Immune-mediated hemolytic anemia (IMHA), from diagnosis to therapeutics

Dana N. LeVine, DVM, PhD, DACVIM (Small Animal Internal Medicine)[1]

[1]College of Veterinary Medicine, Auburn University, Auburn, AL, USA; dnlevine@auburn.edu

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Primary immune-mediated hemolytic anemia (IMHA) is a common and clinically aggressive autoimmune hematologic disorder of dogs. The central pathophysiology is the presence of anti-erythrocyte antibodies which target red blood cells (RBCs) for destruction. Resultant anemia and inflammation can trigger many secondary and systemic pathologies in these patients, the most severe being thromboembolic events. Tragically, the mortality rate of IMHA approaches 80%. Early and definitive diagnosis is imperative so that therapies can be implemented early in the disease process. Treatment strategies should be targeted at immunosuppression, preventing thrombosis, and restoring red cell mass.

1. Pathogenesis

In canine primary IMHA, loss of immunologic self-tolerance results in formation of autoantibodies which target erythrocytes. The underlying mechanisms contributing to the initial immunodysregulation are largely unknown, however. As numerous breeds exhibit predilection for IMHA, hereditary or genetic factors likely have a role in disease development in some cases.[1] Certain allelic variants of major histocompatibility complex DLA genes are associated with several canine immune-mediated conditions including IMHA.[2, 3] Curiously, aberrations in T-regulatory lymphocytes – which are classically associated with many autoimmune diseases in people and dogs – have not yet been identified in canine IMHA.[4] In secondary, or associative, IMHA an underlying condition triggers disease onset. Multiple mechanisms may result in alloantibodies in these cases, such as erythrocytes being targeted as "bystanders" to the immunologic response or pathogen-self antigen mimicry. In cases of primary IMHA, an unidentified trigger may similarly be pushing dogs already genetically predisposed to autoimmunity into clinical immune-mediated disease.

The immunodysregulation in IMHA ultimately permits development of antibodies from autoreactive B-lymphocytes which bind RBCs, however, the main erythrocyte membrane target in IMHA remains unknown.[5] Both IgG and IgM autoantibodies are observed in IMHA dogs, with IgG predominating; erythrocytes may also be opsonized with complement.[6, 7] The main endpoint for antibody-coated erythrocytes is destruction, which may occur through both extravascular and intravascular mechanisms. In the extravascular destruction pathway, macrophages of the reticuloendothelial system phagocytize the opsonized RBCs through Fc or complement receptor dependent means.[8] The majority of phagocytic destruction occurs in the spleen, but can also transpire by macrophages in the liver and bone marrow. Interestingly, cytokines associated with macrophage and monocyte

activation are increased in dogs with IMHA compared with control dogs and in IMHA non-survivors compared with survivors.[9]

In the intravascular hemolytic pathway surface bound Ig activates the complement membrane attack complex (MAC).[8] The MAC destabilizes erythrocyte membrane and osmotic balance, resulting in cell rupture directly in the vasculature. While both mechanisms of hemolysis likely occur concurrently in dogs with IMHA, extravascular hemolysis predominates in ~95% cases.[8] Autoantibodies may also crosslink RBCs, creating rafts of agglutinated erythrocytes which can obstruct small vessels and capillary beds.

Destruction of the red cell mass has numerous downstream pathologic consequences. The resultant anemia may be severe and manifest acutely, causing hypoxemia with broad negative systemic effects. A marked inflammatory response accompanies the hemolysis, which can become so exuberant that dogs develop systemic inflammatory response syndrome.[10] Growing evidence indicates hemolysis is also a potent activator of the hemostatic system in IMHA. Activated macrophages release tissue factor, which can initiate the coagulation cascade. Additionally, erythrocyte membrane fragments and cytoplasmic components (such as ADP and hemoglobin) activate platelets or stimulate neutrophils to release neutrophil extracellular traps (NETs), which are prothrombotic.[11, 12] NETs can also directly inhibit intravascular blood flow and provide a binding site for circulating hemostatic cells and factors.[13] Cumulatively, these prothrombotic mechanisms can result in thromboembolic events such as pulmonary thromboemboli (TE). In fact, fatal TE are the most common cause of natural death in IMHA dogs.

