# I HATE IMHA: IS THERE ANYTHING NEW?

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Immune-mediated hemolytic anemia (IMHA) results in the premature destruction of RBCs through a type II hypersensitivity reaction. It is a common presentation in small animals, particularly in middle-aged female dogs. Many popular breeds are over-represented for IMHA, including Cocker Spaniels, Springer Spaniels, Poodles, and both Bearded and Rough-Coated Collies. American Cocker Spaniels represent a significant majority of IMHA cases. Other breeds commonly affected by IMHA include the Bichon Frise, miniature Schnauzer, spitz breeds, and some sheepdogs, such as the Old English. IMHA is rare in cats. IMHA results in severe anemia, predisposes patients to thromboembolism due to its associated pro-inflammatory processes, and causes significant morbidity in small animal patients. It has a general mortality rate of approximately 50% including both primary (idiopathic) and secondary types with idiopathic IMHA having a mortality rate of 28-83%. Idiopathic IMHA has no determinable cause and comprises the majority of cases while secondary IMHA results as a consequence of another pathology including neoplastic, bacterial, viral, or parasitic disease. Secondary IMHA may be induced by the use of some drugs and vaccines.

Healthy canine RBCs have an average lifespan of 100-120 days and are removed from circulation at the end of their lives via macrophages in the liver and spleen that recognize age-related membrane antigens on the cell surface. IMHA occurs when autoantibodies, including immunoglobulins G, M, and A, are produced against the animal's own RBC membrane antigens, regardless of the age or health of the cells. The immunoglobulins attach directly or indirectly to various components of the RBC membrane, activating the complement cascade and membrane attack complex. This causes premature destruction through extravascular hemolysis, intravascular hemolysis, and intravascular RBC agglutination. Autoantibodies are usually well-managed in healthy animals through the action of suppressor T cells, so it has been posited that animals with IMHA may have poor suppressor T function.

IMHA results in two different types of hemolysis, intravascular and extravascular. When the autoantibody cascade is triggered, massive cell lysis occurs as RBC membranes are damaged and an influx of extracellular fluid causes them to rupture while still in circulation. This is called *intravascular hemolysis* and results in the release of free hemoglobin into the bloodstream, causing hemoglobinemia (wine red serum or plasma that does not become clear with centrifugation) and hemoglobinuria (dark wine-red urine). Intravascular hemolysis is more likely to occur in cases of IMHA that are mediated by IgM, due to its ability to fix complement. Intravascular hemolysis can also result in renal injury due to the toxic effects of free hemoglobin. If the complement and membrane attack systems are not activated, IMHA is less severe and RBCs are destroyed in the liver and spleen through the mononuclear phagocyte system (MPS). This occurs outside the vasculature and is hence termed *extravascular hemolysis*. Hemoglobinemia and hemoglobinuria are not seen in extravascular hemolysis, since the cells are destroyed outside the blood vessels via normal mechanisms. Hemoglobin released through RBC destruction extravascularly is recycled into the bilirubin pathway. It is important to understand that extravascular hemolysis is a normal physiologic process and one of the mechanisms for removal of healthy but aged RBCs from the circulating pool. However, in cases of hemolytic anemia, this normal process reaches pathologic proportions.

As previously stated, primary IMHA is idiopathic. *Secondary IMHA* is caused by an immunologic response to foreign antigens (bacterial, neoplastic, protozoal, rickettsial, or viral) that infect healthy RBCs, thereby "tagging" them for removal. Other mechanisms, such as vaccines, environmental factors, pharmacologic interaction, and alloantibody production have also been explored as etiologic roots of secondary IMHA. Vaccine-induced IMHA has been documented in several studies, but no specific vaccine has been implicated or firmly linked to the disease. One theory holds that vaccination is a trigger that may activate the MPS, heighten a subclinical inflammatory condition, or perhaps deregulate the precise balance of the immune system. In many studies, the patients had been vaccinated within 30 days of developing IMHA. However, more research is necessary before a conclusive link can be determined, to ascertain which vaccines pose a risk (if any), and which breeds are consistently affected. Currently, the benefits of vaccination far outweigh the risks of IMHA. In addition, there is some evidence that there may be a seasonal or regional element to IMHA cases in some parts of the United States. Drugs such as acetaminophen, cephalosporins, NSAIDs, penicillins, phenylbutazone, tetracyclines, and trimethoprim-sulfa have also been implicated as causative agents of secondary IMHA. Proposed mechanisms of action include drug metabolites or their degradation products adhering to the RBC membrane, inducing a complement attack or cell removal by the MPS that results in hemolysis. IMHA may also be caused by *alloantibodies* (those unique to individuals of a species that react negatively to antigens from the same species) directed against elements of the RBC membrane. This most commonly results due to transfusion of incompatible blood products and neonatal isoerythrolysis in both dogs and cats, although neonatal isoerythrolysis is much more common in cats.

