Oh Bo, You're Yellow!

A Case of Immune-Mediated Hemolytic Anemia in a Dog

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Introduction:

Immune-mediated hemolytic anemia is both the most common hemolytic, and autoimmune, disorder of canine patients. Due to an overall mortality rate of approximately 50%, it continues to plague veterinary medicine, despite new and improving approaches to treatment.[10] Immune-mediated hemolytic anemia, or IMHA, refers to a plethora of clinical etiologies that leads to antibody mediated destruction of the body's own red blood cells. Typically a type II hypersensitivity reaction, IMHA can present as a primary (idiopathic) illness or secondary to an underlying cause. Most IMHA patients tend to present with a few non-specific clinical signs including lethargy, loss of appetite, vomiting, and diarrhea.[3] However, presenting clinical signs more specific to IMHA include tachycardia, tachypnea, exercise intolerance, pale or icteric mucous membranes, petechiation, systolic heart murmur, cranial abdominal organomegaly, red-tinged urine, and yellow-tinged feces.[7]

A diagnosis of IMHA is made by proving the presence of immune-mediated hemolysis, a process characterized by anti-erythrocyte antibodies. No singular diagnostic finding is pathognomonic for IMHA, so multiple tests should be considered when IMHA is suspected. Although a positive result of a direct Coombs' test is commonly considered the main diagnostic criteria for IMHA, there are other test results and diagnostic findings that can strongly indicate IMHA. The presence of spherocytosis on a blood smear, autoagglutination via a slide agglutination test, evidence of hemolysis (hemogobinemia, hyperbilirubinemia, hemaglobinuria, bilirubinuria), and regenerative anemia with a hematocrit of less than 30% all corroborate a presumptive diagnosis of IMHA.[3,5,8] In order to ascertain treatment goals, primary IMHA must be distinguished from secondary IMHA. This is achieved by performing more in depth diagnostics, including advanced diagnostic imaging and infectious disease panels, all in an effort to identify any potential underlying disease that could predispose to IMHA.[7]

Predisposing factors related to IMHA include breed, age, and gender. IMHA occurs more commonly in middle-aged female dogs than it does in male dogs or cats of any age. A genetic component may also be present due to breed predisposition and a tendency for familial occurrence in Collies, American Cocker Spaniels, English Springer Spaniels, and Poodles.[3] Fatal risk factors of IMHA include increased concentration of serum bilirubin, hypoalbuminemia, and thrombocytopenia.[7] Since prognosis is guarded with high mortality rates, it is common for patients to not live long enough for treatment to improve their condition.[3] Thus, death or euthanasia usually occurs within the first two to three weeks of treatment, most commonly related to disseminated intravascular coagulation or thromboembolic complications.[1,6,10] Even with patient survival, relapse may occur, so monitoring for these clinical signs may be necessary for the remainder of the pet's life.[5,9]

History and Presentation:

Bo, a 10-year-old male neutered Standard Poodle, presented to the Mississippi State University College of Veterinary Medicine Animal Health Center Emergency Service on January 21st, 2019 for evaluation of acute collapse and suspect IMHA. Two days prior to presentation, Bo became inappetent and lethargic, and his owners started noticing a dark red tint to his urine. By the morning of presentation, Bo's condition had progressed to include dull mentation and ataxia, most notably when he was outside. He then presented to his primary veterinarian around 11 a.m. for collapse. There, he was noted to have a thready pulse, rapid heart rate, and icterus. A blood sample was obtained for a serum chemistry and complete blood count. The serum chemistry revealed a moderately increased blood urea nitrogen (47.1 mg/dL), a markedly increased alkaline phosphatase (515 u/L) and gamma-glutamyl transferase (126 u/L), and a total bilirubin (>29.7 mg/dL) and triglycerides (>500 mg/dL) too high to read. The complete blood count revealed a moderately low hematocrit (14.9%), a moderate leukocytosis (29.2 K/uL), and a mild thrombocytopenia (96 K/uL). A 20-gauge intravenous catheter was placed in Bo's right cephalic vein, and he was administered 12 milliliters

of dexamethasone SP followed by a 0.9% NaCl fluid bolus. A Leptospirosis snap test, result for which was negative, was also run in house before referral. Bo was currently on Previcox for previously diagnosed arthritis and was prescribed Denosyl and Azodyl for increased liver and kidney values at his routine checkup three days prior to presentation.