In addition to erythrocytes in peripheral circulation being destroyed, in some circumstances erythroid precursors in the bone marrow may also be targeted. Such immunologic destruction of the erythroid progenitors may occur in conjunction with active hemolytic disease, or as a standalone disease entity termed precursor-targeted immune-mediated anemia (PIMA). PIMA has a distinct clinical presentation compared to IMHA dogs, with patients having severe, non-regenerative anemia and lacking many of the other clinical abnormalities and comorbidities of IMHA patients.[14]

Diagnostic and therapeutic approaches to IMHA center around the core pathologic features of disease, with diagnostic algorithms targeted towards identifying evidence of immune-mediated hemolysis and treatments focusing on blunting autoimmune RBC destruction and thrombosis, while providing appropriate transfusion support.

2. Clinical Features

While dogs of any breed may develop primary IMHA, many breeds appear predisposed including Poodles, Cocker Spaniels, Old English Sheep Dogs, English Springer Spaniels, and Miniature Schnauzers.[8] Gonadectomized dogs are more likely to develop IMHA than intact dogs and female dogs have a higher incidence than males. IMHA tends to arise most commonly in middle aged dogs, with median age of onset being 6 years.[1]

Presenting clinical signs reflect the underlying hemolytic anemia and corresponding physiologic adaptations. Common findings include pallor, tachypnea, tachycardia, generalized weakness, and anemia-associated cardiac murmurs. Depending on the duration of hemolysis, dogs may have icterus and/or icteric urine; dogs with intravascular hemolysis may have brown to rust discolored urine due to hemoglobinuria. Palpable organomegaly, reflecting spleno- and hepatomegaly, is found in ~40% of dogs and likely represents expanded extramedullary erythropoiesis and/or activated macrophage populations.[1] Dogs with TE, depending upon organ systems involved, may present with acute respiratory distress, neurologic signs, or pain.

3. Diagnosis

3.1 General Clinicopathologic Abnormalities

IMHA dogs classically have severe, regenerative anemias. The anemia is characterized by decreased HCT (often <20%), increased reticulocyte count, elevated MCV, and potentially decreased MCHC.[1] Prominent regeneration is reflected on blood smear analysis by significant polychromasia, anisocytosis, macrocytosis, and increased circulating nRBCs. While most dogs have mounted a regenerative response by presentation, in some cases hemolysis can occur peracutely with rapid destruction of RBC mass. As such, in ~30% cases there may be minimal evidence of regeneration at presentation as the bone marrow requires 2-4 days to accelerate/expand erythropoiesis.[15]

Spherocytes and agglutination are hallmark features of an IMHA blood smear and direct evidence of immune-mediated hemolysis. Spherocytes form when macrophages attempt to remove opsonized RBCs from circulation. Rather than entire RBC being phagocytized, membranous fragments are removed from the erythrocyte which then re-enters circulation. Restructuring of the damaged cell membrane results in a spheroid shape, the spherocyte. A threshold of >5 spherocytes/x100 oil field is highly supportive for IMHA and >3 spherocytes/x100 oil field should raise clinical concern.[15] Agglutination results from crosslinking of RBCs by autoantibodies. In cases with significant agglutination, RBC rafts can be observed macroscopically on glass of vacutainers. Microscopically, agglutination can closely mirror rouleaux – performing a saline agglutination test (SAT) is strongly advised to confirm agglutination. One part blood diluted in 4 parts saline is the recommended minimum dilution ratio for the SAT and absence of RBC dispersion is consistent with agglutination.[15, 16] Washing RBC in saline can further decrease the likelihood of false positive SAT results.[17] True agglutination can also cause marked, potentially supraphysiologic increases in MCV as hematology analyzers count RBC aggregates as single, gigantic erythrocytes. Note, while spherocytosis and agglutination are classically associated with IMHA, spherocytosis is only observed in 67-87% of cases and agglutination in 40-89% of dogs.[18-20] Ghost erythrocytes can also be found due to intravascular hemolysis but are only observed in 5-10% of cases.

Robust inflammatory leukograms and thrombocytopenia are common. Despite IMHA being a sterile inflammatory condition, marked neutrophilia, left-shift, and neutrophilic toxic change can be found. Thrombocytopenia is evident in ~70% cases and likely arises by multiple mechanisms.[18] Dogs with severe thrombocytopenia may have concurrent immune-mediated platelet destruction (Evan's Syndrome), though true Evan's is likely rare. Much more commonly, platelet consumptive processes (*i.e.* disseminated intravascular coagulation) drive the thrombocytopenia.

