Prothrombotic mechanisms and anticoagulant therapy in dogs with immune-mediated hemolytic anemia

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Abstract

Objective: – To review the pathophysiology of thrombosis in hemolytic disease, and the efficacy of thromboprophylaxis in dogs with immune-mediated hemolytic anemia (IMHA).

Data Sources: – Computerized searches of Pubmed, INDEX VETERINARIUS, and the journal database of the Veterinary Information Network, and a manual search of bibliographies of published manuscripts.

Human Data Synthesis: – Experimental data suggest that hemolysis leads to the induction of the potent procoagulant tissue factor on monocytes and endothelial cells and subsequent activation of coagulation. In addition, damaged red cells, activated platelets, and small cell-derived membrane vesicles called microparticles may contribute to coagulation by providing membrane surfaces containing exposed anionic phospholipids that serve as docking sites for prothrombinase (factor Va-factor Xa) and tenase (factor VIIIa–factor IXa) complexes of the coagulation cascade. Some microparticles also contain tissue factor, further fueling coagulation. Thromboprophylaxis for hemolytic disease in people primarily targets the coagulation cascade rather than platelets, as most thromboemboli are of venous rather than arterial origin. The use of unfractionated heparin is closely monitored to ensure therapeutic levels are reached.

Veterinary Data Synthesis: – Thromboembolic disease is a major factor affecting survival in dogs with IMHA. It is likely that hemolysis contributes to the prothrombotic state. Thrombosis occurs in both veins and arteries, with pulmonary thromboembolism (a venous thrombus) occurring very commonly. Evidence suggests that tissue factor mediates the development of the prothrombotic state. Heparin, and the anti-platelet agents aspirin, and clopidogrel have been used for thromboprophylaxis in dogs with IMHA. However, a lack of validated therapeutic endpoints and controlled studies make it difficult to determine if survival is affected or if 1 drug is more effective than another.

Conclusions: – Prospective clinical trials comparing individually adjusted heparin or other anti-coagulant drugs to anti-platelet drugs are needed to make evidence-based recommendations for thromboprophylaxis in dogs with IMHA.

(J Vet Emerg Crit Care 2013; 23(1): 3–13) doi: 10.1111/j.1476-4431.2012.00824.x

Keywords: aspirin, clopidogrel, hemostasis, heparin, microparticle, tissue factor

Abbreviations

APTT	activated partial thromboplastin time
AT	anthrombin
DIC	disseminated intravascular coagulation
GAG	glycosaminoglycans
hIVIG	human intravenous immunoglobulin G
IMHA	immune-mediated hemolytic anemia
LMWH	low-molecular-weight heparin
NO	nitric oxide
PS	phosphatidylserine
PT	prothrombin time
PTE	pulmonary thromboembolism
TF	tissue factor
TFPI	tissue factor pathway inhibitor
tPA	tissue plasminogen activator
UFH	unfractionated heparin
ULDA	ultralow-dose aspirin
vWF	von Willebrand factor

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Introduction

Immune-mediated hemolytic anemia (IMHA) is an important cause of severe anemia, morbidity, and mortality in dogs.[1–5] Complement- or antibody-mediated RBC destruction results in hemolysis during IMHA. Immune-mediated red cell destruction has been associated with the administration of drugs, infectious agents, neoplasia, and other immune-mediated diseases, such as lupus.[6–15] In most patients, the inciting cause is not identified and the immune-mediated anemia is deemed idiopathic. The mortality rate for idiopathic IMHA in dogs is unacceptably high, ranging from 21% to 83%.[1–5,16–19]

Most dogs with idiopathic IMHA die or are euthanized in the acute phase of disease.[4,5,16,20] Thromboembolic disease appears to be a major factor that affects short- and long-term survival.[4,5,16,20,21] The majority of dogs with idiopathic IMHA are in a hypercoagulable state at the time of diagnosis,[17,20,22] and it is likely that hemolysis is an important instigating factor. Therapeutic interventions and supportive measures may further exacerbate the development of thrombosis.[23–28] It is likely that preventing thrombosis is as important as controlling hemolysis if survival rates are to be improved.

The choice of thromboprophylactic agent for human patients at risk for thrombosis is determined by the pathophysiology of thrombus formation for the underlying disease.[29] Drugs like heparin, Coumadin, and direct inhibitors of factor Xa target coagulation. These drugs are used to prevent deep vein thrombosis and pulmonary thromboembolism (PTE) because activation of coagulation is the primary mechanism of venous thrombus formation.[29,30] Drugs that target platelets, such as aspirin and clopidogrel, are used to prevent most types of arterial thrombosis because platelets play a primary role in thrombus formation for these diseases in people.[29,30] In some instances, drugs that target both coagulation and platelets are used.[29] The pathophysiology of thrombosis in dogs with IMHA is the subject of recent studies.[31–34] Heparin, ultralow-dose aspirin (ULDA) and clopidogrel have been used for thromboprophylaxis in dogs with fulminant IMHA.[5,18,20,35,36] However, it is difficult to make definitive conclusions regarding their individual and comparative efficacy due to a paucity of prospective-controlled clinical trials and the high likelihood of inadequate dosing of heparin, and possibly aspirin, in individual patients. The purpose of this review is to discuss potential mechanisms of thrombosis and to review available data regarding the efficacy of thromboprophylaxis in dogs with idiopathic IMHA.

