Immune-mediated hemolytic anemia in the canine

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Class of 2018

Clinicopathologic Conference

Presented March 16th, 2018

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Introduction

Immune-mediated hemolytic anemia (IMHA) is among the most common autoimmune conditions affecting the dog (1). Immune-mediated hemolytic anemia (IMHA) occurs when antibodies and complement molecules of the immune system target red blood cells for destruction via macrophage phagocytosis in the spleen or liver, termed extravascular hemolysis, or through complement-mediated cytolysis within the circulation, termed intravascular hemolysis (3). IMHA may be primary, as an autoimmune or idiopathic disease, or secondary, from causes ranging from parasitic to neoplastic. Immune-mediated hemolytic anemia can also occur in the face of other diseases such as immune-mediated thrombocytopenia. IMHA is the most common immune-mediated disease of dogs with predisposed breeds including Cocker Spaniels, Springer Spaniels and Old English Sheepdogs (3). It is usually seen in middle-aged to older dogs and has no sex predilection.

History and presentation

Bonnie is an 11 year old female spayed Cairn Terrier that recently moved from Houston, Texas to Oxford, Mississippi, two weeks prior to presentation. She is up to date on vaccinations that she received in February of 2017 and is on Revolution for parasite prevention. Bonnie has had a very healthy life and no pertinent medical history aside from surgery on both patellas. On December 25th, Bonnie's owner noticed that she was not acting herself as she began to lose interest in going on walks, her appetite was decreasing, and she overall was not acting normally. She had one episode of vomiting prior to presentation, but she was still eating some and drinking at home. Bonnie's owner reported that recent bowel movements were "dark and hard in appearance." She was taken to Crossroads Animal Clinic in Oxford on December 26th, 2017. She was severely anemic with a hematocrit of 19.2% and a low red blood cell count of 2.96 M/uL.

She was also thrombocytopenic with platelets at 107 K/uL on bloodwork; however, a manual count was not performed. A grade 2/6 heart murmur was also noted. During this visit, she was started on a tapering dose of prednisone at 1.0 mg/kg (5 milligrams orally twice daily for five days, then one tablet once daily for five days then one tablet every other day until gone) and doxycycline at 10.0 mg/kg (100 milligrams orally every 12 hours). She was also prescribed Vitamin K at 3.0 mg/kg (50 milligrams orally every 24 hours) until the results of her coagulation profile returned. By December 28th, Bonnie was more lethargic. She began to exhibit polyuria and polydipsia and her appetite did not improve while on steroids. She had not had a bowel movement since December 26th. A complete blood count repeated at Crossroads revealed worsening anemia with a hematocrit of 12.6% and a worsening red blood cell count of 1.73 M/uL. She was also persistently thrombocytopenic with bloodwork revealing a platelet count of 67 K/uL. A manual platelet count was not performed prior to presentation. She was then referred to MSU College of Veterinary Medicine.

Upon initial presentation to the MSU-CVM Emergency Service on December 28th, 2017, Bonnie was quiet but alert. She had a heart rate of 160 beats per minute with a grade 3/6 heart murmur, a respiration rate of 40 breaths per minute with normal bronchiovesicular sounds bilaterally, and a temperature of 101.0 degrees Fahrenheit. Her mucous membranes were pale with a capillary refill time of less than two seconds. An ECG revealed a normal sinus rhythm. She weighed 8.1 kilograms with a body condition score of 6/9. Upon rectal palpation, dry, dark feces was noted consistent with melena. The rest of her physical examination was unremarkable. A complete blood count revealed a hypochromic anemia and normal manual platelet count of 187 K/ul. Her chemistry revealed mild hyponatremia (140.7 mmol/L), mild hyperglycemia (152 mg/dl), moderately increased blood urea nitrogen (29 mg/dl), severely increased liver enzymes

(ALT 600 U/L and ALP 208 U/L), moderate hypoproteinemia (4.8 g/dl) and mildly increased creatinine kinase (344 U/L). A 4DX snap test ruled out heartworm disease, Lyme disease, Erhlichiosis and Anaplasmosis. Thoracic radiographs revealed a mild, diffuse unstructured interstitial pattern likely due to aging changes. She had a normal cardiac silhouette. Abdominal radiographs revealed intervertebral disc disease at L6-L7 and spondylosis deformans of multiple lumbar vertebrae.

