

# Hypercoagulation in Canine IMHA

Ari Jutkowitz, VMD, DACVECC

Michigan State University

East Lansing, MI

Immune-mediated hemolytic anemia (IMHA) is one of the most common hematologic disorders seen in dogs. Hemolysis results from the binding of immunoglobulins to red blood cell surface antigens, causing cell lysis by complement intravascularly or phagocytosis within the liver or spleen. IMHA carries a guarded prognosis with mortality rates that have ranged between 29% and 77%.<sup>1-8</sup>

Thromboembolic disease (TE) is a frequent complication of IMHA and appears to be a major contributor to morbidity and mortality.<sup>9,10</sup> In studies of dogs with IMHA that underwent necropsy, TE was identified in 60-80% of cases.<sup>8-11</sup> Sites most commonly affected were the pulmonary, portal, and splenic vasculature, with many dogs having thrombi in multiple organs.<sup>9-12</sup> Although exact mechanisms for the prothrombotic state have not been elucidated, increased concentrations of procoagulant factors such as tissue factor and fibrinogen, decreased concentrations of antithrombin and fibrinolytic factors, vasculitis, enhanced platelet reactivity, the presence of antiphospholipid antibodies, liberation of RBC stroma, circulating microparticles, blood transfusion, and administration of steroids have all been hypothesized to play a role in the development of TE.<sup>9-15</sup>

The antemortem prevalence of thromboembolic disease in dogs with IMHA has not been well described due to difficulties involved in making an accurate diagnosis. However, preliminary data from 110 dogs treated for IMHA at the Michigan State University Veterinary Teaching Hospital between 2004 and 2007 suggested that 34% had a clinical diagnosis of TE during their hospital stay.<sup>16</sup> Clinical diagnosis of pulmonary thromboembolism (PTE) was made on the basis of hypoxemia, thoracic radiographs excluding other respiratory diseases, and laboratory evidence of a prothrombotic state. Clinical diagnosis of portal vein thrombosis (PVT) was based upon the presence of ascites, vomiting or diarrhea, ultrasonographic findings consistent with altered portal blood flow or visualized thrombus, and laboratory evidence of a prothrombotic state. Of these dogs with a clinical diagnosis of TE, 51% had pulmonary thromboembolism (PTE) alone, 8% had portal venous thrombosis (PVT) alone, and 41% had both PTE and PVT.

The development of TE appears to significantly contribute to the morbidity and mortality of IMHA.<sup>5,9,10,12</sup> In our data, survival to discharge in dogs with TE was significantly lower than in dogs without TE (49% vs 81%) and median duration of hospitalization was longer (7 days vs 4 days).<sup>16</sup> However, 5 of 5 dogs with suspected PTE and confirmed PVT (by ultrasound or CT angiography) that were treated with thrombolytic therapy and thromboprophylaxis with warfarin survived > 1 year from the time of diagnosis. This suggests that accurate and prompt identification and treatment of TE may result in improved survival in this compromised patient population.

Definitive diagnosis of PTE remains challenging. Radiographic changes suggestive of PTE may include interstitial or alveolar infiltrates, small volume pleural effusion, regional oligemia resulting from reduced pulmonary blood flow distal to the thrombus, wedge-shaped pulmonary opacities, and enlarged or truncated pulmonary arteries. Unfortunately, radiographic changes associated with PTE in dogs are neither sensitive nor specific and may be absent in some cases.<sup>12,17</sup> Ventilation-perfusion (V-Q) scintigraphy has been evaluated in experimental PTE in dogs and was reported to be helpful in supporting a diagnosis of PTE in one dog with IMHA.<sup>10</sup> However, V-Q scanning is not widely available and the need for a 24 hour isolation period at a nuclear medicine holding facility makes this technique unfeasible in animals requiring oxygen therapy and critical care monitoring. In human medicine, CT angiography (CTA) is considered the test of choice for diagnosing pulmonary embolism. Recently, we developed pulmonary and portal angiographic techniques using a 16 slice multidetector CT unit that have been successfully used to detect PTE and/or PVT in dogs with IMHA.

Documentation of the pro-thrombotic state that is responsible for the TE disease is also challenging, and has traditionally been based upon detection of increased fibrinolysis (increased FDPs and D-dimers) and decreased endogenous anticoagulants (antithrombin) rather than rate of clot formation. Thromboelastography (TEG) has shown promise in demonstrating hypercoagulability in dogs with IMHA.<sup>18,19</sup> Recently, our group has documented hypercoagulability as assessed by TEG in this population and demonstrated resolution of the prothrombotic state once hemolysis has ceased. (unpublished data)

Because of the close association between TE and mortality in dogs with IMHA, thromboprophylaxis is commonly instituted. Low-dose aspirin, clopidogrel (Plavix), and parenteral unfractionated heparin are the drugs most frequently used in veterinary patients at this time. Despite the frequent use of these drugs in dogs with IMHA, no large scale prospective randomized clinical studies exist and the use of various drug and dosing regimens remains controversial.