Normal Hemostasis

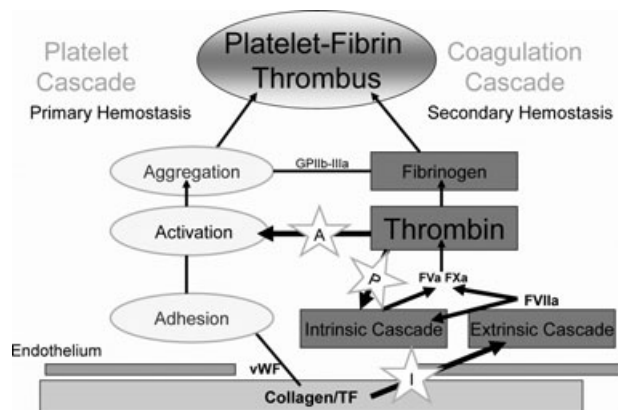Primary hemostasis refers to the formation of the platelet plug, while secondary hemostasis refers to activation of

Figure 1: Formation of a platelet-fibrin thrombus during normal hemostasis. TF, tissue factor, vWF, von Willebrands factor, GPI-IbIIIa, glycoprotein IIbIIIa, F, factor I, initiation, A, activation, P, propagation. See text for explanation.

the coagulation cascade and the formation of a fibrin network. Clinically, it is useful to separate hemostasis into these 2 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 (Figure 1).[37] During hemostasis, a platelet-fibrin clot is formed via 3 overlapping phases, initiation, amplification, and propagation (Figure 1).[29,38,39] The cell-based model of coagulation has been recently reviewed.[39] Endothelial damage initiates the formation of a platelet plug through binding of platelets to subendothelial collagen, which is facilitated by von Willebrand factor (vWf). Tissue factor (TF) within the blood vessel wall simultaneously activates the coagulation protease cascade.[37] The extrinsic cascade is comprised of TF and factor VIIa. The extrinsic cascade initiates coagulation during normal hemostasis and in many prothrombotic states. Tissue factor is normally absent from the vascular space, being expressed by cells surrounding blood vessels such as pericytes and subendothelial fibroblasts.[29,40] The initiation phase of coagulation is localized to TF-bearing surfaces.[29,38] 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, or microparticles.[41,42]

Circulating microparticles are derived from cell membranes of RBCs, platelets, megakaryocytes, endothelial cells, neutrophils, and monocytes.[42–44] 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 the endothelium. Evidence suggests that activated platelets release phosphatidylserine (PS) exposing microparticles.[44] Interestingly, deficiencies in platelet exposure of PS and

microvesiculation result in bleeding tendencies in people and dogs.[45–47] Microparticles derived from monocytes and endothelial cells and possibly platelets also express TF.[41,44] 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 (also referred to as factor IIa).

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[29,38,48] The intrinsic pathway consists of high-molecular-weight kininogen, prekallikrien, and the serine proteases factor XII, factor XI, factor IX, and factor VIII. Thrombin activates factor VIII and factor XI.

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.[29,38,48] 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.[49,50] Although factor XII deficiency does not increase the risk of hemorrhage, factor XII-/- mice have reduced thrombosis in a variety of models.[51]

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.[40,52] Platelets and other cells release microparticles that enhance clotting by providing a membrane surface for the assembly of the prothrombinase and tenase complexes.[43,53] 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, formerly antithrombin III) inhibits factors Xa, IIa, VIIa, IXa, XIa, and XIIa. The activity of AT is dramatically increased after binding heparan sulfate, which is expressed on the surface of endothelial cells.[29]

Fibrinolysis occurs gradually after clot formation. Activated endothelium and monocytes produce tissue plasminogen activator (tPA), which converts plasminogen to plasmin. Annexin A2 on the surface of endothelial cells facilitates the localization of tPA and plasminogen in close proximity to each other. Tissue plasminogen activator activity is dramatically increased for plasminogen bound to fibrin, thus localizing plasmin production to the region of a blood clot. Plasmin is an endopeptidase that cleaves fibrin which destabilizes the clot and results in the production of fibrin degradation products. Plasmin also inactivates factors V, VIII, IX, and XI, cleaves complement component C3, enhances conversion of factor XII to XIIa and conversion of prekallikrein to kallikrein. Inhibitors of plasmin generation and activity include plasminogen activator inhibitors, α-2 antiplasmin, α-2 macroglobulin, and other protease inhibitors.[48,54] Increased procoagulant, decreased anticoagulant, and impaired fibrinolytc activity may shift the hemostatic balance toward thrombosis.

