Thromboembolic Disease: Physiology of Hemostasis and Pathophysiology of Thrombosis*

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ABSTRACT: Thromboembolic disease results from pathologic alteration of the normal hemostatic response. This disorder is underdiagnosed in veterinary medicine but is a significant contributor to the clinical signs and disease processes in many veterinary patients. Hemostasis is a complex physiologic process regulated by the endothelium, platelets, the coagulation cascade, and the fibrinolytic system. Thrombosis may occur with a failure of any part of this system that leads to a prothrombotic state. This article reviews normal coagulation and fibrinolysis and the derangements in this system that can lead to thrombosis.

The term *thrombus* refers to an aggregation of platelets and other blood components that causes partial or complete vascular obstruction. An *embolus* is a clot, or other plug, broken off from one position and brought, under the influence of blood flow, to lodge in a distal vessel. The term *thromboembolic disease* encompasses disorders involving both thrombi and blood clot emboli. Thromboembolic disease is currently the most common cause of mortality in adult human medicine in the United States; it is predominantly associated with ischemic heart disease. Although humans and animals differ in terms of many predisposing causes, researchers are currently identifying many veterinary diseases that put animals at risk for thromboembolic complications (see the box on page 651). Necropsy results show that thromboemboli are far more common than have been suspected on a clinical basis. Although some thromboemboli may result in subclinical abnormalities, significant and unrecognized pathologic disorders are probably induced in certain patients. Advances in the understanding of the underlying pathophysiologic process resulting in thrombosis, as well as advances in the ability to modulate this system and treat pathologic sequelae, make this an exciting area of veterinary critical care.

*A companion article appears on p. 660.*
NORMAL COAGULATION AND FIBRINOLYSIS

In normal hemostasis, a complex system acts to maintain the blood as a fluid within the intact vascular space while allowing clot formation in areas of damaged vascular endothelium. Normally, multiple mechanisms are involved with both the promotion and the inhibition of blood coagulation to preserve this delicate balance. These mechanisms include endothelial factors, platelets, the coagulation cascade, and the fibrinolytic system. A thorough understanding of normal hemostasis is necessary to understand the pathologic manifestations that are ultimately responsible for clinical thromboembolic complications.

Damage to the Endothelium

The endothelium consists of a layer of epithelial cells that line the inner surface of the circulatory system. These cells act as an interface between the blood and the interstitium, and they mediate diffusion, absorption, secretion, and containment.7,4 The endothelium is intricately involved in modulation of hemostasis. The intact cells provide a substrate that is not conducive to clot formation and secrete many substances that are involved in vasoregulation and prevention of thrombosis.5,9

When the endothelium is damaged, however, a pro-thrombotic tendency dominates, and clot formation is favored, which reduces blood loss. Neurogenic mechanisms, as well as endothelin release from the endothelial cells, promote vasoconstriction and cause local blood flow to slow, which allows more intimate contact of the blood cells and platelets with the vessel wall.2,10 In addition, the basement membrane of the vascular wall is revealed, with the resultant exposure of subendothelial collagen, fibronectin, proteoglycans, and von Willebrand’s factor. These substances make up a highly thrombotic substrate that stimulates platelet adherence and aggregation, which leads to the next step in the coagulation process. Tissue factor released from the damaged endothelial cells also causes direct activation of the extrinsic arm of the coagulation cascade, thus favoring thrombosis.2,11

Platelets

Platelet involvement in the coagulation process consists of adhesion, activation, and aggregation. Platelets initially adhere to the damaged endothelium by long pseudopods but then attach more strongly, primarily through von Willebrand’s factor and glycoprotein receptors (Figure 1) but also through mediation with fibrinogen.2,12-13 The adhesion of platelets to vessel walls

![Figure 1—Platelet activation. Exposure of the subendothelial collagen and von Willebrand’s factor (vWF) allow initial platelet adherence. Bound platelets undergo activation consisting of a shape change and the release of alpha and delta granules. Release of granule contents stimulates additional platelet aggregation. Platelets become cross-linked through fibrinogen bridging, with further stabilization of fibrinogen to fibrin.](image_url)
results in activation of the platelets. The platelet surface undergoes morphologic changes, with release of cytoplasmic granules and synthesis of thromboxane A₂ and platelet-activating factor.