Bonnie's packed cell volume upon presentation was 14%, with total solids of 6 g/dl. Her coagulation panel had a normal prothrombin time and a decreased partial thromboplastin time of 6.9 seconds. Due to her historical worsening clinical signs and anemia, a blood transfusion needed to be performed. She was blood typed prior to the transfusion and was confirmed to be DEA 1.1 positive. A slide agglutination test was negative. A Coombs' test was weakly positive. Her corrected reticulocyte count was at 3% indicative of regeneration. Bonnie was initially started on prednisolone at 1.8 mg/kg (15 milligrams orally every 24 hours), cyclosporine at 6.25 mg/kg (50 milligrams orally every 12 hours), Cerenia at 1 mg/kg (0.8 milliliters) intravenously every 24 hours), pantoprazole at 1 mg/kg (40 milligrams (2.0 milliliters) intravenously every 12 hours), doxycycline at 6 mg/kg 50 milligrams every 24 hours), and Plasmalyte fluids at maintenance of 20 milliliters per hour. Her fluids were discontinued the next day and Cerenia was switched to oral with one 2 mg/kg tablet orally every 24 hours. Omeprazole at 1 mg/kg (10 milligrams orally every 12 hours) was added, as well as clopidogrel at 1.5 mg/kg (12 milligrams orally every 24 hours). On day five, metronidazole at 7 mg/kg (60 milligrams orally every 12 hours) was added, as well as sucralfate 1 gram in a slurry every 6 hours due to her continued melena and possible gastric ulcer. Two days later prednisolone and clopidogrel were discontinued due to the persistent melena and possible gastrointestinal ulcer. Her appetite

continued to be decreased and mirtazapine at (0.6 mg/kg (15 milligrams orally per day) was added to the treatment regimen. Enoxaparin at 0.8 mg/kg (6.32 milligrams (0.06 milliliters) subcutaneously every 6 hours) was also added to prevent thromboembolism. Doxycycline was discontinued on day 9. Gabapentin at 5/5 mg/kg (50 milligrams orally every 12 hours) was added on day 10 to help with pain elicited on palpation of her back.

On the night of 12/28/17, a blood transfusion of 120 milliliters of whole blood was given over five hours without any complications. She was blood typed prior to the transfusion and was confirmed to be DEA 1.1 positive. The donor was DEA 1.1 positive. Two hours post transfusion her packed cell volume was at 21%. The next morning (12/29/17) her packed cell volume was 23% and total solids were 5.8 g/deciliter. An abdominal ultrasound performed on 12/29 revealed a small amount of anechoic free fluid throughout the abdomen, gall bladder sludge, and most notably, changes to the splenic head. Fine needle aspirates of the spleen were performed and a small amount of echogenic free fluid at the splenic head was seen following aspiration due to hemorrhage. The renal cortices were hyperechoic wit hyperechoic striations. There was also bilaterally decreased corticomedullary distinction that could be due to diffuse nephrocalcinosis and glomerulo- or interstitial nephritis. Cellular and/or proteinaceous urinary bladder debris was present. Within the left limb of the pancreas, a small, smoothly marginated, hypoechoic nodule measuring 0.31 cm in thickness was seen with differentials including nodular regeneration, pancreatic pseudocyst or neoplasia. Within the mesentery of the mid cranial abdomen and right mid abdomen, there were at least three ovoid, smoothly marginated, hypoechoic structures that were adjacent to the wall of the few segments of the small intestines (the largest being 1.35 cm in thickness). Possible organs of origin include lymph node, mesentery, small intestines, and ectopic spleen with differentials of reactive lymphadenopathy, metastatic neoplasia or ectopic

spleen. Two fine needle aspirates were performed on these nodules and cytology was non-diagnostic. Urinalysis from a voided sample revealed many bacteria and urine specific gravity of 1.013. However, she did not exhibit any signs of a urinary tract infection during her stay.