The Pathophysiology of Thrombosis in IMHA

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.[29,30] 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.[50] Generally speaking, antithrombotic 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).[29,30] Some diseases also result in a generalized microvascular thrombosis. Drugs that target the coagulation cascade, like heparin, are used to reduce levels of thrombin and indirectly decrease platelet activation. In some cases, drugs that target coagulation or platelets are used in combination.[29,55]

Pulmonary thromboembolism, thought to occur due to the release of venous emboli, is very common in dogs with IMHA[1,2,5,16,20] In addition, IMHA is a common underlying disease in dogs with cranial vena cava thromboses.[56] Portal vein, cephalic vein, splenic vein, and hepatic vein thrombosis have also been

described.[36,57] The histopathologic description of blood clots is not included in most published reports of dogs with IMHA. However, fibrin-rich thrombi have been documented.[21,58] Although venous thrombi appear to be common in dogs with IMHA, myocardial, splenic, renal, iliac, and mesenteric artery infarction and pituitary thromboembolism have also been described.[1,2,21,36,59,60] This appears similar to the distribution of thromboembolic disease in people with IMHA, where PTE and venous thrombosis commonly occur, although myocardial and cerebral infarctions have also been documented.[26,61,62] Thus it appears that IMHA may cause generalized thromboembolic disease of both veins and arteries, with PTE (a venous thrombus) being a very common clinical manifestation of thrombosis.

The presence of both venous and arterial thrombosis suggests that dysregulation of both coagulation and platelets occurs in dogs with IMHA. Recent studies using thromboelastography have verified that the majority of dogs with IMHA are in a generalized hypercoagulable state.[17,22] Indeed, dogs with IMHA exhibit excessive platelet activation,[31,34,63] and activation of coagulation is evidenced by the common findings of decreased AT, thrombocytopenia, prolongation of activated partial thromboplastin time (APTT) and prothrombin time (PT), elevated D-dimers (a marker of fibrin degradation),[1,16,20,36] and the finding of widespread fibrin deposition at necropsy.[21] Many dogs with IMHA meet the clinical criteria for disseminated intravascular coagulation (DIC).[1,20] Interestingly, the presence of DIC was not a risk factor for thrombosis in 1 study.[1] However, prolongation of PT, suggesting consumption of factors in the extrinsic pathway, is associated with mortality.[16,60]

The extrinsic pathway is essential in normal hemostasis but also plays a role in the formation of pathologic thrombi.[40,64] In healthy animals, TF expression is tissue specific. Tissue factor is not normally expressed on endothelial cells and cells in contact with blood. However, high levels of TF are expressed by cells in the brain, heart, and lungs, perhaps because hemorrhage into these organs would have devastating consequences.[65] It is interesting to note that these organs are commonly affected by pathologic thrombus formation in dogs with IMHA.[1,2,21,36,59,60] Tissue factor expression on the surface of activated monocytes and endothelial cells may initiate intravascular coagulation in pathologic states associated with thrombus formation.[40] In vitro studies, in vivo animal model studies, and some human clinical studies suggest that TF expressing microparticles may also play an important role in pathologic thrombus formation.[42,43]

Studies of humans and laboratory animals suggest that activated endothelium plays a central role in throm-

bosis associated with hemolytic disease (Figure 2). Free hemoglobin results in the scavenging of nitric oxide (NO).[66] Reduced levels of NO may contribute to increased TF expression in the pulmonary endothelium of mice with hereditary sickle cell anemia and human endothelial cells in vitro.[67,68] Sequestration of NO by plasma hemoglobin also results in platelet aggregation.[66] Cytokine-induced expression of TF on the surface of endothelium is believed to play a role in initiating coagulation in human patients with IMHA.[61] Monocytes obtained from patients with sickle cell anemia have been shown to activate endothelial cells and induce TF expression.[69] Phagocytosis of RBCs is hypothesized to be the mechanism of monocyte activation in these patients. A recent study showed that cytokines associated with macrophage and monocyte activation were significantly more elevated in dogs with IMHA who died as compared to survivors.[70] In addition, whole blood tissue factor gene expression is upregulated in dogs with IMHA.[33] It is possible that inflammatory cytokines, activated monocytes, and free hemoglobin induce endothelial cell TF expression, and that TF expressing microparticles derived from monocytes bind to endothelium and contribute to thrombosis in dogs with IMHA.
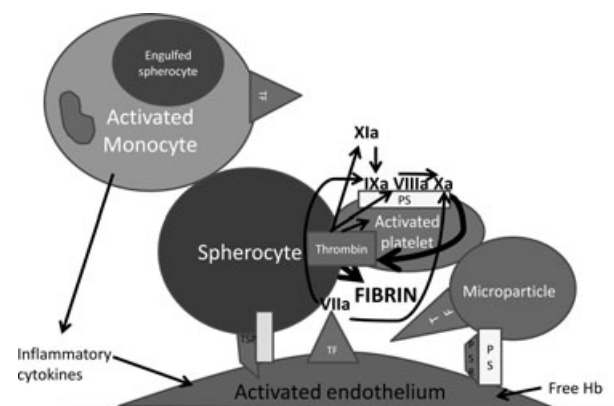

