinins</td>
<td>Prothrombin time, partial thromboplastin time, thrombin time, fibrinogen</td>
</tr>
<tr>
<td>Specific markers of the pathological or therapeutic activation of coagulation, fibrinolysis, platelets, and cellular vascular processes</td>
<td>Screening tests, only measure the deficiencies of clotting processes. Useful in the screening and monitoring of hypo-coagulable state</td>
</tr>
<tr>
<td>Ultrasensitive, capable of detecting minute changes in the components of hemostatic system</td>
<td>Mild sensitivity, detects only major changes in the coagulation processes</td>
</tr>
<tr>
<td>Highly specific, provides information on the native blood (physiological)</td>
<td>Non-specific</td>
</tr>
<tr>
<td>Very small amount of test sample needed</td>
<td>Requires exogenous activation of test samples (nonphysiological)</td>
</tr>
<tr>
<td>Requires special anticoagulant cocktail</td>
<td>Large (&gt;500 μL) sample size needed</td>
</tr>
<tr>
<td>Requires newer technology</td>
<td>Citrated plasma can be used</td>
</tr>
</tbody>
</table>

Currently, simple 125I- and 3H-based radioimmunoassays are available for platelet factor 4, β-thromboglobulin, fibrinopeptide A, Bβ 15-42 related peptide, thromboxane B2, 6-keto-PGF1α, and PGE2. Nonspecific methods such as the enzyme-linked immunosorbent assays and fluorimmunoassays are being developed.

Serotonin and ADP are measurable by HPLC, enzyme immunoassays, and spectrophotofluorometry. Analytic methods for fibrin split products (fragments D and E) and serine protease–inhibitor complexes such as thrombin–antithrombin-III, factor Xa–antithrombin-III, and kallikrein–Cl esterase are also being developed.

Among the applications of molecular markers in the study of hemostatic disorders are the differential diagnoses of coagulation disorders and assessment of therapeutic efficacy of procoagulant and antithrombotic agents. The use of prostaglandin measurements has added a new spectrum to our understanding and monitoring of cardiovascular disorders. Because concentrations of some of these molecular markers are increased before there are any clinically observed manifestations, they are useful for early detection of disorders. Some markers, specifically thromboxanes, are generated during stress. In certain patients, these quantifiable stress-induced changes may be useful in predicting pathological alterations related to a disease or trauma, and early prophylactic measures can be recommended. Cancer is now known to produce both bleeding and clotting disorders. Unlike current methods of coagulation testing, the molecular markers of hemostasis can profile many of the prethrombotic and prefibrinolytic conditions before a pathological manifestation is observed clinically.

Although currently their use is somewhat limited because of their cost, the need to educate users, and other factors, we predict the real future of these tests will be in the diagnosis of hemostatic defects and the management of newer antithrombotic drugs that cannot be monitored by current methods. Some of these molecular markers can also be used in quality control of blood products and plasma-based therapeutic agents.

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