Activated platelets release the contents of alpha and delta (dense) granules. Alpha granules contain fibrinogen, fibronectin, certain coagulation proteins, chemokines, platelet-derived growth factor, and transforming growth factor-β. Delta granules contain ADP, ATP, ionized calcium, histamine, serotonin, and epinephrine. The shape change in the activated platelets exposes a phospholipid surface that eventually provides the site necessary for activation of the intrinsic arm of the coagulation cascade through the action of ionized calcium and activated coagulation factors. The ADP and serotonin released from the delta granules, fibrinogen released from the alpha granules, and thromboxane A₂ and platelet-activating factor synthesized from the activated platelets are powerful stimuli for further platelet aggregation. Thrombin, the end product in the coagulation cascade, is the final proponent of platelet aggregation. Platelets are bound to each other through a fibrinogen linkage. This primary platelet plug is then further stabilized by means of platelet contraction and the thrombin-induced conversion of fibrinogen to fibrin. The formation of the primary platelet plug is known as primary hemostasis.

### Coagulation Cascade

The coagulation cascade consists of a series of transformations of inactive serine proteases into their active forms, with thrombin as the end product. Thrombin then acts on the soluble fibrinogen and converts it to fibrin. This transformation is known as secondary hemostasis.

All reactions require an enzyme (the coagulation factor), a substrate, and a cofactor. In normal hemostasis, the substrate consists of the platelet phospholipid layer, and calcium acts as the cofactor by holding the components together. In this way, clot formation is limited to the area around activated platelets. The coagulation cascade is traditionally divided into the intrinsic, extrinsic, and common pathways (Figure 2). The extrinsic pathway is the main initiator of the clotting process. Tissue factor, produced by damaged tissues or on the surface of endothelial cells or monocytes, activates factor VII and thus stimulates the clotting mechanism. The intrinsic pathway is involved with amplification of the clotting process as well as modulation of fibrinolysis.

The intrinsic, extrinsic, and common pathways act together to produce large amounts of thrombin. Thrombin facilitates the cleavage of fibrinogen to fibrin monomers. These monomers polymerize to form soluble fibrin, which is then cross-linked through the action of factor XIII to insoluble fibrin, thereby stabilizing the clot. In addition, thrombin promotes further platelet aggregation. However, thrombin also provides a negative feedback loop on itself by interactions with thrombomodulin, proteins C and S, and antithrombin III (ATIII).

### Fibrinolytic System

The fibrinolytic component of the hemostatic mechanism is of paramount importance in the normal individual, but it is the least understood aspect of hemostasis for many practitioners. Multiple mechanisms are available to limit the extent of clot formation. Negative feedback on thrombin partially limits the induction of clot formation, but mechanisms to promote clot breakdown (i.e., fibrinolysis) also exist. The initial stimulus that induces the coagulation cascade simultaneously induces the release of tissue plasminogen activator (t-PA) from the endothelial cells. This enzyme works to convert the inactive plasminogen to plasmin, which then proceeds to break down fibrinogen and fibrin, thereby lysing the blood clot and releasing fibrin/fibrinogen degradation products. The conversion to plasmin depends on the presence of fibrin, which
A thrombotic state can occur in any of three components of the hemostatic balance, known as Virchow’s triad: (1) abnormalities in the structure or function of the endothelium; (2) blood stasis, resulting from changes in blood flow; and (3) a hypercoagulable state, resulting from quantitative or qualitative changes in the constituents of blood. Many disease processes affect more than one of these components, which amplifies a predisposition toward thrombosis.