Hyperbilirubinemia is frequently observed, with increased serum bilirubin concentrations being the consequence of increased heme breakdown from the erythrocyte destruction. Hyperbilirubinemia may be reflected as icteric serum/plasma and bilirubinuria. Dogs with predominantly intravascular hemolysis may have hemolyzed serum/plasma and hemoglobinuria. Other serum biochemical abnormalities are highly variable depending upon anemia severity and duration; mildly elevated hepatobiliary enzymes are frequently observed due to hepatic hypoxic insult.[8]

Hemostatic testing can yield variable results depending upon disease stage. Dogs early in clinical disease may have normal hemostatic findings. However, many dogs with IMHA eventually develop a hypercoagulable state which can be identified by thromboelastography (TEG), although this may be, in part, artifactual from the inverse relationship between hematocrit and TEG maximum amplitude.[21] Hypercoagulability primes dogs to develop TE and potentially disseminated intravascular coagulation (DIC). These dogs may have evidence of rapid platelet and coagulation factor consumption and clot formation, including thrombocytopenia, prolonged PT/PTT, elevated D-dimer concentrations, decreased fibrinogen concentration, and decreased antithrombin activity.

3.2 Direct Antiglobulin Tests

Detection of surface-associated antibodies on erythrocytes is critical, supportive evidence for the diagnosis of IMHA. Classically referred to as the Coombs' Test, there are many different assays available to detect anti-erythrocyte antibodies – this family of assays are referred to as direct antiglobulin tests (DAT). Available tests include antiglobulin titer-based methodologies and flow cytometric assays for anti-RBC antibodies performed by referral laboratories and immunochromatographic strip tests which can easily be performed cageside. Despite the wide variety of analytic approaches, there is overall excellent diagnostic concordance between most DAT methods and all are considered reliable assays.[20] However, Coombs' Test should not be considered the "gold standard" stand alone test for diagnosing IMHA as reported diagnostic sensitivities range from 61-82% and specificities of 94-100%[15] As such, a positive DAT should be considered highly supportive for IMHA but a negative result does not exclude IMHA.

3.3 Ancillary Diagnostics

Evaluation of suspected IMHA dogs for possible underlying diseases is strongly encouraged. Diagnostic imaging looking for neoplasia, especially hemic neoplasia, should be

considered.[15] While splenomegaly and hepatomegaly are encountered in ~40% of cases, such organomegaly can be caused by extramedullary hematopoiesis, lymphoid reactivity, and macrophagic system expansion.[1] Abdominal visceral cytology may help differentiate these processes from potential neoplastic conditions such as histiocytic sarcoma. Vector borne disease testing is also highly encouraged. Certain vector borne diseases may trigger associative IMHA – in particular, there is strong evidence of babesiosis being associated with secondary IMHA.[15] In-clinic immunoassays are insufficient as they don't cover all putative infectious triggers, and combining serology and PCR testing significantly increases test sensitivity.[22]

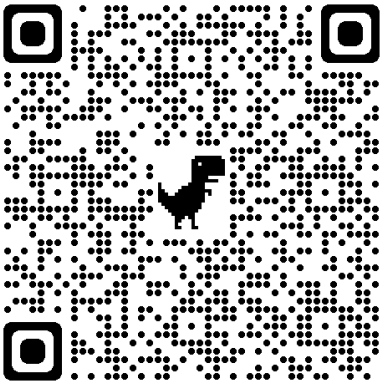

Fig. 1. Scan to access ACVIM Consensus Diagnostic Algorithm for IMHA

3.4 Differential Diagnoses

Many hematologic and hepatobiliary diseases can clinically overlap with IMHA; differentiating these conditions from IMHA can be diagnostic challenging. While IMHA is the most common hemolytic anemia of dogs, oxidative toxins can also trigger extravascular and intravascular hemolysis. Such exogenous toxins include certain heavy metals (Zn, Cu, Fe), propylene glycol, naphthalene, acetaminophen, *Allium* genus plants, and skunk spray. In rare cases strong endogenous acids, like ketoacids and uremic family acids, may induce Heinz body formation in RBCs and subsequent hemolysis. Although rare, inherited erythrocyte enzyme deficiency disorders – such as pyruvate kinase deficiency and phosphofructokinase deficiency – can also result in hemolysis. While these other hemolytic anemias would mimic many features of IMHA, including robust regenerative anemia and hyperbilirubinemia, they are less likely to show evidence of an immune-mediated component (ex. minimal spherocytosis, no agglutination, DAT negative). A spectrum of hepatobiliary conditions can also cause marked hyperbilirubinemia and anemia, mimicking some traits of IMHA dogs. However, these anemias are generally non-regenerative and lack evidence of an immune-mediated process.

