Immune-mediated hemolytic anemia (IMHA) is a condition in which autoantibodies bind to endogenous antigens on the erythrocyte membrane, leading to complement or antibody mediated red blood cell destruction. These antigens can be created as part of the normal immune response to an abnormal antigen (i.e. band-3, peroxiredoxin-2, or epitope created by an infectious disease) or due to an abnormal immune response to a normal antigen. Agents that have been identified to lead to the formation of new epitopes on the surface of red blood cells include drugs, (ex. sulfonamides) infectious agents (ex. Babesia spp, Ehrlichia spp, Anaplasma phagocytophilum), vaccination and neoplasia. Recent studies, however, have failed to confirm an association between recent vaccination and the development of IMHA in dogs. In most cases (approximately 70%), an underlying cause is not identified and the dog is classified as “idiopathic” or having primary IMHA. Thromboembolism is the most common and severe manifestation of the disease process, with studies identifying both arterial and venous thromboembolism on necropsy. The highest mortality rate occurs in the acute phase of the disease process and thromboembolic events are a major contributor to the mortality rate.

Studies in dogs with IMHA have demonstrated increased platelet activation as well as changes in traditional assessments of coagulation such as PT/PTT, fibrinogen, D-dimers and antithrombin. These tests are useful for identifying dogs that are hypocoagulable, however, most dogs with IMHA are in a hypercoagulable state, as demonstrated through the use of thromboelastography. Some factors that contribute to the prothrombotic state in dogs with IMHA include: activation of coagulation, decreases in anticoagulants, impaired fibrinolysis and treatment.

Key factors in the activation of coagulation in dogs with IMHA include the expression of tissue factor (TF) on the surface of monocytes, microparticles and the endothelium. Microparticles are small vesicles of membrane derived from other cell types (i.e. erythrocytes, platelets, leukocytes) that have undergone apoptosis or activation. Activation of TF allows binding to factor VII, forming the FVII:TF complex, which initiates coagulation via the extrinsic pathway. Multiple factors contribute to the expression of tissue factor on the cell membrane. The phagocytosis of red blood cells (as occurs during IMHA) activates monocytes, releasing cytokines. These cytokines increase TF expression on the endothelium. The destruction of red blood cells also leads to an increase in free hemoglobin, which can decrease nitric oxide production from the endothelium (promoting platelet aggregation and vasoconstriction) and promote TF expression on the endothelium. Tissue factor on the surface of microparticles (derived from monocytes) can also bind directly to the endothelium and possibly also contribute to thrombosis.

Another key factor in the hypercoagulable state in dogs with IMHA is the creation of a procoagulant membrane surface. When the membranes of erythrocytes, platelets and the endothelium are activated by hypoxia, microparticles and other damaged cells, enzymes move anionic phospholipids to the outer surface of the membrane. Anionic phospholipids can
bind to the positively charged domains of factors IIa, FVIIa, FIXa and FXa, initiating coagulation.\textsuperscript{30} Microparticles from platelets and red blood cells also express anionic phospholipids, initiating coagulation in a similar way to intact cells.\textsuperscript{24,29}

In addition to the activation of coagulation, dogs with IMHA also have decreased levels of anticoagulants and changes in fibrinolysis. Decreases in antithrombin can be secondary to consumption (from the generation of thrombin), decreased production (acute phase protein), loss (through the urinary or gastrointestinal tract) or degradation.\textsuperscript{31} The conversion of plasminogen to plasmin allows the breakdown of fibrin into fibrin degradation products. Increased levels of plasminogen activator inhibitor (PAI-1), which inhibits fibrinolysis, has been documented in some humans with antiphospholipid syndrome.\textsuperscript{32} Whether or not increases in PAI-1 occur in dogs with IMHA requires additional investigation.

The agent used for thromboprophylaxis depends on the pathophysiology of clot formation. In conditions of low shear stress (i.e., venous circulation), thrombi consist primarily of fibrin and red blood cells due to the activation of coagulation.\textsuperscript{33} Anticoagulants such as unfractionated heparin (UF), low-molecular weight heparin (LMWH), Coumadin and newer direct Xa inhibitors are a logical choice in this situation.