Role of the Endothelium

Endothelial cells normally produce antiplatelet, anticoagulant, and fibrinolytic factors that aid in the prevention of clot formation in the undamaged vascular space (Figure 3). The three main antiplatelet factors are nitric oxide, prostacyclin, and adenosine diphosphatase. Nitric oxide is an endogenous gas that is produced by endothelial and other cells through the action of nitric oxide synthase. Nitric oxide causes vasodilation as well as inhibition of platelet activation. Prostacyclin, another molecule produced by the endothelium and induced by stimulation with thrombin, bradykinin, and histamine, also inhibits platelet activation and aggregation and mediates vascular smooth muscle relaxation. Nitric oxide and prostacyclin work synergistically to reverse platelet aggregation that has already occurred. Adenosine diphosphatase is an enzyme present on the endothelial surface that degrades ADP to AMP. Because of the binding of ADP as it is released from the activated platelets, it is no longer available to act as a potent stimulator of platelet aggregation, and thus this aspect of the coagulation system is limited.

PATHOPHYSIOLOGY OF THROMBOSIS

Thrombotic complications in veterinary medicine result from pathologic alterations in normal hemostatic balance. Alterations favoring clot formation or inhibiting clot breakdown result in pathologic overactivation of the coagulation system. Alterations leading to a thrombotic state can occur in any of three components of the hemostatic balance, known as Virchow’s triad: (1) abnormalities in the structure or function of the endothelium; (2) blood stasis, resulting from changes in blood flow; and (3) a hypercoagulable state, resulting from quantitative or qualitative changes in the constituents of blood. Many disease processes affect more than one of these components, which amplifies a predisposition toward thrombosis.

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endothelial cell through endocytosis. The thrombin is degraded, and the thrombomodulin is recycled to the membrane surface. Thrombomodulin also binds activated coagulation factor X to prevent its activation of prothrombin, and it induces a conformational change in thrombin to enhance its activation of protein C. Protein S, also synthesized by the endothelial cells, works in conjunction with protein C as an anticoagulant by inactivating factors V and VIII. When ATIII is coupled with its cofactor heparan as an accelerator, it is a potent anticoagulant molecule. Heparan sulfate is produced by and expressed on the surface of endothelial cells as well as on the subendothelium. Heparan sulfate can act as a cofactor for the thrombin–antithrombin complex to inactivate factors IXa, Xa, XIa, and XIIa. TFPI is a protease inhibitor that is normally produced primarily by the liver; endothelial cells also produce this inhibitor, especially in inflammatory conditions. In combination with factor Xa, TFPI inhibits the tissue factor–factor VIIa complex, thereby blocking the extrinsic arm of the coagulation cascade. The endothelial cells are partially responsible for activation of the fibrinolytic system through the production of t-PA.9

Disruption of the vascular endothelium for any reason leads to a chain of rapid reactions that result in vascular constriction, formation of hemostatic plugs, and an attempt at vascular repair. These reactions occur to prevent blood loss, maintain homeostasis, and prevent injury to the host. When this damage is diffuse or severe, however, the endothelium loses its ability to regulate the balance between normal hemostasis and pathologic thrombosis. The antiplatelet, anticoagulant, and fibrinolytic mechanisms just discussed rely on a healthy endothelium for appropriate regulation. A dysfunctional endothelium causes these mechanisms to fail, and the coagulation process can proceed unchecked. In addition, disruption of the endothelium results in exposure of the thrombotic basement membrane and upregulation of substances that favor thrombosis, including production of tissue factor by endothelial cells. The combination of decreased antithrombotic mechanisms and increased prothrombotic mechanisms together allows endothelial damage to be an extremely potent stimulator of thrombosis.11,16

Blood Stasis

Alterations in blood flow leading to stagnation or turbulence of blood, such as is seen with heart disease, shock, or prolonged recumbency, provide another way in which a prothrombotic tendency can develop. In normal animals, a constant, low-level, systemic activation of clotting factors operates. The physiologic anticoagulant mechanisms, hepatic clearance of activated coagulation factors, and sufficient blood flow to allow dispersion and dilution of these activated factors normally prevent pathologic thrombus formation. Blood stasis results in disruption of laminar flow, inhibition of coagulation factor dilution and clearance, and inhibition of coagulation inhibitor dispersion. Stasis may also cause local tissue hypoxia and endothelial damage. These derangements together promote thrombus formation.2