Figure 2: Proposed mechanism of thrombosis in IMHA. TSP, thrombospondin, PSR, phosphatidylserine receptor, PS, phosphatidylserine, TF, tissue factor, Hb, hemoglobin. Tissue factor expression is induced on the surface of vascular endothelium by inflammatory cytokines or free hemoglobin. Tissue factor expression on the surface of activated endothelium, monocytes, platelets, and microparticles initiates coagulation via the extrinsic cascade. Amplification and propagation occur as in normal hemostasis. Surfaces of activated platelets, spherocytes, and microparticles express adhesion proteins that localize surfaces bearing phosphatidylserine to endothelial receptors and the site of thrombus formation. Anionic phospholipids such as phosphatidylserine also provide binding sites for prothrombinase and tenase complexes, and may contribute to vascular occlusion.

Normal membranes have an asymmetric distribution of phospholipids, with anionic phospholipids, such as PS located in the inner leaflet. Loss of asymmetry and exposure of PS on the outer surface occur after senescence and activation of RBC and platelets, respectively.[47] Activated platelets, damaged RBC and microparticles are procoagulant because they have anionic phospholipids, such as PS, on their surfaces.[43,53,71,72] Anionic phospholipids facilitate assembly of the prothrombinase and tenase complexes.[47] PS-positive RBCs also bind to macrophage PS receptors. PS receptor-mediated binding induces signaling that results in phagocytosis. Recent in vitro studies have shown that similar PS receptors are constitutively expressed on the surface of endothelial cells.[73] Endothelial cell PS receptor expression is upregulated in response to inflammatory cytokines, hypoxia, and free hemoglobin.[67] Erythrocyte PS also binds to thrombospondin, a protein that is present in subendothelial matrix.[74] The importance of PS in normal hemostasis is illustrated by Scott syndrome. This bleeding disorder has been described in dogs and people and is caused by failure to transport PS to the surface of activated platelets and to shed membrane-derived PS expressing microparticles.[45,47]

Increased PS expression on the surface of RBCs occurs in hemolytic diseases associated with thrombosis, including sickle cell anemia, beta-thalassemia and hereditary spherocytosis.[47,75] Reticulocytes also express increased levels of cell surface PS, and it has been postulated that reticulocytosis contributes to the risk of thrombosis in hemolytic disease.[76,77] The risk of thrombosis is correlated with the magnitude of PS expression on the surface of RBC in humans with sickle cell anemia but not in mice with hereditary spherocytosis.[75,78] In vitro studies show that PS is an important mediator for RBC binding to thrombospondin in sickle cell anemia, but that other ligands mediate RBC binding in hereditary spherocytosis.[78,79] Thus, the mechanism of RBC binding to endothelium and subendothelial matrix may vary with the pathophysiology of the underlying disease. It is possible that reticulocytosis or antibody-induced spherocyte formation results in excessive RBC binding to endothelial cells, thrombosis, and vascular occlusion in human or canine patients with IMHA (Figure 2). Interestingly, the absence of reticulocytosis was associated with an increased risk of death in 1 study of dogs with IMHA.[2] The reason for the increased mortality in these patients is unknown, but may reflect a poorer prognosis when immune-mediated destruction is directed at red cell precursors.

Microparticles derived from activated platelets and RBCs have PS exposed and may play an important role in facilitating thrombosis.[80,81] Microparticles derived from monocytes and endothelial cells also express TF that can further activate endothelium and the extrinsic coagulation pathway.[42,43,80] High circulating levels of microparticles are associated with thrombosis in people with sickle cell anemia and other diseases, such as cancer.[43,44,82] Triggers for increased RBC microvesiculation include complement attack, sheer stress, and oxidative injury, conditions that are present in dogs with IMHA.[32,43] Circulating RBC, endothelial or WBC microparticle levels have not been measured in dogs with IMHA, however platelet microparticles were found to be increased in 1 study.[63] It is possible that microparticles contribute to the prothrombotic state and fibrin formation in these patients (Figure 2). Microparticles, along with agglutinating red cells may also contribute to the direct occlusion of vasculature and worsening thrombosis. Autoagglutination is common in dogs with IMHA and is associated with decreased survival in some[3,5,10] but not other studies.[1]