On presentation, Bo was dull and depressed. He weighed 35.7 kilograms and was overweight with a body condition score of 7/9. He had a temperature of 100.9° F, a respiratory rate of 24 breaths per minute, and he was tachycardic with a heart rate of 180 beats per minute. Pulses were bounding and asynchronous. Mucus membranes were tacky and icteric with a capillary refill time of less than 2 seconds. Bo was dehydrated at 8% with a moderate reduction in skin turgor. Cardiothoracic auscultation revealed no crackles, wheezes, or murmurs, but a sinus arrhythmia was appreciated. Visible skin of the ear pinna was severely icteric. Abdominal palpation was soft and non-painful with no appreciable organomegaly or masses. Peripheral lymph nodes were of normal size and character. There was mild discomfort exhibited on flexion of his hindlimbs. The remainder of the physical exam was within normal limits.

A triage examination revealed occasional ventricular premature contractions on electrocardiogram consistent with Bo's asynchronous pulses. An osillometric blood pressure was performed and was within normal limits. Thoracic and abdominal FAST scans were performed, and no free fluid was noted. An accurate pulse oxygen level was unable to be determined due to the level of icterus of Bo's mucus membranes.

Diagnostic Approach/Considerations:

Based on Bo's weak, icteric condition and previous bloodwork performed by his primary veterinarian, the decision was made to obtain a blood sample for an initial packed cell volume, total protein, and slide agglutination test. Bo's anemia had worsened, with a packed cell volume of 14% and a normal total protein

of 7.5 g/dL. His slide agglutination test was positive with macroagglutination appreciated. At this time, IMHA was suspected and a coagulation profile and complete blood count were submitted. The coagulation profile was within normal limits (PT: 8.2 seconds, PTT: 10.4 seconds). The complete blood count revealed a severely decreased hematocrit of 10.7% and thrombocytopenia (43 K/ul), along with a moderate leukocytosis (32.8 K/ul), and a mild neutrophilia (26.6 K/ul) and monocytosis (2296 /ul). Pathologist review of the blood submitted for complete blood count revealed a significant amount of obvious and readily identifiable agglutination, suggestive of IMHA. Polychromasia and anisocytosis were also noted, however spherocytes were not seen.[5] A reticulocyte count was conducted to determine the character of Bo's anemia. The reticulocyte count was increased at 2.3%, indicating a regenerative anemia. To rule out the possibility of secondary IMHA due to tick borne disease, a 4DX snap test was performed, and results were negative. Although Bo's age and breed pointed towards idiopathic IMHA, to fully rule out a secondary etiology, and due to the late hour of Bo's presentation, further diagnostics were planned for the following morning. These included thoracic and abdominal radiographs, and abdominal ultrasound. Overnight, Bo continued to exhibit cardiac arrythmias with an inconsistent heart rate fluctuating between 170 and 300 beats per minute, so an electrocardiogram was submitted to IDEXX Laboratories for cardiologist review around midnight. Assessment of Bo's cardiac arrythmia revealed a sinus arrythmia with frequent atrial premature contractions, short paroxysms of supraventricular tachycardia, and intermittent single ventricular premature contractions. Considerations for the atrial premature contractions seen on electrocardiogram included atrial dilation, variations in autonomic tone, intra-abdominal disease, myocarditis, electrolyte abnormalities, and metabolic disease. Ventricular premature contraction considerations also included underlying cardiac or systemic disease. Given Bo's history, his cardiac arrhythmias were determined to most likely be secondary to IMHA.

Pathophysiology:

Immune-mediated hemolytic anemia is characterized by the destruction of erythrocytes coated with complement, immunoglobulin, or a combination of both. Canine cases of IMHA are most commonly due to erythrocyte binding of IgG and complement.[4] In idiopathic cases of IMHA, autoantibodies attack normal erythrocytes. Secondary IMHA leads antibodies to attack erythrocytes antigenically altered by the underlying cause of disease. This type II hypersensitivity leads to hemolysis either within the vasculature or extravascularly within the liver or spleen, and intravascular erythrocyte agglutination. Extravascular hemolysis occurs when IgG marked erythrocytes become phagocytized by specific macrophages, primarily found in the spleen, that possess multiple constant fragment (Fc) segment receptors meant to bind IgG. This form of ethrophagocytosis results in splenomegaly, and can overflow to the liver, thus resulting in hepatomegaly as well. Intravascular hemolysis occurs due to IgM synthesis and subsequent complement system activation. Following IgM binding, serine protease C1 can bind to erythrocytes as well, to activate the complement cascade. This generates the membrane attack complex (C8C9C5bC6C7) to lyse erythrocytes. C3b, a form of complement, attaches to erythrocytes and is removed by partial erythrophagocytosis, resulting in spherocytes. IgM binding to erythrocytes also causes agglutination by antiglobulin attaching to the antierythrocyte antibodies.[3,5] To prevent a response against host tissue in humoral immunity, regulatory T cells (Tregs) normally play a role of T cell and B cell suppression by way of cytokines and transforming growth factor beta production, inhibitory molecules, and destruction of target cells.[11] However in IMHA patients, peripheral immune response fails, and regulatory T cells become poorly controlled.[10]

Treatment and Management:

Immunomodulation is the cornerstone of immune-mediated hemolytic anemia treatment. Immediately upon arriving at a diagnosis of IMHA, glucocorticoids should promptly be administered in effort to dampen the destructive immune response. Commonly used glucocorticoids, such as oral

prednisolone, oral prednisone, or intravenous dexamethasone, decrease immunoglobulin affinity for erythrocytes and modify macrophage constant fragment receptors to inhibit immunoglobulin and complement recognition.[5] A second immunomodulatory drug may need to be started simultaneously on presentation if the patient is exhibiting immediately life-threatening clinical signs or if the packed cell volume has decreased by at least 5% within the last 24 hours. Additionally, some dogs may need a secondary immunosuppressive medication if they show an inadequate response to a single immunosuppressive agent. Common signs of inadequate response include requirement of multiple blood transfusions, persistent agglutination past the first week of treatment, or adverse reaction to glucocorticoid administration.[9] Commonly used secondary immunosuppressive medications include azathioprine, a purine analogue that alters DNA and RNA synthesis and immune functions; and cyclosporine, a calcineurin inhibitor that alters lymphocyte and macrophage activation to suppress cell-mediated immune responses.[5] Less commonly, leflunomide, a pyrimidine biosynthesis inhibitor that halts T and B cell proliferation by inhibiting tyrosine kinase, can be used. However it has been shown to be less effective than azathioprine or cyclosporine. Use of three or more immunosuppressive drugs in a non-responsive patient is contraindicated with IMHA treatment due to increased possibility of adverse effects. Human intravenous immunoglobulin may be used as a salvage attempt in patients who do not respond to a two immunosuppressive medication protocol. Human intravenous immunoglobulin decreases phagocytic activity by blocking macrophage constant fragment receptors. Immunoglobulin has been seen to increase packed cell volume recovery time and decrease the need for blood transfusions, but its use is not routinely recommended.[9]

Supportive care for IMHA patients should include anticoagulant therapy, gastroprotectants, and fluid therapy.[5] Glucocorticoid use and increased rate of hemolysis both have the potential to create a hypercoagulable state in IMHA patients leading to thrombogenesis, specifically pulmonary thromboembolism. [6] Evidence of coagulation can be determined by thrombocytopenia, prolonged

coagulation times, and widespread fibrin present at necropsy. High levels of circulating inflammatory cytokines and free hemoglobin may contribute to endothelial cell tissue factor expression inducing thrombogenesis, as well as disseminated intravascular coagulation.[2,3] Thus, medications such as heparin, an anticoagulant that prevents adherence of fibrin and platelets to thrombin, should be prophylactically administered to all dogs presenting with IMHA.[5] However, this may be contraindicated in patients presenting with severe thrombocytopenia. Anticoagulant therapy with clopidogrel or aspirin may also be considered due to tendency of venous thromboembolism formation in fibrin rich, low blood flow scenarios in dogs.[2,9]

Acute onset of anemia combined with anorexia predisposes IMHA patients to gastric ulceration due to poor gastrointestinal perfusion. Gastroprotectants, including histamine blockers, such as famotidine; prostaglandin analogues, such as misoprostol; proton pump inhibitors, such as omeprazole; and medications to coat ulcers, such as sucralfate, may be utilized if there is risk of gastric ulceration or bleeding. Although placement of intravenous catheters for fluid therapy may contribute to thrombi formation with IMHA, it should be done, as the advantages outweigh the disadvantages in helping to stabilize critical patients. Blood transfusions may be necessary in patients exhibiting a moderate to severe anemia (6-23% packed cell volume) or potential for tissue hypoxia.[5] Packed red blood cell transfusions, no more than 7-10 days old, are ideal. However, whole blood can be used as an alternative.