The night of 12/29, her packed cell volume was at 20% and total solids were 6.0 g/deciliter. Bonnie had two bowel movements. The first was darker feces but firm. The second bowel movement contained melena, alerting us to sustained internal bleeding, raising our suspicion of a gastrointestinal ulcer. On December 30th her PCV was at 25% and her total solids were 5.8 g/deciliter. A second CBC revealed moderate anemia and thrombocytopenia with a manual platelet count of 64 K/ul. The chemistry revealed persistent increased liver enzymes, increased bilrubin and hypoproteinemia. On 12/31, Bonnie's PCV was at 22% with total solids of 5.8 g/deciliter.

Bonnie's PCV on the morning of 1/1/18 was 17% and total solids were 5.8 g/deciliter. Her mucous membranes were paler than previously noted. A complete blood count revealed moderate anemia with resolved thrombocytopenia as her manual platelet count was normal at 208 K/ul. A chemistry revealed increased BUN, increased liver enzymes (decreased since previous chemistry), elevated bilirubin, hypoproteinemia, hypocalcemia and hypocholesterolemia. Her PCV that afternoon was 16% and total solids were 5.4 g/deciliter.

Due to her decreasing packed cell volume and clinical signs, a second blood transfusion was performed. One hundred and twenty milliliters of whole blood was administered over four hours with no complications. Two hours post transfusion her PCV was at 22% and 24%. Her packed cell volume was checked twice daily until she was discharged, ranging between 20-25%. On 1/2/18, her PCV was at 25% that morning and total solids were 5.8 g/deciliter Her PCV that night was 23%. On 1/3/18 her PCV was at 21% that morning (total solids 5.4 g/deciliter) and that

night her PCV was at 22% with total solids of 5.4 g/deciliter. Bonnie had bowel movements on 1/3 and 1/4/18 that were loose and consisted of melena. On 1/4/18 her PCV was at 22% (total solids 5.4 g/deciliter) and that afternoon her PCV was down to 20%. On 1/5/18 her PCV that morning and afternoon were both at 21%. She also had a bowel movement that was dark but starting to form back to normal consistency. Due to her PCV not consistently improving, it was decided that a repeat abdominal ultrasound needed to be performed.

A repeat ultrasound (compared to one week previously) revealed resolution of the previous abdominal fluid. Within the left craniodorsal abdomen, a smoothly marginated, ovoid, approximately 2.73 cm in thickness, heterogeneous mass was found that was hypoechoic to the surrounding mesentery. Within a portion of the duodenum, there was a thin, linear, hyperechoic region within the mucosa. Abutting several segments of the small bowel were multiple smoothly marginated, ovoid, (1.23 cm x 0.84 cm being the largest), hypoechoic nodules with some causing loss of wall layering of the smooth bowel segment. Two fine needle aspirates of the craniodorsal mass and one of a nodule abutting a segment of small bowel were obtained. A small amount of free fluid was present post aspiration; however, follow up aFAST revealed resolution of the fluid. There was possible evidence of a gastrointestinal ulcer due to the presence of gas tracking to the nodules.

On 1/6/18 Bonnie's PCV was at 21% that morning and 20% that night. On 1/7/18, PCV was at 24% with total solids at 5.8 g/deciliter that morning. It remained steady throughout the day. On 1/8/18, her PCV was at 24% that morning and total solids at 5.8 g/deciliter. Bonnie looked clinically well and her packed cell volume remained stable. A third complete blood count revealed a regenerative anemia that was improving with a manual platelet count of 144 K/ul. Bonnie was sent home on 1/8/18 on cyclosporine at 6.3 mg/kg (50 milligram orally every 12

hours), Cerenia at 2 mg/kg (16 milligrams orally every 12 hours), omeprazole at 1mg/kg (16 milligrams orally every 12 hours), metronidazole at 7 mg/kg (50 milligrams orally every 12 hours), sucralfate 1 gram (orally in slurry every 6 hours), gabapentin at 5.5 mg/kg (50 milligrams orally every 12 hours), mirtazapine at 0.6 mg/kg (15 milligrams every 24 hours), and fenbendazole 100mg/ml (3.6 milliliters orally) for one more dose. Instructions included returning to her rDVM on 1/10/18 for a recheck of her blood work and then to return to MSU on 1/29 to assess her response to therapy.