The excessive activation of coagulation in dogs with IMHA is accompanied by decreased levels of anticoagulants. Decreased AT activity is common in these patients.[20,58] Decreased AT likely contributes to the prothrombotic state, although it is of interest that no association with AT activity and mortality was found in 1 study.[20] Impaired fibrinolysis, characterized by increased levels of plasminogen activator inhibitors, antibodies to annexin A2, and other mechanisms, contribute to the prothrombotic state in some thrombotic diseases in people.[54,83] Whether decreases in inhibitors of coagulation other than AT, or an imbalance of the fibrinolytic pathways occurs in human or canine patients with IMHA has not been investigated.

Therapy and supportive measures used in the treatment of dogs with IMHA may also contribute to thrombosis. Microparticles are cleared by phagocytic cells in the spleen that contain receptors for PS or opsonins on the microparticle surface, such as complement.[43] It is possible that immunosuppressive and immunomodulatory therapy directed at inhibition of phagocytosis in patients with IMHA might increase levels of circulating microparticles and contribute indirectly to the prothrombotic state. Indeed, splenectomy and human intravenous immunoglobulin G (hIVIG) are associated with an increased risk of thrombosis in people, and hIVIG administration is prothrombotic in normal dogs.[23,24,84] However, in 1 retrospective study describing splenectomy as an adjunctive therapy for 10 dogs with idiopathic IMHA, no clinical signs of thromboembolic events were observed after splenectomy was performed and 9/10 dogs were alive at 30 days.[85] One dog died during the perioperative period but the circumstances surrounding the death of this dog were not described.

Drugs used in the treatment of IMHA may exacerbate thrombosis by other mechanisms. Glucocorticoids

increase circulating levels of some coagulation factors and decrease fibrinolysis.[86] However, whether glucocorticoids are prothrombotic appears to depend on the underlying disease process in people.[86] It is not known if glucocorticoids are prothrombotic in human patients with IMHA. However, in other human diseases associated with pro-inflammatory states, it appears that they do not exacerbate thrombosis.[86] Thrombotic events are a well-known complication of cyclosporine use in people.[87] Recent in vitro evidence suggests that cyclosporine can induce PS exposure on the surface of RBCs and that the procoagulant effect of cyclosporine can be reduced by blocking PS expression.[28] Whether the use of glucocorticoids or cyclosporine in dogs with IMHA contributes to the risk of risk of thrombosis is not known. Other supportive measures commonly used in the treatment of dogs with IMHA, such as the use of IV catheters, have been associated with thrombosis in people and in dogs.[27,56]

Thromboprophylaxis in IMHA

Unfractionated heparin, ULDA, and clopidogrel have been used as thromboprophylactic agents in dogs with IMHA.[5,18,20,35] Given that venous thrombi and PTE are common, fibrin-rich thrombi have been documented, and TF-induced activation of coagulation likely initiates thrombus formation in hemolysis, the use of drugs that target coagulation is a logical choice for dogs with IMHA. Indeed, heparin and Coumadin are commonly used as thromboprophylactic agents in human IMHA patients at risk for thrombosis, and heparin is the standard of care for prevention of deep vein thrombosis and PTE.[26,29,61]

Unfractionated heparin facilitates the AT-mediated inactivation of thrombin and factor Xa. Factors IXa, XIa, VIIa, and XIIa are also inactivated by this complex.[88,89] Heparin also facilitates release of TFPI from endothelial cells.[88] In addition, in vitro studies using RBCs taken from people with sickle cell disease have shown that PS binds to the heparin-binding domain of the endothelial cell matrix protein, thrombospondin. Clinically relevant doses of heparin inhibit this binding in a concentration-dependent manner.[74] Heparin also inhibits complement. Complement inhibition is thought to contribute to the anti-thrombotic effects of heparin in women with antiphospholipid antibody syndrome.[90] Heparin inhibits hemolysis-induced thrombin generation in vitro.[91] Thus, heparin appears to target many of the pathways that might contribute to the prothrombotic state in IMHA.