Heparin binds to antithrombin (AT) via a high affinity pentasaccharide that is present on approximately 1/3 of heparin molecules.\textsuperscript{34} Molecules of heparin with <18 saccharides (such as low-molecular weight heparin) lack sufficient chain length to bind to both AT and thrombin, and therefore cannot inhibit thrombin.\textsuperscript{35} These smaller molecular weight heparin fragments (LMWH) mediate their anticoagulant effects via AT-mediated factor Xa inhibition.\textsuperscript{36} Inactivation of thrombin has many different effects that limit widespread coagulation, platelet activation as well as immunomodulation. Heparin-mediated thrombin inhibition decreases fibrin formation, inhibits thrombin-mediated platelet activation, and inhibits the formation of factors Va and VIIIa.\textsuperscript{37} Heparin limits production of factors Va and VIIIa because thrombin is required for cleavage of factor VIIIa from von-willebrand factor (vWF) and for the generation of factor V in the amplification process of coagulation.\textsuperscript{38} Heparin therapy also inactivates factors IXa, XIa and XIIa in addition to factors IIa (thrombin) and Xa. Unfractionated heparin also facilitates the release of tissue factor pathway inhibitor (TFPI) from the endothelium, which inhibits the actions of factors X, II and the VII/TF complex.\textsuperscript{22} Finally, heparin can also inhibit complement activation, which is another pathway that contributes to thrombosis in dogs with IMHA.\textsuperscript{22}

Dogs on heparin therapy should have appropriate monitoring, which has its limitations in clinical practice. In human medicine, heparin therapy is monitored by evaluating PTT or more commonly, anti-Xa activity. When PTT is utilized, targeting 1.5–2.5X baseline values is an established endpoint and is predictive of outcome in humans with venous thromboembolism.\textsuperscript{34} The dose of heparin required to prevent venous thromboembolism in dogs has yet to be established.\textsuperscript{39} Another method of monitoring heparin therapy is by measuring anti-factor Xa activity. While monitoring the inhibition of factor Xa makes physiologic sense, there are many problems with this method of monitoring in veterinary medicine. The therapeutic range used in veterinary medicine (0.35–0.7 U/mL) has been extrapolated from human medicine and has not been validated in a canine model to prevent thrombosis.\textsuperscript{15,40} Only a small number of laboratories offer anti-factor Xa testing, and the results are not available immediately, questioning the practical use of this monitoring method in dogs.\textsuperscript{41} Another problem with these monitoring techniques is that PTT does not consistently strongly correlate with therapeutic anti-factor Xa activity in dogs with inflammatory disease.\textsuperscript{15,40-42} In a study by Diquelou et al in which healthy dogs were given 200 U/kg UF subcutaneously, prolongations of PTT by 120–160% corresponded to anti-Xa values of 0.3–0.7 U/mL.\textsuperscript{43} These results have not been supported in clinical cases of dogs with IMHA. In one study, only 1/13 dogs
reached therapeutic anti-factor Xa activity by 48 hours of treatment, and anti-Xa activity did not correlate with PTT. In another study, the only dose of UFH that increased anti-factor Xa activity to 0.3–0.7 U/mL was associated with marked prolongations in PTT and hemorrhage in 4/6 dogs. There is some evidence that individualized dosing protocols and adjusting heparin therapy based on anti-factor Xa activity may improve case mortality and decrease the number of thromboembolic events in dogs with IMHA.

Another potential drawback of heparin therapy is the risk of bleeding. The risk of bleeding in dogs with a normal platelet count who have not developed a consumptive coagulopathy appears to be much less than normal dogs, as dosages up to 712 U/kg q6–8hr did not result in hemorrhage in one study of dogs with IMHA. Doses of 200–300 U/kg of unfractionated heparin subcutaneously have been shown to adequately prolong PTT in normal dogs. Possible explanations for this increase in dose of heparin required in dogs with IMHA include decreases in AT, increased heparin clearance and decreased bioavailability of heparin, which is highly protein bound to acute phase proteins that are increased in dogs with IMHA.

Low molecular weight heparin (LMWH) has a similar mechanism of action to UFH, however, the polysaccharide chain is long enough to interact and inhibit factor Xa, but not thrombin. Since LMWH does not mediate its pharmacologic effects via thrombin inhibition and does not bind to other proteins or endothelial cells it has more predictable pharmacokinetics than UFH. While many humans prescribed LMWH are not monitored, anti-factor Xa testing is recommended in critical patients. LMWH has similar problems to UFH in that the dose required to prevent thrombosis is unknown, and anti-factor Xa monitoring is difficult in veterinary patients. Given the lack of standardized and readily available monitoring, subtherapeutic dosing is common.

