What's New in the Pathophysiology of Thrombosis in Patients with Predisposing Underlying Disease?

Linda Kidd, DVM, PhD, DACVIM
Western University College of Veterinary Medicine
Pomona, CA

Review of coagulation

Primary hemostasis refers to the formation of the platelet plug, while secondary hemostasis refers to activation of the coagulation cascade and the formation of a fibrin network. Clinically it is useful to separate hemostasis into these two stages because disorders of primary and secondary hemostasis have distinct clinical presentations and causes. However, during normal hemostasis, activation of coagulation and platelets occurs simultaneously. Formation of the platelet-fibrin clot is occurs during three overlapping phases, initiation, amplification and propagation. Endothelial damage initiates the formation of a platelet plug through binding of platelets to subendothelial collagen, which is facilitated by von Willebrand's factor. Tissue factor (TF) within the blood vessel wall simultaneously activates the coagulation protease cascade through the extrinsic cascade. The extrinsic cascade is comprised of TF and factor VIIa. The extrinsic cascade initiates coagulation during normal hemostasis and in many prothrombotic states. The intrinsic cascade is not currently believed to be involved in initiation of coagulation in vivo. Tissue factor is normally absent from the vascular space, being expressed by cells surrounding blood vessels such as subendothelial fibroblasts. Thus, the initiation phase of coagulation is localized to TF bearing surfaces. During this phase, coagulation is initiated by exposure of TF to plasma due to endothelial damage, or expression of TF on the surface of activated endothelial cells, monocytes and/or microparticles.

Circulating microparticles are derived from cell membranes of RBCs, platelets, megakaryocytes, endothelial cells,neutrophils and monocytes. They express cell surface molecules that are derived from their cell of origin, and are able to interact with, and induce cell signaling in other cell types, including endothelial cells. Evidence suggests that activated platelets release phosphatidylserine (PS) exposing microparticles. PS is normally present on the inside of cell membranes. Activation of platelets and microparticle formation results in PS exposure on their external surface. PS has procoagulant effects as it provides the docking site for coagulation factors during the process of coagulation. Interestingly, deficiencies in platelet exposure of PS and microvesiculation result in bleeding tendencies in people and dogs. Microparticles derived from activated monocytes and endothelial cells and possibly platelets also express TF. Thus microparticles are thought to play a role in pathologic thrombosis as well. Regardless of its source, exposure of TF to plasma factor VII/VIIa initiates coagulation and results in the production of a small amount of thrombin.

The amplification phase of coagulation occurs mainly on platelets. Thrombin activates platelets, and platelet associated factor V. Factor Va acts as a cofactor for factor Xa. Together they form the prothrombinase complex that converts prothrombin to thrombin and this results in the production of more thrombin. The intrinsic pathway consists of high molecular weight kininogen, prekallikrien, and the serine proteases factor XII, factor XI, factor IX and factor VIII. In addition to platelets and factor V, thrombin also activates factor VIII and factor XI of the intrinsic cascade.

The propagation phase is driven by thrombin activation of the intrinsic pathway downstream of factor XII, and is thought to occur primarily through thrombin induced activation and formation of the tenase complex (factors VIIIa- IXa) and factor XIa. The TF-factor VIIa complex also activates the intrinsic cascade through activation of factor IX. Formation of the tenase complex, and subsequent further activation of factor X and V, results in further generation of thrombin. Of note, factor XII is not necessary for normal hemostasis as evidenced by the fact that factor XII deficient cats, people, and mice do not exhibit bleeding tendencies. Although factor XII deficiency does not increase the risk of hemorrhage, factor XII-/- mice have reduced thrombosis in a variety of models.

Large amounts of thrombin are produced in the propagation phase. Thrombin catalyzes the conversion of fibrinogen to fibrin, and activates the transglutaminase factor XIII which then cross-links fibrin and stabilizes the clot. In addition to its role in the propagation of the coagulation cascade and formation of fibrin, thrombin is a potent activator of platelets and endothelial cells via cleavage of protease-activated receptors.

Platelets and other cells release microparticles that enhance clotting by providing a membrane surface for the assembly of the prothrombinase and tenase complexes. Thus, platelets and the clotting cascade work together in the generation of a blood clot.

The major inhibitor of the extrinsic pathway is tissue factor pathway inhibitor (TFPI). TFPI is expressed by endothelium and binds to its surface. There are also small amounts of TFPI in the circulation. Another anticoagulant expressed by activated endothelium is thrombomodulin. When thrombomodulin binds thrombin its substrate specificity changes and it becomes an anticoagulant protein by activating protein C. Activated protein C with its cofactor, protein S, cleaves and inactivates factors Va and VIIIa. Antithrombin (AT) inhibits factors Xa, IIa, VIIa, IXa, XIa and XIIa. The activity of AT is dramatically increased after binding heparan sulphate, which is expressed on the surface of endothelial cells.

Fibrinolysis occurs gradually after clot formation. Activated endothelium and monocytes produce tissue plasminogen activator (tPA), which converts plasminogen to plasmin. Plasmin is an endopeptidase that cleaves fibrin which destabilizes the clot and results

in the production fibrin degradation products. Inhibitors of plasmin generation and activity include plasminogen activator inhibitors, α-2 antiplasmin, α-2 macroglobulin and other protease inhibitors.

Increased procoagulant, decreased anticoagulant and impaired fibrinolytic activity may shift the hemostatic balance towards thrombosis.