Importantly, UFH heparin therapy requires diligent and appropriate monitoring for each individual patient. Monitoring therapy based on anti-factor Xa activity or APTT is standard in human medicine.[92] Standardized dosing results in highly variable levels of anticoagulation

in normal dogs and individualized monitoring has been recommended.[93] Unfortunately, what constitutes appropriate monitoring has not been established for dogs with IMHA and is not consistently performed in studies or in clinical practice. This has led to difficulties in determining whether heparin is efficacious or even detrimental in these patients. Measuring anti-factor Xa activity in individual patients is the gold standard for determining whether therapeutic UFH heparin levels are reached in human medicine. In human medicine APTT ratios are correlated with, and can be substituted for measuring anti-factor Xa activity after appropriate calibration.[94] Targeting therapy to prolong APTT to 1.5–2.5× control values is an established physiologic endpoint in human patients receiving UFH. APTT is predictive of clinical outcome in human patients with venous thrombosis treated with UFH but its predictive value with other thrombotic disease may not be as consistent.[95] The range of anti-factor Xa activity needed to prevent thrombosis in dogs with IMHA has not been established. Furthermore, validated anti-factor Xa activity assays for dogs are not available through most major veterinary diagnostic laboratories. Target anti-factor Xa activity level in canine patients has been extrapolated from human studies and laboratory animal studies that correlate the occurrence of venous thrombosis with anti-factor Xa activity. In an in vitro study using dog plasma spiked with heparin, APTT prolongation to 1.5–2.5× the mean APTT resulted in anti-factor Xa activity within a therapeutic range reported for humans,[96] and in a study of normal dogs treated with heparin, APTT correlated with anti-factor Xa activity.[97] However, predicting whether plasma anti-factor Xa activity is within a target range based on prolongation of APTT in individual normal dogs is difficult.[93,98] Furthermore, the relationship between APTT and heparin activity in normal dogs depends on the reagent used in the APTT assay.[99] This is similar to the case in people, where a therapeutic heparin concentration can result in an APTT ratio of 1.6–6.2, depending on the reagent used in the APTT assay.[100]

In diseased patients, APTT can be affected by additional factors.[92] High fibrinogen levels, commonly present in dogs with IMHA, can interfere with the assay.[20,96] Large doses of UFH are required to prolong APTT in the presence of high fibrinogen.[96] The presence of antiphospholipid antibodies, which occur in some human patients with IMHA, artificially prolong APTT.[94] antiphospholipid antibodies do not appear to play a large role in the pathogenesis of canine IMHA.[20] Increased circulating levels of factor VIII can also shorten APTT compared to anti-factor Xa activity.[95] Glucocorticoids, commonly used to treat IMHA, increase factor VIII levels, however, their effect on APTT in dogs with IMHA is not known.[86] Unlike anti-factor Xa

activity, APTT prolongation is dependent on thrombin activity. The anti-thrombin activity of UFH is due to the presence of high-molecular-weight multimers that are large enough to complex with AT and thrombin. Lower-molecular-weight multimers have anti-factor Xa activity but have a reduced ability to complex with thrombin. High-molecular-weight multimers are metabolized more rapidly than low-molecular-weight multimers. Therefore, APTT values may suggest that heparin levels are subtherapeutic when anti-factor Xa activity, due to the persistence of low-molecular-weight multimers, is adequate.[95] In 1 study of dogs with IMHA, adjusting heparin doses based on prolongation of APTT to 1.5–2× baseline (or high end of the reference range if baseline values were prolonged), did not appear to correspond to anti-factor Xa activity within the established therapeutic range.[58] However it was difficult to determine if target APTT ratios were reached, as they were not described for most of the dogs in that study. Only 1 dog had anti-factor Xa activity within the target range, and that dog's APTT was described as within the reference range. In contrast, the natural log of APTT was linearly related and strongly correlated with anti-factor Xa activity in another study of dogs with IMHA.[36] In a study of dogs with a variety of underlying diseases that increase the risk of thrombosis, the effect of high doses of UFH on APTT prolongation as compared to anti-factor Xa activity was unpredictable.[101] Taken together, these studies illustrate that adjusting heparin therapy based on APTT prolongation does not consistently correlate with anti-Xa activity in dogs.

Monitoring heparin therapy is also important due to the risk of bleeding associated with its use. However, it appears that the risk of bleeding associated with UFH therapy in dogs with IMHA might be less than in normal dogs. Although doses of 500 IU/kg SQ BID cause hemorrhage in some normal dogs, hemorrhage was not observed in dogs with IMHA receiving doses from up to 725 IU/kg TID to QID.[35,98] Reduced AT or bioavailability of UFH may explain the lack of hemorrhagic tendencies in dogs with IMHA. It should be noted that dogs with severe thrombocytopenia or markedly prolonged APTT were excluded from these studies and that the risk of hemorrhage from heparin therapy would be expected to be increased in these patients as it is in people.[35] Supplementation of AT also decreases the dose of heparin required for anticoagulation in people.[95] Studies correlating target range of anti-factor Xa activity, APTT, AT activity, and the physiologic outcome of thrombosis in dogs with IMHA treated with UFH are necessary to establish appropriate guidelines for therapy. It is clear that administering UFH to dogs with IMHA without monitoring is not appropriate.