3.5 ACVIM Consensus Statement on the Diagnosis of Immune-Mediated Hemolytic Anemia

To vet the published body of diagnostic evidence for IMHA and refine an evidence-based approach to diagnosis, in 2019 the American College of Veterinary Internal Medicine published a consensus statement on the diagnosis of immune-mediated hemolytic anemia in dogs and cats.[15] Based upon the evaluated literature, the consensus panel developed a diagnostic algorithm for IMHA which centers on identifying clinical evidence of: 1) anemia, 2) anti-erythrocyte antibodies, and 3) hemolysis. The algorithm provides a stepwise diagnostic ladder which culminates in 4 possible diagnostic outcomes for patients: Diagnostic for IMHA, Supportive of IMHA, Suspicious for IMHA, and Not IMHA. **The algorithm can be accessed by the QR code in Figure 1.** Briefly, the first level is identifying

anemia. The second level is identifying either a positive SAT with RBC washing or >2 signs of an immune-mediated component to the anemia (spherocytes, positive SAT without washing, or positive DAT). The third level is evidence of hemolysis (hyperbilirubinemia/icterus/bilirubinuria, hemoglobinemia/hemoglobinuria, or ghost cells). Patients identified as Diagnostic for IMHA or Supportive of IMHA are clinically considered dogs with IMHA. Patients Suspicious for IMHA may be considered a tentative diagnosis – additional diagnostics are strongly recommended in these patients to screen for other diseases before IMHA therapies are instituted. Dogs identified as Not IMHA by the algorithm should be evaluated for other causes of their anemia.

4. Treatment

Following diagnosis, prompt and multifaceted therapy is essential for giving patients the best chance for disease remission and survival. Comprehensive treatment regimens should center on three key approaches: 1) immunosuppression, 2) thromboprophylaxis, and 3) blood product transfusion. To refine a more standardized and evidence-based approach to treating canine IMHA, in 2019 the American College of Veterinary Internal Medicine published the consensus statement on the treatment of immune-mediated hemolytic anemia in dogs.[23] The consensus statement is the culmination of a critical assessment of the published efficacy of different therapeutic modalities for IMHA. Based upon the available literature, the consensus panel crafted evidence-based treatment algorithms use of a) immunosuppressive therapy (Figure 2), b) antithrombotic therapy (Figure 3), and c) relapse management (Figure 4).

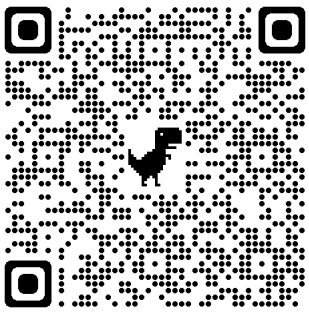

Fig 2. Scan to access ACVIM Consensus Immunosuppressive Therapy Algorithm

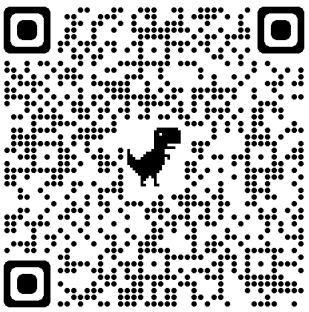

Fig 3. Scan to access ACVIM Consensus Antithrombotic Therapy Algorithm

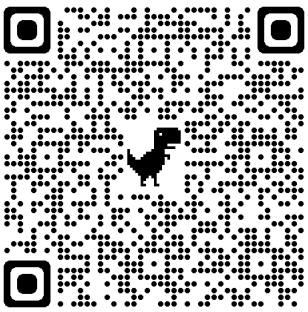

Fig 4. Scan to access ACVIM Consensus Relapse Therapy Algorithm

The main treatment objectives in dogs with active IMHA are a) stabilization of HCT and b) mitigation of thromboembolic events. Concerning initial monitoring, patient HCT should be evaluated daily – stabilization and then gradual increase of HCT to >20% is considered an appropriate initial response therapy.[8] Patients that fail to respond to first line immunosuppressive therapy or rapidly decompensate warrant additional, concurrent therapies, including addition of a second immunomodulatory drugs or transfusion. Dogs that positively respond to interventions can be shifted to weekly, then monthly, monitoring

of HCT until red cell mass normalizes. When HCT >30% for at least 2 weeks, gradual withdrawal of immunosuppressive therapies may begin. To mitigate relapse, therapeutic withdrawal occurs over the course of 4-8 months depending upon immunosuppresants used and clinical response.