Not all hemolytic anemias are immune mediated. In patients with hemolysis, the differential diagnosis must include not only immune mediated etiologies but non-immune mediated as well. Other causes of hemolytic anemia include inherited defects (such as pyruvate kinase deficiency or phosphofructokinase deficiency), hypophosphatemia due to refeeding syndrome or diabetic ketoacidosis, toxicity (zinc, acetaminophen, naphthalene, onion, garlic, and other oxidating agents), and microangiopathic hemolytic anemia. Zinc toxicosis typically results due to ingestion of U.S. pennies minted after 1983, when the metallic composition was altered. Repeated use of the anesthetic agent propofol has also been reported to cause Heinz body anemia in cats. *Microangiopathic hemolytic anemia* is a condition in which RBCs are physically damaged during circulation due to vessel occlusion, abnormal vascular morphology, or fibrin shearing. As the red blood cells pass through tortuous, occluded, or inflamed vessels, they are fragmented, much like a hardboiled egg passed through an egg slicer or cheese through a grater. Splenic torsion, neoplasia (such as hemangiosarcoma), heartworm disease, fibrin deposits (as in DIC), vasculitis, liver disease, intravenous catheterization, or vessel damage result in schistocytes that are removed from circulation by the MPS. These damaged RBCs are readily identified on a blood smear. Treatment of microangiopathic hemolytic anemia includes identification and correction of the underlying disease.

When determining an immune mediated versus other cause of hemolytic anemia, a thorough history and complete signalment are essential. Clinical signs are a direct result of tissue hypoxia due to reduced oxygen carrying capability—if fewer red cells are circulating, oxygen delivery will be compromised, although many patients will also demonstrate clinical signs associated with elevated bilirubin and/or hemoglobin clearance and generalized inflammation. Patients present a spectrum of clinical signs with varying severity; some are profoundly affected, while others are only affected during exertion or exercise, and many compensate well to a point before becoming severely clinically ill. Clinical signs associated with IMHA include weakness, lethargy, collapse, altered mentation, anorexia, tachycardia, tachypnea, hepatosplenomegaly, and heart murmur. Gastrointestinal signs such as nausea, vomiting, and diarrhea are frequently noted. Clients may also report discolored or "port-wine" colored urine. Upon physical examination, pallor, icterus, fever, and lymphadenopathy are common. Icterus is usually noted in the mucous membranes first and becomes global as bilirubin levels increase. A heart murmur due to turbulent blood flow is also common, usually a low grade II-III/VI and often noted when the PCV falls below 20%.

Distinguishing between primary and secondary IMHA is essential for effective treatment, yet there is no single laboratory test that is pathognomonic for IMHA or that will determine type. Diagnosis is generally made based on signalment, history, clinical signs, and a battery of laboratory assays. The diagnosis of IMHA is supported by findings that include anemia, evidence of hemolysis, evidence of immune involvement, and no other definitive cause of anemia. To differentiate between primary and secondary disease, thorough screening for an underlying causative agent or disease is necessary and may require imaging in addition to laboratory tests.