Hypercoagulability

Hypercoagulability is the third component of Virchow’s triad. Either quantitative or qualitative alteration in the procoagulant and anticoagulant substances that modulate the hemostatic system can favor thrombosis. This occurs through increased levels or activity of procoagulant substances, decreased levels or activity of anticoagulant or fibrinolytic substances, or increased levels of fibrinolytic inhibitors. Primary (i.e., genetic) hypercoagulability caused by defective or deficient synthesis is well established in many
human disorders but is less frequently reported in veterinary medicine.\textsuperscript{17} In secondary (i.e., acquired) hypercoagulability, changes in blood constituents result from an underlying systemic disease process. Acquired hypercoagulable states are more commonly identified than primary or genetic states in veterinary medicine.\textsuperscript{4–6} ATIII deficiency is likely the most well known of the hypercoagulable states. ATIII deficiency may stem from defective synthesis by the liver; increased consumption of ATIII, as with disseminated intravascular coagulation (DIC); or extracorporeal loss, as with glomerular disease. ATIII is an α₂-globulin produced by the liver that is thought to account for approximately 80% of the anticoagulant effect of plasma.\textsuperscript{18} ATIII acts to bind thrombin or other activated serine proteases, including factors IXa, Xa, XIa, and XIIa as well as kallikrein.\textsuperscript{19} Through binding, these factors are inactivated. This reaction is slow but dramatically accelerated by the action of heparan sulfate\textsuperscript{9} or heparin,\textsuperscript{19} both of which act as cofactors for ATIII.

Proteins C and S are vitamin K–dependent serine proteases produced by the liver and endothelial cells, respectively (Figure 4). They play a significant role in modulation of the normal hemostatic system. The thrombin–thrombomodulin complex activates protein C. Protein C, with protein S acting as cofactor, is a potent anticoagulant and profibrinolytic substance; it also has antiinflammatory properties. Proteins C and S work together to inhibit the generation of thrombin through inactivation of factors Va and VIIIa, which thereby limits this potent stimulator of thrombosis. Decreased thrombin levels result in reduced platelet activation, neutrophil recruitment and activation, and cytokine production. These proteins also act to inhibit PAI-1.\textsuperscript{20,21} Decreased levels or defective synthesis of protein C or S results in increased levels of activated clotting factors and decreased fibrinolytic function.\textsuperscript{22,23} Alterations in the quantity and function of proteins C and S have been documented in humans with evidence of hypercoagulability, such as that produced by DIC, trauma, vitamin K deficiency, L-asparaginase treatment, and malignancy.\textsuperscript{17}

Diminished or defective fibrinolysis is increasingly being recognized as an extremely important component in the pathophysiology of thrombosis. It is currently thought to be the most common inducer of hypercoagulability in human medicine, but it remains the least well understood. Physiologic clots are normally rapidly broken down by the body’s fibrinolytic system.\textsuperscript{24} The persistence of pathologic thrombi in any disease state suggests a failure of this mechanism. Defective fibrinolysis can be seen with decreased levels of the natural fibrinolytic components t-PA, urokinase, and plasminogen. More often, increased levels of the fibrinolytic inhibitor PAI, which result in reduced conversion of plasminogen to plasmin, have been implicated. Cytokine influence, mainly tumor necrosis factor and interleukin-1, also cause increased production of the fibrinolytic inhibitors PAI, α₂-antiplasmin, and thrombin-activated fibrinolytic factor.\textsuperscript{25–28} Through this mechanism, any disease involving a systemic inflammatory response, such as sepsis, severe pancreatitis, and heatstroke, has the potential to inhibit fibrinolysis.