Oral anticoagulants such as warfarin, inhibit recycling of Vitamin K, thus preventing the carboxylation of the glutamic acid residues of factors II, VII, IX, X and anticoagulants proteins C, S and Z. Similar to heparin, warfarin has unpredictable pharmacokinetics and pharmacodynamics and bleeding is a complication. Dosing is also based on an international normalized ratio (INR), which takes into account variations in the laboratory thromboplastin reagent as well as the laboratory technique in obtaining PT. The recommended therapeutic range for INR in humans depends on the condition, but varies between 2–3. No value has been validated to prevent thromboembolism in veterinary medicine.

Another class of drugs that target factor Xa is the direct factor Xa inhibitors Rivaroxaban and Apixaban. These drugs are orally administered and are unique in that they do not require AT for activity. In human studies, these drugs have proven to be non-inferior to warfarin and LMWH for the prevention of venous thromboembolism in humans undergoing orthopedic procedures, for long-term prevention of venous thromboembolism and for prevention of stroke in humans with non-valvular atrial fibrillation. The effects of rivaroxaban on coagulation parameters have been investigated experimentally in healthy dogs and cats, however, its clinical use has been limited. As more studies emerge on the pharmacokinetics, dynamics and safety of these medications in healthy and critically ill patients, the use of these medication is likely to increase.

Under high sheer stress conditions (i.e., arterial circulation), thrombi form secondary to platelet activation and are considered “platelet rich.” Therefore, strategies to prevent platelet aggregation include direct agonist receptor inhibitors (i.e., Clopidogrel), indirect intracellular platelet inhibitors (i.e., aspirin) and newer integrin $\alpha_{IIb}\beta_3$ inhibitors.

Aspirin exerts its pharmacological effect by inhibiting cyclooxygenase, which halts the production of thromboxane A2, a powerful platelet agonist. Ultra-low dose aspirin (0.5 mg/kg
q12–24 hrs) inhibits thromboxane production, while sparing endothelial production of prostacyclin, which limits platelet aggregation. There are several studies evaluating the effect of aspirin on normal platelet aggregation in healthy dogs. Two studies have identified that doses of 0.5 mg/kg every 12–24 hours was effective in inhibiting platelet aggregation. Ultimately, the optimum antithrombotic dose in dogs with IMHA is unknown and prospective, randomized controlled trials are needed before conclusions can be drawn about dose, use with other antithrombotic medications and outcome. Varying results in regards to aspirins antiplatelet effect is likely due to methodology (use of whole blood vs. platelet rich plasma, agonist used), breed variability and aspirin resistance.

Clopidogrel (Plavix®) is a prodrug that is activated in the liver by cytochrome P450 enzymes. Its metabolite irreversibly binds to the ADP receptor on the platelet surface and decreases the release of granule contents that initiate platelet aggregation (ADP, serotonin, fibrinogen, vWF) and decreases ADP mediated activation of the fibrinogen receptor. A study by Brainard (2010), demonstrated that Clopidogrel at doses of 1 mg/kg/day significantly reduced ADP induced platelet aggregation at 60–180 minutes post dosing.

There have been a few studies comparing different antiplatelet and anticoagulant medications for treatment of dogs with IMHA. A study by Orcutt et al, evaluated individually dosed UFH (based on anti-Xa activity) compared to ULDA (0.5 mg/kg/day) and found no significant difference in survival or the number of thromboembolic events between the 2 treatment groups. A recent study by Mellett et al (2011), found no significant difference in survival, number of hemorrhagic events or transfusion requirements between patients treated with clopidogrel, clopidogrel plus aspirin or aspirin alone.

Given the lack of evidence for a superior treatment, Michigan State University is currently performing a prospective study of dogs with IMHA that are randomized to receive heparin or aspirin in hospital and are discharged with aspirin for continued thromboprophylaxis at home. In dogs that do not have prolongations in PT/PTT (a criteria for inclusion), we initiate treatment with either heparin (100 U/kg IV bolus, followed by a CRI of 30–60 U/kg/hr) or aspirin (0.5 mg/kg PO q12). PTT is monitored daily and adjusted daily to achieve a 1.5–2.5x prolongation in PTT. At discharge, dogs are prescribed aspirin at 0.5 mg/kg q12, which is continued throughout the treatment period (weaning over 5–6 months). In dogs that have a prolonged PTT (and are not included in the study) and a reasonable platelet count, we typically institute either aspirin (0.5 mg/kg q12 hr) or clopidogrel (2 mg/kg/day) therapy. In dogs with documented thromboembolic disease, we begin treatment with an antiplatelet and anticoagulant drug and/or institute thrombolysis.

References