A complete blood count with slide review and reticulocyte count is necessary to diagnose anemia (hematocrit < 25-30%) and determine whether it is regenerative or non-regenerative. CBC findings in IMHA include regenerative anemia, leukocytosis with left shift, lymphocytosis, thrombocytopenia, and an elevated reticulocyte percentage. In most patients, IMHA induces a regenerative anemia, so a blood smear should show evidence of anisocytosis, nucleated RBCs, polychromasia, and reticulocytosis. Reticulocytes are immature RBCs that have been released into circulation with organelle remnants intact (ribosomes, mitochondria, and polyribosomes) and minus the nucleus; it is these organelles that take up stain and give them their distinctive appearance when stained with new methylene blue. They appear in response to anemia and hemolysis. In addition, spherocytosis is suggestive of IMHA and has been identified in 89-95% of dogs with IMHA. Spherocytes are RBC that appear small, circular, darker than normal RBCs, and devoid of central pallor due to an altered membrane shape caused by damage from the MPS. During slide review RBCs should be examined for any evidence of inclusions or parasites.

Additional blood tests include a chemistry panel, coagulation testing, saline agglutination testing, and the Coomb's test. Common derangements noted on the chemistry panel include hyperbilirubinemia, hyperglobulinemia, azotemia, and increased liver enzyme values (particularly alanine transaminase). Serial chemistry assessment may be necessary to track liver and renal function. Coagulation testing is important because IMHA predisposes patients to hypercoagulability and hence DIC; at a minimum, PT and aPTT testing are indicated but if DIC is suspected, assessment of D-dimers, other FDPs, and antithrombin are optimal. Urinalysis is also recommended. Patients with non-regenerative anemia may also benefit from bone marrow aspirate or biopsy. Approximately 30% of canine IMHA patients have non-regenerative anemia. Infectious disease testing, such as PCR assays for tick borne and rickettsial illness, is also recommended.

Slide agglutination testing is rapid, inexpensive, and positive in about 40-89% of canines with IMHA. In patients with IMHA, autoagglutination of the RBCs is mediated by antibodies IgG and IgM. To perform the test, a drop of anticoagulated whole blood is mixed with 4 drops of 0.9% sodium chloride on a clean glass microscope slide. The sample is gently mixed by rocking 2-3 times before being examined both with the naked eye and under a microscope.

Autoagglutination (cell clumping) that is noted with the naked eye is referred to as "macroscopic" agglutination while that seen microscopically is referred to as "micro" agglutination. The slide agglutination test will only be positive if there is a sufficient quantity of antibody coating the RBCs to cause agglutination. The test is also useful to differentiate agglutination from Rouleaux formation (normal cell stacking that is not mediated by antibodies). Rouleaux formation will be dispersed by saline, but agglutination will not. In severe cases of IMHA, agglutination will be noted in blood regardless of anticoagulation, so blood tubes should always be checked for agglutination prior to saline testing. If the test is equivocal, the blood can be diluted 1:4 with sterile normal saline and the cells washed 3 times.

A patient may have IMHA but not have sufficient circulating antibodies to induce the degree of agglutination that will cause a positive saline slide agglutination test. In these cases, a Coomb's test may be helpful. The diagnosis of IMHA is supported by a positive Coombs' test, also known as the antiglobulin test, and it has potential utility in differentiating between primary or secondary IMHA but cannot be conducted if severe agglutination is present. There are two types of Coomb's test: the direct and indirect. The direct Coomb's tests for antibodies or complement that are attached to RBCs, while the indirect tests for RBC antibodies in the patient's serum. Coomb's testing is not conclusive and requires careful interpretation since sensitivity ranges from 60-90%.

Treatment of IMHA is predicated upon immunosuppression, typically accomplished with glucocorticoids and secondary immunosuppressive agents. If secondary IMHA is suspected, therapy must also be administered for the underlying condition(s) that may have triggered hemolysis (for example, tick borne or rickettsial disease). The approach to therapy is organized around two phases, the acute and maintenance phase. The acute phase of therapy lasts until remission is achieved. Following remission, maintenance therapy may be required for months to years. The therapeutic approach should be determined on a case by case basis. Nursing staff in emergency rooms and ICUs will most commonly be involved in the initial diagnosis and stabilization of patients in the acute phase or in the treatment of patients who have relapsed following remission.