4.1 Immunosuppression

Immunosuppression is the mainstay of managing an IMHA patient, with the aim being to suppress the immune system adequately enough to attenuate or stop immune-mediated erythrolysis. Glucocorticoids are the recommended first line immunosuppressants, being highly effective in attenuating multiple components of the immune response and the most rapidly acting immunosuppressant. For dogs with IMHA, they can block macrophagic Fc mediated RBC phagocytosis, decrease complement-mediated erythrolysis, lower autoantibody production, and decrease pro-inflammatory cytokine concentrations.[8] For dogs in which there is a failure to respond to initial therapy or disease relapse, addition of a second immunomodulatory agent may be required. Unfortunately, most immunosuppressive agents are mechanistically non-specific therapies and are associated with a spectrum of adverse side effects, some of which can greatly decrease quality of life for IMHA dogs.

For initial glucocorticoid immunosuppression, in relatively clinically stable IMHA dogs the following drugs and doses are recommended:

- Prednisone / prednisolone: 2-3 mg/kg/day PO or 50-60 mg/m^2/day for dogs >25kg
    - Once daily administration may be associated with fewer adverse effects[24]
- Dexamethasone SP: 0.2-0.3 mg/kg/day IV if vomiting or inappetent

For clinically stable dogs that have started glucocorticoid therapy **but do not have controlled disease by 7 days of therapy**, addition of a second immunomodulatory disease is advised. In addition, **dogs that have life-threatening disease at presentation** are recommended to begin glucocorticoid therapy and a second immunomodulatory drug concurrently. Such life-threatening criteria include a) severe and/or rapidly progressing anemia, b) probable need for multiple transfusions, and c) presence of negative prognostic factors (see section below). Second agents should also be considered in large dogs (>25 kgs) due to their greater intolerance of steroid side-effects.[25] Unlike glucocorticoids, many of the second-line medications take up to two weeks for their immunosuppressive activity to manifest.[23] One study in healthy dogs did demonstrate cyclosporine efficacy in 24 hours, but this has not been assessed in sick patients.[26] Second-line immunomodulatory drugs to consider include:

- Azathioprine: 2 mg/kg/day PO for 14 days, followed by q48h dosing
- Cyclosporine: 5 mg/kg PO q12h
- Mycophenolate: 10 mg/kg IV or PO q12h

Note, while addition of second line immunomodulatory therapies is recommended in these situations, there is no evidence that these medications improve outcome in dogs with IMHA compared to glucocorticoid therapy alone.[23] Likewise, there is inadequate reported data to guide which second-line therapy may be therapeutically superior.[23] A recent multicenter retrospective study identified a higher relapse rate with corticosteroids alone than with corticosteroids and a second agent, but there was no effect on time to PCV stabilization, duration of hospitalization or mortality rate.[27] While some retrospective studies suggest superiority of one agent, others contradict them, and all are marred by the typical shortcomings of retrospective studies. Large, multicenter prospective studies to compare second line immunosuppressive treatments are direly needed. Client acceptance of the potential side effects of each medication and client finances currently help guide selection of second immunosuppressive agents in each case. Addition of a third immunosuppressive agent should be avoided as this is usually associated with increased risk of opportunistic infections and overlapping drug toxicity, and a clear benefit has not been established.[23]

4.2 Thromboprophylaxis

Dogs with IMHA are frequently hypercoagulable and prone to development of TE and DIC. With fatal thrombosis being the leading cause of natural death in IMHA, prompt institution of thromboprophylaxis is essential.[23] Anti-thrombotic therapy can be separated into two approaches – anticoagulant therapy or antiplatelet therapy. Since thrombosis from IMHA is primarily venous, and such thrombi are typically fibrin rich, the consensus statement prioritized use of anticoagulant medications. However, the cell-based model of hemostasis establishes that platelets play a key role in secondary hemostasis as well as primary hemostasis and likely contribute to venous thrombosis.[28, 29] Thus, we believe antiplatelet agents are also likely beneficial in dogs with IMHA. [23]