When it comes to secondary IMHA specifically, appropriate antimicrobials should be administered while awaiting diagnostic test results pertaining to possible infection.[9] Administration of doxycycline or tetracycline covers a wide variety of infections, such as ehrlichiosis in dogs and hemobartonelosis in cats. All previous medications should be halted in case the patient developed IMHA secondary to a drug administration. Additionally, due to the reported possibility of IMHA being linked to a recent vaccination event, discontinuation of the current vaccine schedule should be considered.[9]

In regard to continued management of the disease, if the patient remains stable and the packed cell volume is maintained above 30%, the glucocorticoid dose should be decreased by 25% at two weeks post initiation of treatment. Decreases of 25% in the glucocorticoid dose should be performed after each three-week period. If a secondary immunosuppressive is being used as well, the dose should be unchanged until glucocorticoid treatment is discontinued. Following discontinuation of glucocorticoid use, secondary immunosuppressive use should be tapered off in a similar fashion. Four to eight months should be expected for complete resolution of immunosuppressive treatment. Each reduction milestone should be preceded by a measurement of packed cell volume, presence of spherocytes and/or agglutination, and assessment of serum bilirubin to guarantee continued response to treatment. Even with survival or complete resolution of IMHA, relapse is possible. Immunosuppressive medications should be tapered at a slower rate if relapse occurs prior to resolution of the disease. If multiple subsequent relapses occur, lifelong immunosuppressive therapy may be considered to maintain remission.[9] As a last resort, refractory IMHA cases may be subject to splenectomy consideration. Removal decreases antibody production by eliminating a source of B cells and macrophages, potentially reducing immune-mediated burden.[5]

In Bo's case, he was admitted to the intensive care unit for overnight treatment and monitoring. An intravenous fluid bolus of PlasmaLyte A was administered initially. While in hospital, Bo was started on maropitant citrate for nausea, pantoprazole for gastroprotection, doxycycline for possible secondary infection, clopidogrel for anticoagulation, enoxaparin for prophylaxis, atenolol for cardiac arrhythmia, and methadone for pain control. Throughout the night, he received intravenous Plasmalyte A fluid therapy. Due to his low hematocrit, Bo was also administered 470 milliliters of a sodium chloride diluted packed red blood cell transfusion.

Outcome:

Overnight, Bo's condition steadily declined. His cardiac electrical activity was continuously monitored through the night via electrocardiogram due to persistent cardiac arrhythmias. During his 2 a.m. walk, hematuria was noted. Bo's respiratory rate and effort increased throughout the night. Around 4:30 a.m., his respiratory rate significantly increased, and he began having more frequent ventricular premature contractions. A thoracic FAST scan was performed, but no abnormalities were seen. An SpO2 was also performed measuring 90% on room air. Bo was administered flow-by oxygen therapy and his SpO2 subsequently saturated to 100%. At this time, he appeared to stabilize with resolution of his cardiac arrythmias on electrocardiogram. However, he seemed uncomfortable and had severe tachypnea. Bo became markedly weak and was unable to rise by himself, so assistance was required for another walk around 6:30 am. At this time, worsening hematuria was noted upon elimination. On return to his kennel, Bo became apneic and went into cardiopulmonary arrest at 6:40 a.m. Cardiopulmonary resuscitation was performed but unsuccessful, and Bo was pronounced dead at 6:55 a.m. on the morning of January 22nd, 2019.

His body was submitted for necropsy examination which revealed a necrosuppurative myocarditis with interstitial fibrosis, pulmonary edema, extramedullary hematopoiesis of the liver and spleen, bridging portal fibrosis of the liver, splenomegaly, myodegeneration of skeletal muscle, reactive bone marrow, and severe, diffuse icterus of soft tissues. Pathologist assessment of Bo's myocarditis and fibrosis suggested systemic disease as the potential etiology. Although immune-mediated disease has been reported as a potential cause of myocarditis, a definitive etiologic diagnosis could not be determined.[1] In addition, a potential underlying disease process contributing to hemolysis was unable to be identified, suggestive of idiopathic immune-mediated hemolytic anemia in Bo's case.

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