Alterations in primary hemostasis may also predispose toward thrombosis. Excessive platelet aggregation and activation can lead to a hypercoagulable state.\textsuperscript{29,30} Heart disease, diabetes mellitus, neoplasia, nephrotic syndrome, and certain drug therapies are known to cause platelet hyperaggregability.\textsuperscript{29,31–33} Conditions with increased platelet numbers can include reactive thrombocytosis (e.g., secondary to endocrine disorders, infections, trauma, surgery, and certain drug therapies), neoplasia, and essential thrombocythemia.\textsuperscript{34–37} Platelet aggregation and activation are usually normal in patients with reactive thrombocytosis, and thrombotic risk is not thought to be elevated.\textsuperscript{34} With essential thrombocythemia, however, a greater risk of thrombosis is well documented in human medicine.\textsuperscript{36,37} This disease is infrequently reported in veterinary medicine, and its relationship to thrombosis remains to be fully elucidated.

**CONCLUSION**

The intricacy of the coagulation and fibrinolytic systems allows exact regulation of hemostasis in healthy animals. This same complexity, however, exposes
numerous physiologic pathways to potential disruption. Many disease states interfere with the body’s regulation of hemostasis and lead to a prothrombotic state. A thorough understanding of the normal hemostatic process is necessary to fully appreciate the multiple causes of abnormal regulation of coagulation that may result in a pathologic condition.

REFERENCES


ARTICLE #1 CE TEST

The article you have read qualifies for 1.5 contact hours of Continuing Education Credit from the Auburn University College of Veterinary Medicine. Choose the best answer to each of the following questions; then mark your answers on the postage-paid envelope inserted in Compendium.

1. Necropsy results reveal that thromboemboli are thought on the basis of clinical suspicion.
   a. more common than   c. as common as
   b. less common than   d. none of the above
2. __________ released from damaged endothelial cells results in activation of the coagulation cascade.
   a. von Willebrand’s factor  c. Tissue factor
   b. Calcium                    d. Endothelin

3. Normal stimulators of platelet aggregation include all of these factors except
   a. ADP, fibrinogen, nitric oxide.
   b. fibrinogen, thromboxane A₂.
   c. thromboxane A₂, fibrinogen.
   d. nitric oxide.

4. The end product of the coagulation cascade is
   a. fibrinogen, insoluble fibrin.
   b. soluble fibrin, thrombin.
   c. fibrinogen, insoluble fibrin.
   d. insoluble fibrin, thrombin.

5. Virchow’s triad includes all of the following components except
   a. stasis of blood, platelet aggregation.
   b. dysfunctional endothelial cells, hypercoagulability.
   c. platelet aggregation, dysfunctional endothelial cells.
   d. hypercoagulability, dysfunctional endothelial cells.

6. The endothelium produces all of the following anticoagulant factors except
   a. protein C, thrombomodulin.
   b. protein S, TFPI.
   c. protein C.
   d. thrombomodulin.

7. A hypercoagulable state may result from all of these factors except
   a. excessive platelet aggregability.
   b. decreased activated protein C levels.
   c. deficient thrombin generation.
   d. increased fibrinolytic inhibitors.

8. Which statement about ATIII is true?
   a. ATIII is a protein produced by endothelial cells.
   b. ATIII accounts for approximately 50% of the anticoagulant effect of plasma.
   c. ATIII’s anticoagulant effect is accelerated by the action of heparan sulfate or heparin.
   d. ATIII functions through binding activated vitamin K–dependent clotting factors II, VIII, IX, and X.

9. The most common cause of acquired hypercoagulability in humans is
   a. defective fibrinolysis.
   b. defective thrombomodulin production.
   c. protein C deficiency.
   d. protein S deficiency.

10. A thorough understanding of the physiology of thrombosis is important because
   a. as veterinary critical care progresses, veterinarians will frequently be challenged with this clinical entity.
   b. the pathogenesis of thrombosis is multifactorial and complex.
   c. specific areas of the hemostatic system can be pharmacologically targeted to effectively manage patients with thromboembolic predispositions.
   d. all of the above.