Just as with secondary immunosuppressant agents, there is a lack of well-designed prospective studies comparing all available antithrombotics in IMHA dogs. The IMHA consensus statement recommended individually-adjusted, but not constant dose, unfractionated heparin therapy (UFH).[23] This recommendation was based on one prospective study of 15 IMHA dogs receiving either constant dose or individually dose-adjusted (based on anti-Xa monitoring) UFH. The outcome of dogs receiving individually-adjusted UFH was superior to that of many other IMHA reports with 88% of those dogs alive at 180 days post-diagnosis; in contrast, only 14% of dogs in the constant dose group were still alive.[30] Individualized UFH therapy can be challenging as most clinics do not readily have access to anti-Xa monitoring. Nomograms to adjust UFH therapy using an aPTT assay or thromboelastography have been described.[31] The recommended starting dose of UFH is 150 U/kg -300 U/kg SQ q6h or 900 U/kg/24 hours CRI following a 100 U/kg bolus; it is imperative to monitor efficacy ideally using factor Xa inhibition assay and titrate dose based upon results.[8, 23, 30] Constant dose UFH should be avoided. Alternatively, oral direct Xa inhibitors like rivaroxaban (1-2 mg/kg/day) may be equally as effective and can be dosed orally without monitoring.[28] Canine vessel occlusion models have demonstrated equal or

superior efficacy of Xa inhibitors compared to UFH.[32] Rivaroxaban was tolerated in one study of dogs with IMHA, but its efficacy was not compared to UFH.[33] Low molecular weight heparins (LMWH) provide another alternative but have not been compared to UFH or rivaroxaban in IMHA.[8, 23]

Antiplatelet agents are also recommended in combination with anticoagulants or can be used alone if anticoagulant therapies are not feasible in a given patient. For dogs exhibiting the highest risk of thromboembolic disease or clinical TE, antiplatelet therapy should be administered concurrently with anticoagulant regimens. Given that thirty percent or more of dogs do not respond to low dose aspirin, clopidogrel (2-4 mg/kg PO q24h) therapy is prioritized.[23, 34, 35] If aspirin is utilized it should be dosed at 1 - 2 mg/kg PO q24h, but this dose may be insufficent.[8, 23, 36] Interestingly, a recent retrospective study found that clopidogrel compared to a multi-agent antithrombotic protocol was not associated with different thrombotic outcomes.[27] Similarly, in this study there was no difference in risk of definitive thrombus development when using anti-platelet compared to anti-coagulant drug protocols.[27] However, in this study, the clopidogrel monotherapy group may have had less severe disease.

4.3 Transfusion

While administration of blood products is common for IMHA patients, not all dogs require a transfusion and blood administration should be balanced between the clinical needs of the patient relative to risk of adverse transfusion reactions. Transfusion should be considered when there is clinical evidence of anemia-associated hypoxia compromising physiologic functions – these signs may include weakness, tachypnea, tachycardia, hypothermia, and hypotension. While there is no specific HCT threshold for initiating a transfusion, dogs with HCT >20% are unlikely to have comprised organ function due to anemia and dogs with <12% likely have significant hypoxemic stress.[8, 23] If transfusion is warranted, administration of packed RBCs (pRBCs) is advised over whole blood since IMHA dogs are usually normovolemic.[23] Data from a retrospective study suggests that younger blood improves outcomes in dogs with IMHA. [37] In contrast, a more recent prospective study demonstrated no difference in morbidity or mortality in IMHA dogs randomized to receive ≤7 days or ≥ 21 days stored RBC units; however, this study was underpowered.[38] The IMHA consensus recommends transfusing with units ≤10 days old if possible.[23, 37] Before transfusion administration, dogs should be blood typed preferentially with immunochromatographic typing strips in which agglutination does not interfere with test interpretation or, if using a tube based assay, autocontrols with recipient plasma and recipient red cells should be performed and compared against the donor reactions. The authors prefer to crossmatch dogs receiving serial transfusions even before 72 hours after the initial transfusion.[39]