Glucocorticoids are a first-line therapy for IMHA due to their inhibition of the macrophages of the MPS. Therapy begins with immunosuppressive doses of prednisone, prednisolone, or dexamethasone. Doses higher than 1-2 mg/kg prednisone/prednisolone per os every twelve hours are not associated with a better outcome. Prednisone, a precursor drug to prednisolone, does not yield as much serum drug as prednisolone in feline patients, so prednisolone should be administered in cats. If the patient cannot tolerate oral drug administration, intravenous dexamethasone can be administered, bearing in mind that it is more potent than prednisone. If the patient's disease is severe, autoagglutination is profound, and no response has been noted to glucocorticoids, a second line immunosuppressant drug such as mycophenolate mofetil, azathioprine, cyclosporine, leflunomide, or chlorambucil can be added. Most patients also receive GI protectants (e.g., omeprazole, pantoprazole, misoprostol) to prevent gastrointestinal ulceration, bleeding, and to manage associated GI signs. In addition, IMHA is a hallmark disease for thromboembolic events. The use of anticoagulating agents and platelet aggregate inhibitors (e.g., heparin, aspirin, enoxaparin, and clopidogrel) is warranted. If there is a favorable response, indicated by a rise and stabilization in hematocrit and reticulocyte count over 1-2 weeks, immunosuppressant drugs are tapered slowly to prevent recurrence of hemolysis. When tapering drugs, the most expensive or most problematic in terms of side effects should be weaned first, followed by tapering of the remaining drug(s) to the minimum effective dose and interval. This can take 3 to 6 months and should be guided by serial hematocrit and CBC assessments.

Management of severe IMHA cases may involve a combination of immunosuppressive drug therapy in addition to blood transfusion(s) or hemoglobin-based oxygen carrying solutions (HBOCs). Multiple transfusions of packed red blood cells may be required over a period of days to weeks while immunosuppressive therapy takes effect. Blood typing and crossmatching are recommended but can be difficult to interpret due to autoagglutination and hemolysis. If blood transfusions are indicated, there is a substantial increase in the risk of pulmonary thromboemboli (PTE) and acute renal failure. If available, HBOCs can be used in lieu of traditional blood products to provide an increase in circulating hemoglobin for tissue oxygenation. HBOCs present some advantages in comparison with packed red blood cells, including a lower risk of PTE found with traditional blood products and a lower risk of transfusion reactions from donor RBCs. They are shelf stable and do not require refrigeration like traditional blood products; however, their availability is very limited in the Unites States. Other treatment for IMHA includes IV fluid therapy in patients who are dehydrated, hypovolemic, or with hemoglobinuria. Splenectomy is considered a last resort in patients with extravascular hemolysis that have not responded to traditional medical therapy. Nutritional support may also be required in patients with persistent anorexia.

Recent innovations in treatment for patients with severe IMHA include the administration of human immunoglobulin G (IgG), which has been effective in small cohorts of dogs that are refractory to standard therapy. Human IgG is thought to bind complement, consequently diverting attacks on cellular targets, and is also thought to inhibit cytokine release from monocytes, modulate B and T cell clones, inhibit cytotoxic T cells, and downregulate antibody production. The small size of the IgG molecule allows for unimpeded movement between the vasculature

and body tissues. Equine IgG has been used in dogs as the cost is significantly less than human IgG; canine IgG is not widely available. The risk of PTE may be increased with the use of IgG; concurrent treatment with LMWH or mini-dose aspirin may be indicated. In addition, use of exogenous products, such as human or equine IgG, may predispose the patient to a hypersensitivity reaction. Newer drugs, such as leflunomide and mycophenolate mofetil, may have a quicker onset of action than older first line drugs such as azathioprine but more research is needed into their efficacy and overall utility. Unfractionated heparins and low molecular weight heparins such as enoxaparin have proven safe in dogs with IMHA and are easier to use than warfarin. There is renewed interest in low dose aspirin therapy.