Aspirin irreversibly inhibits the formation of thromboxane A<sub>2</sub> thus inhibiting platelet aggregation. It has traditionally been used in disease states associated with arterial thromboembolic disease such as heart disease, as the composition of arterial thrombi tend to be more "platelet-rich" than venous thrombi. However, several human studies have reported a decrease in the occurrence of TE in patients at high risk for venous TE when they have been prescribed low dose aspirin in addition to other antithrombotic drugs. Additionally, inhibition of platelet aggregation with aspirin appeared to be beneficial in one retrospective study evaluating treatment protocols in 151 dogs with IMHA.<sup>7</sup> The optimal aspirin dose in dogs with IMHA has not been determined. However, a comparison of

aspirin doses in normal dogs showed that 0.5 mg/kg q12 hours was more effective than 0.5 mg/kg q24 hours or 10 mg/kg q24 hours at inhibiting platelet aggregation.<sup>20</sup>

Clopidogrel inhibits ADP receptors P2Y<sub>12</sub> on the platelet membrane, offering a different mechanism for platelet inhibition than does aspirin. A daily dose of 1 mg/kg was shown to effectively inhibit platelet aggregation in normal dogs.<sup>21</sup> Clopidogrel was also evaluated in a small prospective study and resulted in similar short-term survival rates when compared to low dose aspirin.<sup>22</sup> Whether clopidogrel offers additional benefits over aspirin in dogs with IMHA remains to be determined.

Heparin inhibits secondary hemostasis through activation of antithrombin (AT) and subsequent inhibition of the proteases (factors II, IX, X, XI, XII) necessary for the formation of a clot. Because fibrin-rich pulmonary and venous thrombi are most common in IMHA, drugs like heparin that target coagulation would appear to be the most logical choice for clot prevention. However, unfractionated heparin was not shown to be beneficial in retrospective studies<sup>27</sup> although the dose administered (75-125 U/kg subcutaneously every six to eight hours) in these studies was lower than that shown to prolong activated partial thromboplastin time (aPTT) in healthy dogs.<sup>23</sup> In dogs with IMHA, heparin doses of 300 u/kg every 6 hours were insufficient to achieve therapeutic anti-Xa activity (>0.35 u/ml) in a majority of clinical cases, suggesting that significantly higher doses may be required in this patient population.<sup>24</sup> It is also clear that titration of heparin to a therapeutic endpoint is most appropriate due to variations in individual response to heparinization. Dogs with IMHA that had their heparin doses individually adjusted based upon anti-Xa activity demonstrated significantly longer survival times when compared with dogs on fixed dose heparin.<sup>25</sup> However, the optimal test for monitoring heparinization and the appropriate therapeutic endpoints that should be employed are not well established in dogs. Anti-Xa activity appears to be a likely candidate, but is not widely available at most institutions and therapeutic endpoints are currently extrapolated from human patients. Activated partial thromboplastin time (aPTT) is readily available, but has shown questionable correlation with anti-Xa activity in dogs. Further studies comparing therapeutic endpoints and outcome in clinical patients are necessary.

The current protocol at our institution is a 150 U/kg intravenous bolus of unfractionated heparin followed by 30-60 U/kg/hr constant rate intravenous infusion. The heparin dose is then adjusted daily in 10 U/kg/hr increments, to achieve target prolongation of aPTT (1.5-2.5x upper limit of reference interval). Aspirin (0.5 mg/kg q12h) is added in the event of failure to achieve a target aPTT by day 2. In a pilot study, 26 consecutive dogs with IMHA were admitted to the hospital and treated with this heparin protocol before being transitioned to oral low-dose aspirin before discharge. In this population, no significant TE or bleeding complications were reported and 60 day survival was 100%. (unpublished data)

## Conclusion

Despite the frequency with which TE is suspected in dogs with IMHA, definitive diagnosis is rare. Consequently, effective preventative and therapeutic options may be withheld due to concerns about side effects such as bleeding. However, current evidence suggests that thromboprophylaxis is an important consideration in the management of dogs with IMHA. Further studies are required to better define the optimal drugs, dosages, and monitoring strategies in this patient population.

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