Low-molecular-weight heparin (LMWH) has been suggested as an alternative to UFH in dogs because of more predictable pharmacokinetics and similar efficacy and safety as compared to individually adjusted UFH therapy in people with deep vein thrombosis and other thrombotic disease.[88] This translates to a reduced need for monitoring anti-factor Xa in most human patients, although monitoring is still recommended for critically ill patients.[88,92] APTT is not used to monitor LMWH because the smaller heparin molecules are not large enough to inhibit thrombin while complexed to AT. Many veterinary practitioners administer a standardized dose of LMWH to dogs based on human pharmacokinetic data or anecdote. However, it has been shown that the pharmacokinetics of enoxaparin in normal dogs differs from people.[102] In addition, dalteparin administered at a commonly used dose to dogs at risk for thrombosis failed to increase anti-factor Xa activity from baseline.[101] Furthermore, high doses of dalteparin were required to achieve target anti-factor Xa activity that corresponded to halting consumption in a canine model of DIC.[103] Establishing that target anti-factor Xa activity has been reached during therapy for individual canine patients receiving LMWH has been recommended.[102] Determining whether LMWH decreases the risk of thrombosis in dogs with IMHA and an appropriate target range to achieve this goal requires further study.

By targeting coagulation, heparin indirectly inhibits platelet activation. Platelet activation is increased in dogs with IMHA.[31,34,63] The mechanism of platelet activation is not known, although it is likely that endothelial cell activation and thrombin-induced activation play a role. Ease of use and lack of need for intensive monitoring make platelet inhibitors, such as aspirin and clopidogrel, attractive thromboprophylactic agents for dogs with IMHA. Aspirin is a cyclooxygenase inhibitor. Thromboxane A_2 is produced by platelets in a COX-1 dependent manner. Thromboxane A_2 is a potent platelet agonist.[104] Ultra low doses of aspirin inhibit platelet thromboxane A_2 production while preserving COX-1 dependent prostacyclin (which is antithrombotic) production from endothelial cells.[104] The dose of aspirin needed to achieve this effect in normal dogs or dogs with IMHA has not been established. The dose of 0.5 milligrams of aspirin per kilogram per day has been shown to inhibit normal canine platelet aggregation in vitro when ADP and collagen are used as agonists.[105] However up to 30% of dogs did not exhibit an anti-platelet effect depending on the parameter measured, suggesting that the dose may be inadequate to inhibit platelet function in some individuals. Another study showed that dosing every 12 hours was more effective at inhibiting platelet aggregation than administering aspirin every 24 hours in normal dogs

using this dose.[106] A dose of 0.5 mg/kg of aspirin/day does not increase the risk of gastrointestinal ulceration in normal dogs treated with immunosuppressive doses of prednisone.[107] Whether concurrent use of prednisone and aspirin in dogs with IMHA increases the risk of gastrointestinal ulceration has not been determined, however anecdotally it does not appear to be common. No prospective studies comparing ULDA to no thromboprophylaxis have been performed. In a recent study where 29/30 dogs with idiopathic IMHA received ULDA (0.5 mg/kg/day) 58.6% were alive 6 months later.[17]

Clopidogrel has been shown to inhibit platelet aggregation in dogs.[108a] Clopidogrel irreversibly inhibits the platelet P2Y12 ADP receptor. ADP is a potent activator of platelets.[104] A recent prospective study showed that dogs with IMHA treated with clopidogrel had comparable short-term survival to dogs treated with ULDA (0.5mg/kg/day) or clopidogrel combined with ULDA.[18] The overall mortality rate at 90 days for this study was 21%, and thrombotic events were suspected or confirmed in 3/5 fatalities.

Whether UFH is a superior thromboprophylactic agent to ultralow-dose aspirin or clopidogrel for canine IMHA is not clear. The authors of a retrospective study using historical controls concluded that dogs receiving azathioprine, prednisone, and UFH had reduced survival compared to patients receiving azathioprine, prednisone, and ULDA, or patients receiving azathioprine, prednisone, UFH, and ULDA.[5] Of note, the dose of UFH used in that study was 75 U-125 U/kg SQ q 6–8 h. No adjustment in the dose was made based on APTT or anti-factor Xa activity.[5] A dose of 200 IU/Kg SQ q 6 h of UFH results in target anti-factor Xa activity and APTT prolongation in normal dogs.[97] However, recent studies have shown that 300 IU SQ/kg q 6 h resulted in more than half of dogs with IMHA having anti-factor Xa activity below the target range.[36] Furthermore, it appears that the dose of heparin required to reach target anti-factor Xa activity is highly variable in dogs with IMHA. A prospective controlled clinical trial by Helmond et al.[35] showed that doses ranging from 150 U/kg to 566 U/kg QID were necessary to achieve target anti-factor Xa activity of 0.35–0.7 U/mL in individual dogs.[35] In the Helmond study, survival was significantly greater in dogs given individually adjusted doses compared to a constant dose of 150 U/kg QID. Only 1/8 dogs died in the individually adjusted dose group while 6/7 dogs in the constant dose group died. The authors hypothesized that differences in the dose required for target anti-factor Xa activity in normal dogs compared to dogs with IMHA may be due to reduced bioavailability due to the presence of inflammatory mediators. Indeed, the bioavailability of UFH

is affected by binding to numerous circulating proteins, endothelial cells, and macrophages.[88]