Pathophysiology/Anatomical considerations

Immune-mediated hemolytic anemia is a pathologic process that results in the premature destruction of red blood cells when antibodies target red blood cells of all ages (3). The normal lifespan of the canine red blood cell is 100-120 days. Removal of mature red blood cells occurs through the liver and spleen by the mononuclear phagocyte system, or MPS. Fc receptors on macrophages in the liver and spleen bind to the Fc component of antibody coating the red blood cell membrane causing phagocytosis and destruction of the red blood cell. The mononuclear phagocyte system senses antibodies that are directed against senescent membrane antigens and clears them from circulation (3).

Immune-mediated hemolytic anemia is considered a type II hypersensitivity reaction, where anti-red blood cell antibodies including IgG, IgM and IgA, attach directly or indirectly to parts of the red blood cell membrane. This attachment can cause hemolysis, either intravascular or extravascular, and red blood cell agglutination. With severe immune reactions, large numbers of antibodies attach to the red blood cell membrane and activation of the complement cascade stimulates the membrane attack complex. The membrane attack complex causes direct damage to the cell membrane with an influx of extracellular fluid into the red blood cell and rupture of the

cell while in circulation. This process is termed intravascular hemolysis, resulting in hemoglobinemia and hemoglobinuria, occurring with IgM-mediated disease due to IgM being better than IgG at fixing complement. In less severe cases of IMHA, with minimal complement-mediated cell wall damage, antibody attachment with IgG leads to destruction of red blood cells by the mononuclear phagocyte system. This process occurs outside of circulation, termed extravascular hemolysis. Hemolgobinemia and hemoglobinuria are not present with extravascular hemolysis because red blood cell hemoglobin enters the bilirubin metabolic pathways instead of spilling into the circulation (3).

Immune-mediated hemolytic anemia can be divided into primary (idiopathic) or secondary. Primary immune-mediated hemolytic anemia occurs as an autoimmune disorder with no detectable underlying cause. It is the predominant form of IMHA (3). During primary IMHA, autoantibodies are produced against the patient's red blood cell membrane antigens, specifically targeting glycophorin, a glycoprotein that spans the plasma membrane. In a normal animal, autoantibodies are prevented from reacting with the host tissue by suppressor T cells. However, it has been hypothesized that animals with IMHA have poorly regulated suppressor T cell function and/or overstimulated immune systems that allow the autoantibodies to attach to normal cells and cause red blood cell destruction (3). Secondary IMHA occurs when the surface of the red blood cell becomes altered by an underlying disease process, pathogen, xenobiotic, or toxin. Causes studied include bacterial, viral, infectious (parasitic), drug-induced, inherited red blood cell defects, and neoplastic disorders. Other causes include onion, garlic, zinc, bee-stings, and vaccinations. Studies have shown that patients vaccinated within 30 days of presentation have a temporal association to developing IMHA (3). No specific vaccine has been found to cause IMHA directly; however, it is believed that vaccines are a nonspecific trigger that activate

macrophages, heighten a low grade inflammatory condition or deregulate the balance of the immune system. Drugs such as sulfa antibiotics, penicillin's, cephalosporins, levamisole, insulin, acetaminophen, tetracycline, phenylbutazone, dipyrone, quinidine and chlorpromazine have been identified as causes of IMHA with limited proof (3). The major proposed mechanism for penicillin and cephalosporin associated causes is adhesion of the drug or drug breakdown products to the red blood cell membrane, inducing complement attack or cell removal by the mononuclear phagocyte system. Sulfonamides, quinidine, insulin, acetaminophen and tetracycline may induce IgM antibody production (3). The drug-antibody complex binds to red blood cell membranes and initiates complement activation causing intravascular hemolysis (3).

Immune-mediated hemolytic anemia is a disease more common in dogs than cats. Primary IMHA can occur in any breed; however, spaniel breeds, poodles, Old English sheepdogs, Irish setters and collies are overrepresented (3). Studies have found IMHA to be more present in females (3). The mean age of acquiring the disease is 6 years; however it can develop between the ages of 1-13 years old. Some studies have shown increased incidences of IMHA during warmer months that may be attributed to underlying infectious or tick-borne diseases (3). Studies have suggested a variety of prognostic factors associated with mortality in IMHA including severity of anemia, presence of autoagglutination, thrombocytopenia, leukocytosis, absence of erythrocyte regeneration, increased serum alkaline phosphatase, increased total bilirubin, and decreased serum concentration of albumin (14).