If available, therapeutic plasma exchange (TPE) has emerged as a promising therapy for IMHA. TPE is also known as plasmapheresis and has been documented as a treatment for immune disease in dogs since the 1980s; however, until recently veterinary medicine lacked access to the instrumentation necessary for efficient therapy. The increased availability of extracorporeal therapeutics in specialty veterinary medicine (TPE, charcoal hemoperfusion, intermittent hemodialysis, continuous renal replacement therapy, etc.) has led to further exploration of the clinical utility of these techniques. In TPE, the patient's circulating plasma is removed and partially replaced with donor plasma using a centrifugation or membrane-based techniques. The patient must be referred to a specialty center for therapy. A 2021 studied published in the Journal of Veterinary Internal Medicine evaluated TPE in 17 canine patients with IMHA, ITP, and IMHA/ITP against a control group of dogs treated with conventional therapy for the same hematologic immune disorders; 71% of dogs treated with TPE achieved remission. Eighty-three percent of dogs treated for IMHA with TPE responded to therapy versus 65% in the control group. Eighty-two percent of dogs treated with TPE in the study survived to discharge (this group included patients with ITP and IMHA with ITP).

Serial PCV monitoring is important to gauge efficacy of drug therapy or determine the efficacy of transfusion medicine. However, it is important to remember that the patient's overall clinical picture can be more significant than isolated PCV values and trends coupled with physical examination findings should guide therapy (treat the patient, not the number). Patients that are clinical for severe anemia (bounding pulses, pallor, tachypnea, fatigue, depression) require rapid intervention. Nursing concerns include monitoring for signs of worsening anemia, monitoring for transfusion reactions, monitoring for adverse reactions to immunosuppressive medications, monitoring for DIC or sepsis, and monitoring for the increased respiratory effort that may be indicative of PTE. Patients receiving immunosuppressive doses of glucocorticoids and potent secondary immunosuppressives are not immunocompetent and therefore at an increased risk for infection and sepsis. Close attention should be paid to IV catheter sites, wounds, medical device insertion sites, and incisions if present.

IMHA induces a profoundly hypercoagulable state, so patients with IMHA are at an increased risk of thrombosis, particularly pulmonary thromboembolus (PTE) and sudden death. Clinical signs of PTE include acute dyspnea, tachypnea, cyanosis, hypoxemia, and hypothermia. Physical exam findings of PTE are variable but may include pulmonary crackles, tachypnea, tachycardia, and poor pulse quality. Thoracic radiographic findings are variable; however, hypovascular lung regions and/or alveolar infiltrates on dorsoventral or ventrodorsal projections may be seen along with right-sided cardiomegaly, pleural effusion, and main pulmonary vessel enlargement. Thoracic radiographs may also appear normal despite severe respiratory insufficiency. Diagnostic tests to confirm PTE include arterial blood gas (ABG), Doppler ultrasound of the main pulmonary vessels, pulmonary scintigraphy, and pulmonary angiography. Common ABG findings include decreased $PaO_2$, increased alveolar–arterial oxygen concentration difference, and decreased $PaCO_2$. A normal ABG does not rule out PTE. Treatment of PTE includes oxygen therapy, anticoagulant therapy with heparin, low molecular weight heparin (LMWH), platelet inhibitors, or low-dose aspirin, and fibrinolytic drugs such as streptokinase or tissue plasminogen activator (TPA). Repeated coagulation assessment is necessary to target anticoagulant therapy. Heparin or warfarin will not dissolve an existing clot but may help prevent the formation of additional microthrombi. Dissolution of existing clots may occur with administration of fibrinolytic drugs, but there are serious complications associated with their use, including hemorrhage. The prognosis for patients with severe PTE is generally poor.

Nursing management of the IMHA patient requires attention to detail and vigilant monitoring for signs of respiratory distress and potential PTE, evidence of worsening anemia, adverse transfusion reaction, signs of infection or sepsis, drug side effects, renal insufficiency due to intravascular hemolysis, unaddressed nutritional needs, and dehydration. Signs of dehydration should be addressed immediately in patients with IMHA as they are at a higher risk of venous stasis and hence thrombosis. Central venous catheters facilitate blood sampling but should be avoided if possible to decrease the risk of thromboemboli. Hemodynamic monitoring should include pulse quality, blood pressure, lactate, pulse oximetry, and urinary output. Electrolyte analysis, blood chemistries, and CBC testing should be performed to ensure that the patient is responding favorably to treatment. As patients with IMHA are on immunosuppressive drug therapy, signs of infection such as unexplained fever should be addressed immediately. Daily

intravenous catheter inspection is recommended for assessment of patency, possible phlebitis, thrombosis, or infection that necessitate an immediate catheter change.

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