4.4 Additional Therapeutics

IMHA dogs with ongoing hemolysis despite glucocorticoid therapy and second-line immunosuppressive agents may warrant either intravenous immunoglobulin (IVIG) therapy

or splenectomy. Before considering these therapies, steps should be taken to ensure the diagnosis of IMHA is correct, that drug dose and administration is appropriate, and therapeutic drug monitoring should be performed if available for the patient's second-line agent.[23] IVIG should be considered prior to splenectomy. Mechanistically, administration of human IVIG supersaturates the Fc receptors on macrophages. With receptors saturated, macrophages cannot bind autoantibodies on RBCs and thus cannot phagocytize erythrocytes. Human IVIG product can be administered at dose of 0.5 – 1.5 g/kg over an 8-12 hour period; only a single transfusion is recommended due to concern for risk of immunologic responses to a second transfusion.[23, 39] The efficacy of IVIG therapy to blunt hemolysis is variable, but instances of sudden attenuation of erythrolysis are reported.[23] As opposed to its established efficacy in ITP, evidence documenting IVIG's efficacy in IMHA is lacking. This is likely due to the prothrombotic potential of IVIG in an already hypercoagulable condition. If IVIG is to be utilized, it should be combined with anticoagulant therapy.

Splenectomy may be considered in those dogs requiring continuous immunosuppressive therapy to prevent relapse, those suffering from frequent relapses, or those intolerant to medical therapy.[23] As splenic macrophages are most implicated in extravascular RBC destruction in IMHA, splenic removal can acutely diminish extravascular hemolysis.[8] All published studies assessing splenectomy in dogs with IMHA lack control groups, thus efficacy of splenectomy is hard to gauge. A recent retrospective case series found that of 7 dogs which underwent splenectomy for IMHA, 4 dogs had partial (2) or complete (2) disease remission.[40] Before splenectomy is performed, thorough vector-borne disease screening is recommended.

For dogs with associative IMHA, treatment of the underlying cause is also imperative. Resolution of the inciting trigger could potentially lessen the severity of clinical disease and in some cases, especially infectious agents, may promote disease resolution and prevent disease relapse.

4.5 Discontinuation of Therapy

In dogs that successfully respond to therapy, deciding when and how to discontinue therapeutics can be challenging as withdrawal of medications too quickly can result in relapse. While there are no uniform metrics for when to begin therapeutic tapering, slow withdrawal could be considered after HCT has been >30% for over 2 weeks without ongoing evidence of hemolysis (spherocytosis, agglutination). Glucocorticoids should be tapered first by reducing dose 20-25% every 3 weeks; patients should be monitored for relapse during this time window. With complete withdrawal of glucocorticoids, secondary immunosuppressive agents can be stopped promptly or tapered, per consensus guidelines, but the authors usually elect to taper these medications. Antithrombotics can also be discontinued with glucocorticoid therapy, but UFH, LMWH, and oral Xa inhibitors should be weaned vs. abruptly discontinued to prevent rebound hypercoagulation.[41]

Follow-up CBC monitoring is encouraged to screen for early signs of disease recurrence. Reported relapse rates for dogs that survive initial hemolytic crisis range from 11-15%; some dogs may require recurrent or lifelong therapy for disease control.[23] Approach to relapse should follow the algorithm in Figure 4. Caution should be taken to ensure that a true relapse is occurring and that anemia is not now secondary to medication-induced gastrointestinal bleeding or bone marrow suppression.[23]

4.6 Predictive Factors and Prognosis

Many clinical and clinicopathologic variables have been identified as prognostic survival predictors in canine IMHA that can guide earlier institution of second line immunosuppressants.[1] In an effort to make a more clinically useful predictive model, Whelan *et al*. designed the Canine Hemolytic Anemia Objective Score (CHAOS) which includes the following patient metrics: age, temperature, agglutination, albumin concentration, and bilirubin concentration.[42] Higher CHAOS is associated with poorer survival, with dogs having CHAOS ≥ 3 being 4x more likely to die before hospital discharge and 3.5x more likely to not survive 30 days post-diagnosis.[10, 42] Unfortunately even with the most aggressive of therapies, IMHA has an unfavorable prognosis with reported mortality rates ranging from 50-80%.[1]

5. Summary

Canine IMHA is a disease with an unacceptably high mortality rate and high treatment-associated morbidity. Outcome improvement requires a better understanding of disease pathogenesis to allow for development of more targeted immunomodulatory therapy. Furthermore, prospective randomized multi-institutional clinical trials are needed to determine whether secondary immunosuppressive agents improve outcomes, and if so, which agent (if any) is superior. Similar trials are also needed to determine the best antithrombotic regimen.

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