If the onset of the anemia is acute or slow, clinical signs can be minimal until the anemia progresses in severity. Tachypnea, tachycardia, and increased cardiac output can compensate for anemia only to a certain extent. Findings in a patient's history with IMHA can include collapse, weakness, exercise intolerance, lethargy, anorexia, tachypnea, dyspnea, vomiting and diarrhea.

Polyuria and polydipsia are occasionally present (3). Clinical signs can be minimal at rest but worsen during periods of stress or exercise.

Findings on physical examination include pale mucous membranes, tachypnea, splenomegaly, hepatomegaly, icterus, pigmenturia (hemoglobinuria or bilirubinuria), fever, and lymphadenopathy (3). Cardiovascular changes include tachycardia, S3 gallop and systolic murmur. A grade 2 or 3 of 6 systolic hemic murmur is detected in animals with packed cell volume of less than 15-20% caused by anemia-associated blood turbulence (3). Approximately 50-70% of dogs with immune mediated hemolytic anemia have concurrent thrombocytopenia (Evans syndrome) (3). Uncommonly, systemic signs including polyarthritis and glomerulonephritis may be identified.

Immune-mediated hemolysis can be caused by alloantibodies directed against red blood cell membrane components. Alloantibodies are antibodies produced by an individual that react with antigens in another member of the same species. Causes of alloimmune hemolytic anemia include blood group incompatibility transfusion reactions and neonatal isoerythrolysis. Causes of non-immune-mediated hemolytic anemia must be differentiated from immune-mediated hemolytic anemia. Common causes of non-immune mediated hemolytic anemia include inherited defects, toxins, hypophosphatemia, zinc, and microangiopathic hemolytic anemia (3).

Differentials in young animals with hemolytic anemia include hereditary disorders such as pyruvate kinase deficiency (in Basenjis commonly) or phosphofructokinase deficiency in spaniels (3). Alaskan malamutes and miniature schnauzers can harbor hereditary red blood cell osmotic fragility. Toxin exposure can include zinc (ingesting pennies after 1983, zinc oxide diaper ointment, sunscreen), acetaminophen, onion, garlic and oxidant agents leading to Heinz body anemia. Hypophosphatemia is associated with treatment of diabetic ketoacidosis or

aggressive nutritional support in malnourished patients. Microangiopathic hemolytic anemia occurs when red blood cells that are damaged while in circulation are removed by the mononuclear phagocyte system. Causes of microangiopathic hemolytic anemia include heartworm disease, vascular or gastrointestinal neoplasia, heart valve disease, intravenous catheters, splenic diseases or torsion, liver disease and disseminated intravascular coagulation (3).

Diagnostic Approach/Considerations

One must differentiate primary versus secondary immune mediated hemolytic anemia to determine effective treatment. “To diagnose primary IMHA, suggested adequate criteria must be met that include anemia with a hematocrit less than 25-30%, evidence of hemolysis characterized by hemoglobinemia or hemoglobinuria, evidence of antibodies directed against red blood cells with autoagglutination, spherocytosis, or positive results from Coombs’ test, elimination of other underlying causes of anemia and an appropriate response to immunosuppressive therapy” (3). Primary IMHA can be treated with aggressive immunosuppressive therapy. Secondary IMHA will respond to treatment if directed at the underlying cause and may worsen with immunosuppressive therapy. The presence of spherocytosis and autoagglutination, even with a negative Coombs’ test, strongly supports a diagnosis of IMHA (11).

A complete blood count with a reticulocyte count is the most beneficial test to diagnose immune-mediated hemolytic anemia. “The classic patient with IMHA has mild to severe, highly regenerative anemia” (3). A blood smear should be performed to identify signs of regeneration such as reticulocytosis, polychromasia, anisocytosis, and nucleated red blood cells. Immature red blood cells that have extruded their nucleus but contain polyribosomes, ribosomes and mitochondria are called reticulocytes. The number of reticulocytes increases when responding to