Reduced levels of AT also contribute to heparin resistance because the anticoagulant effects of heparin are dependent on AT. AT activity is commonly decreased in dogs with IMHA.[1,20] Heparin resistance in human patients undergoing cardiopulmonary bypass is commonly caused by decreased AT activity.[95] Inadequate anticoagulation despite large doses of UFH is corrected by administration of AT concentrate, or, less ideally, fresh-frozen plasma in these patients.[95]

In contrast, simultaneous administration of AT and heparin is more controversial for human septic patients. Evidence suggests that AT has anti-inflammatory effects by increasing prostacyclin production, inhibiting leukocyte adherence, and decreasing cytokine production through binding glycosaminoglycans (GAG) on the surface of endothelial cells and leukocytes. Heparin may interfere with these anti-inflammatory effects by competing for the GAG-binding sites.[89,110]. Concomitant low-dose standardized heparin administration was believed to abrogate the beneficial effects of supraphysiologic (180% of normal) standardized doses of AT in a phase III clinical trial (the Kybersept trial) of septic human patients.[89,110,111] Whether these findings would be relevant for septic patients given heparin and AT with the goal to maintain AT in the physiologic range to optimize AT's anticoagulant rather than anti-inflammatory effects was not addressed by the study design. In 1 study of dogs with IMHA treated with heparin adjusted to maintain APTT at 1.5–2.5 baseline or control (if baseline values were outside the reference range), administration of 1 dose of 10 mL/kg fresh-frozen plasma had no effect on AT activity or thromboembolic complications.[58] Determining the effect of maintaining AT at physiologic levels on heparin resistance requires further study.

Although AT levels were not measured, the Helmond study suggests that individualizing heparin doses in dogs with IMHA may help overcome heparin resistance and improve outcome significantly in these patients. Furthermore, preliminary data from a retrospective study of dogs with IMHA suggest that individualized heparin dosing adjusted based on anti-factor Xa activity may reduce thrombotic events and mortality compared to low-dose aspirin.[b] Prospective studies comparing IAD UFH with clopidogrel or aspirin for dogs with IMHA are needed.

Because canine IMHA appears to be a mixed thromboembolic disease, heparin combined with ULDA may also be useful. Indeed, therapies that target both platelet function and coagulation are used in some prothrombotic human patients.[29,112] However, the risk of hemorrhage is increased compared to the use of either therapy

alone, and close monitoring is required. Potentially safer, orally administered inhibitors of coagulation are being used in select human patients at risk for thrombosis.[92,113] Inhibitors of Factor Xa and Factor XIIa and direct inhibitors of thrombin that act independently of AT are also being investigated.[30,92] Therapies that target microparticles are also being developed.[30,114] Further research investigating the mechanisms of thrombosis in dogs with IMHA along with prospective-controlled clinical trials using monitoring methods that are appropriately correlated with clinical outcomes may lead to more effective prophylactic antithrombotic therapies and improve survival in dogs with this devastating disease.

Future Studies/Changes in Practice

Hemolysis may lead to activation of coagulation by macrophage cytokine-induced TF expression with activation of the extrinsic cascade. Abnormal red cells, activated platelets, and microparticles may promote venous stasis and coagulation by providing PS-positive cell surfaces for coagulation and directly causing vascular occlusion. Platelet activation also occurs. It is likely that drugs that target coagulation and platelets can help prevent thrombosis in these patients. However, it is clear that standardized doses of heparin do not result in therapeutic drug levels in most dogs with IMHA. Therefore, conclusions regarding the efficacy of heparin in preventing thrombosis in these patients cannot be made from the current body of veterinary literature. Prospective clinical trials comparing IAD heparin (based on anti-factor Xa activity) to anti-platelet drugs are needed to make definitive evidence-based recommendations regarding appropriate thromboprophylactic therapy in these patients. Newer anticoagulant drugs such as direct inhibitors of factor Xa may also hold promise in increasing survival.

Footnotes

[a] Haviland RL, Pacifico N, Bianco D. Clopidogrel therapy in dogs with immune mediated hemolytic anemia (Abstr.) J Vet Internal Med 2009; 23: 745

[b] Orcutt E, Armstrong P, Helmond S, Smith S. Comparison of individually monitored unfractionated heparin versus low-dose aspirin on survival of dogs with immune mediated hemolytic anemia (Abstr.) J Vet Internal Med 2009 23: 693.

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