Pathophysiology of thrombosis

Thrombosis is defined as the pathologic formation of a blood clot inside a blood vessel. Thrombosis can occur in either arteries or veins. Importantly, the pathogenesis of the generation of an arterial or venous thrombus differs, although this distinction is not commonly considered in small animal medicine. Arterial thrombi form primarily as a consequence of platelet activation under high blood flow conditions in arteries and arterioles, and are described as "platelet-rich." Venous thrombi form under low blood flow in veins and venules and are fibrin-rich due to the activation of coagulation. Recognizing differences in what initiates a thrombus can have therapeutic implications. Generally speaking, anti-thrombotic drugs either prevent the formation of arterial thrombi by targeting platelets (anti-platelet drugs), or they prevent the formation of venous thrombi by targeting the coagulation cascade (anti-coagulant drugs). Myocardial infarction (MI) in people is primarily a result of platelet activation in coronary arteries after artherosclerotic plaque rupture. Coagulation is also activated due to tissue factor exposure. Subsequent thrombin production is believed to further activate platelets. Therapy for acute MI therefore involves anti-platelet therapy such as low dose aspirin, while prevention of pulmonary thromboembolism (PTE) due to deep vein thrombosis (DVT) involves the anticoagulant drugs warfarin and heparin.

It is important to keep in mind that many diseases also result in a generalized microvascular thrombosis of both arteries and veins. In human medicine, drugs that target the coagulation cascade, like heparin, are sometimes used to reduce levels of thrombin and indirectly decrease platelet activation in these instances. In some cases drugs that target coagulation or platelets are used in combination, although the risk of hemorrhage may be increased. For many diseases the pathophysiology of thrombus formation has not been elucidated.

Many diseases are associated with areterial and venous thrombosis in dogs. Common sites for venous thrombosis are the portal vein, the splenic vein and the pulmonary arteries (PTE). Diseases that have been associated with splenic vein thrombosis with or without portal vein thrombosis in dogs include neoplasia, glucocorticoid administration, SIRS, DIC, immune mediated disease (most commonly IMHA) protein losing nephropathy and Cushings disease. Pulmonary thromboembolism is common in dogs with IMHA. The pathogenesis of PTE in dogs with IMHA has recently been reviewed. Pulmonary thromboembolism has also been described in dogs with a history of corticosteroid administration, diabetes mellitus, heartworm infection DIC, endocarditis, Cushings, hypothyroidism, venous catheterization, heart disease, neoplasia, pancreatitis, protein losing nephopathy, sepsis and surgery.

Activation of coagulation or decreased anticoagulation and increased tissue factor expression are believed to play important roles in the formation of venous thrombi. Activation of coagulation and decreased levels of AT are evident in many of the disease processes associated with venous thrombosis in dogs. In addition, increased tissue factor mRNA expression has been identified in the blood of dogs with IMHA. Increased levels of circulating microparticles with exposed PS and microparticles expressing tissue factor are hypotesized to play an important role in initiating thrombosis in many human diseases such as sickle cell anemia and neoplasia. Increased platelet microparticles have been demonstrated in dogs with IMHA and tissue factor microparticles are shed from canine epithelial neoplasia in vitro.

Infarcts (arterial thrombus formation) in the spleen, renal and cerebral arteries have also been described in association splenic and portal vein thrombosis and pulmonary thromboembolism in dogs. Platelet activation has been demonstrated in dogs with IMHA and in models PTE associated with vegetative endocarditis. Platelet activation has also been demonstrated in dogs with heartworm disease. It is likely platelet activation is a major mechanism initiation of thrombus formation for diseases associated with tuburlent blood flow and a high shear rate such as heartworm disease. For other diseases such as IMHA, where endothelial damage, tissue factor expression, likely play a central role, it is possible that activation of coagulation may be primary with platelet activation occurring secondary to thrombin generation. Alternatively both activation of platelets and coagulation may have equal importance in the pathophysiology of thrombus formation in IMHA.

In people, the pathophysiology of Aortic thrombosis (AT) appears to vary with the underlying cause. Aortic thrombosis may occur secondary to mural damage such as artherosclerosis or aneurysm with associated occulsion of the aorta. However AT is also associated with disorders where there is no underlying intimal lesions. These patients have underlying coagulation disorders. Patients with atrial fibrilliation are also at risk for AT due to venous stasis and activation of coagulation in the left atrium. Aortic thrombosis also occurs in patients with vasculitis from various causes. Thus the mechanism of aortic thrombus formation differs depending on the underlying disease in people.

Aortic thrombosis has been described in dogs with protein losing nepropathy, hypothyroidism, aortic neoplasia, diabetes mellitus, hyperadrenocorticism and trauma and in dogs without any identifiable underlying disease. In cats, aortic thrombosis is a well known complication of hypertropic cardiomyopathy.

Venous stasis and activation of coagulation is believed to play a major role in aortic thromboembolism in cats, with thrombus formation being initiated in the left atrium and subsequent embolization to the distal aorta. Decreased antithrombin levels have been

shown in dogs with PLN and was a common finding in a study of dogs with aortic thrombosis. The authors theorized that endothelial damage, hypercoagulability and hypofibrinolysis contribute to the pathogenesis of AT in dogs. In a study of dogs with PLN, hypercoagulability was demonstrated using thromboelastography. Decreased AT and increased fibrinogen were also documented. However dogs with disease not associated with AT had similar coagulation profiles in that study. The relative roles of activation of coagulation and/or platelet activation in dogs with AT are not yet known but likely vary with the underlying disease process as is the case in people.

Discerning whether coagulation or platelet activation play primary roles or if both contribute significantly and simultaneously to the pathogenesis of thrombus and thromboemboli formation associated with individual diseases is important as the pathogenesis may dictate whether drugs that target platelets or coagulation or both are most appropriate. Studies elucidating the pathogenesis of thrombosis associated with each underlying disease will help direct controlled studies to determine which thromboprophylactic agent will be most effective in an individual patient.

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