blood loss or hemolytic diseases They can be seen using new methylene blue which induces the ribosomes to clump into granules. A reticulocyte count is obtained by counting the number of reticulocytes in 1,000 cells and multiplying the resultant percentage by the total red blood cell count to achieve the number of reticulocytes per microliter of blood (3). An absolute reticulocyte count greater than 60,000/µl indicates regeneration. The percentage of reticulocytes per 1,000 cells can be used to determine a corrected reticulocyte percentage via the patient's packed cell volume multiplied by the reticulocyte percentage, divided by the normal packed cell volume in a dog of 45%. If it is greater than 1% in an anemic patient, regeneration is present (3). Approximately one-third of patients with IMHA present with poorly regenerative anemia due to an acute onset, lacking sufficient time to mount an adequate regenerative response (3). Marked spherocytosis is highly suggestive of IMHA. A complete blood count will reveal leukocytosis with a neutrophilic left shift that can alert a clinician to potential tissue damage secondary to anemia hypoxia (3). A serum biochemistry and clotting times should be performed to evaluate the presence of hyperbilirubinemia, disseminated intravascular coagulation and/or underlying diseases. Blood chemistry abnormalities include hyperbilirubinemia and increased liver enzymes, ALT specifically. These can be a result of hepatocellular damage, thromboembolism and ischemia (3). Hyperbilirubinemia may not be present in mild or chronic cases if bilirubin produced does not overwhelm the hepatic bilirubin metabolic pathway. Further diagnostics such as PCR for blood-borne infectious agents, heartworm testing, and fecal examination are warranted.

A slide agglutination test should be performed in which one drop of anticoagulated whole blood is placed on a slide and one drop of physiologic saline is added. Visualization of agglutination, in which antibody molecules are present on the red blood cells, is done both

grossly and microscopically. To differentiate from rouleaux, knowing that persistent agglutination does not disperse with saline is important. A positive saline agglutination test is common in patients with immune-mediated hemolytic anemia. It is possible that a patient may have too low anti-red blood cell antibody levels to cause agglutination. A Coombs' or antiglobulin test can then be performed. The Coombs' test can be direct in which it detects antibodies attached to red blood cells, or indirect in which it detects antibodies to red blood cells in the serum. The direct Coombs' test should be used in a patient with IMHA due to antibodies of most concern being attached to the red blood cells (3). However, it is important to note that the direct Coombs' test has varying sensitivity. Therefore, a negative test result does not rule out a diagnosis of IMHA (3).

Diagnostic imaging should be performed to detect any underlying diseases. Thoracic radiography should be performed to assess for underlying diseases and neoplasia. Abdominal radiography and ultrasound should be performed to assess liver and spleen size, and detect any foreign bodies or mass lesions. If an animal presents with severe tachypnea, it should be evaluated for pulmonary thromboembolism, which is a complication of the hypercoagulable state of IMHA. "Thromboembolism has been cited as the most common complication in dogs with primary IMHA" (12). It is likely that preventing thrombosis is as important as controlling hemolysis if survival rates are to be improved (4). It causes no pathognomonic radiographic changes; therefore, it is difficult to diagnose. "The patient may appear clinically healthy or markedly hypoxic. Depending on the degree of hypoxia, patients may exhibit tachypnea, labored breathing, cough or hemoptysis, sudden collapse or altered mentation (15)". Pulmonary thromboemoblism should be strongly suspected in a dyspneic patient with immune-mediated hemolytic anemia even if normal thoracic radiographs are present. "Pulmonary

thromboembolism, thought to occur due to the release of venous emboli, is very common in dogs with IMHA (4).” The etiology of thromboembolism in IMHA remains unclear. The primary factors involved in the pathogenesis of thrombosis, as defined by Virchow’s triad, are as follows: changes in blood flow, in the balance of procoagulatory and anticoagulatory substances in the blood, and in vessel walls (5). A high number of transfusions has been associated with greater risk of pulmonary thromboembolism in dogs with IMHA (5). The association of thromboembolism with various treatments from immunosuppressive medication, intravenous catheterization, and blood product transfusion complicates the process (12). `

IMHA at the bone marrow level (which is usually non-or inappropriately regenerative) may lead to bone marrow damage and secondary myelofibrosis. “In patients with IMHA, bone marrow analysis typically reveals hyperplasia of the erythroid series” (3). “IMHA is often associated with a strongly regenerative bone marrow erythroid response, but a high percentage of dogs and cats with IMHA do not have peripheral reticulocytosis at the time of diagnosis. (17). Non-regenerative IMHA has been associated with bone marrow hyperplasia or with erythroid maturation arrest (17). “The pathogenesis of non-regenerative IMHA involves both antibody-mediated destruction of bone marrow precursor cells and pathological evens within the bone marrow that result in ineffective erythropoiesis” (17). Further diagnostics for IMHA’s should be performed if it is non-regenerative or poorly regenerative, and/or if all cell lines are affected, include performing bone marrow aspiration cytology and histopathology of the marrow core biopsy (3).

Treatment and Management

Treatment depends on a diagnosis of primary versus secondary IMHA. With primary immune mediated hemolytic anemia, aggressive immunosuppressive therapy is standard

treatment. Prednisolone at 1 mg/kg orally every 12 hours can be given to clinical effect with slow tapering and eventual withdrawal (2); however, many dogs relapse post withdrawal and may require long term therapy. Gastroprotectants should also be administered to dogs receiving high doses of steroids due to potential ulcer formation. If a patient presents with clinical signs indicative of the need for a transfusion, it is recommended to first blood type them, then administer whole blood or packed red blood cells. Reactions to transfusions include tachycardia, tachypnea, fever, vomiting, or diarrhea. It may be necessary for a patient to have more than one transfusion during hospitalization or even later in life if they relapse. If the patient is in the hospital for more than four to five days and requires a second transfusion, cross matching should be performed prior to subsequent transfusions

Glucocorticoids, cyclophosphamide, azathioprine, and cyclosporine are commonly used as second-line treatments. Human intravenous immunoglobulin, leflunomide, clodronate and mycophenolate mofetil have also been used (7). The mechanism of action of human intravenous immunoglobulin occurs by modulation of expression and function of Fc receptors, interference with activation of B and T cells and complement, and a decrease in immunoglobulin production (13). Adverse effects of glucocorticoid use include polyuria, polydipsia, panting, weight gain, delayed wound healing, alopecia, secondary infections, and muscle wasting (7). Advantages of using mycophenolate mofetil over other immunosuppressive drugs are availability of oral and parenteral forms, rapid onset of action and lack of myelosuppression or hepatotoxicity (7). Azathioprine is available for oral use only and adverse effects include myelosuppression, hepatotoxicity, pancreatitis and gastrointestinal upset. Adverse effects of cyclosporine include vomiting and diarrhea, excessive shedding, gingival hyperplasia, papillomatosis, secondary infections, and neoplasia (7). Adverse effects, in addition to expense, of human intravenous

immunoglobulin include anaphylaxis, risk of type II hypersensitivity reactions, hypercoagulability and hypertension (7).

If IMHA is severe enough to require more than steroid therapy, azathioprine can be administered at 2 mg/kg orally every 24 hours (3). Drugs such as azathioprine are put to use in severe disease cases or if the patient's treatment is not resolved with glucocorticoids alone (9). Adverse effects of long term cytotoxic drugs include neoplasia, leukemia, and testicular and ovarian dysfunction (9). Antithrombotic drugs either prevent the formation of arterial thrombi by targeting platelets (anti-platelet drugs), or they prevent the formation of venous thrombi by targeting the coagulation cascade (4). Although development of anemia is what we see clinically, it is important to note that other processes such as thromboembolic disease contribute to the overall morbidity and mortality of the disease (8).To prevent pulmonary thromboemoblism, it is recommended to administer anticoagulants such as aspirin. Because of its availability, lack of need for monitoring, ease of administration, and minimal cost, using ultra low dose aspirin is standard antithrombotic therapy for dogs with primary IMHA (10). It appears that IMHA may cause generalized thromboembolic disease of both veins and arteries, with pulmonary thromboembolism being a very common clinical manifestation of thrombosis (4).

It is imperative to make owners aware that this disease process can relapse and their pet may need multiple transfusions and/or lifelong therapy. Although administration of glucocorticoids provides the first line of defense in IMHA, combination treatment is warranted for nonresponsive dogs, dogs with intolerance to high dosages of glucocorticoids, and dogs that require long-term treatment (6). Mortality rates for idiopathic IMHA range from 22 to 80% with higher mortality rates early in the disease process (7).

"One difference in the treatment of IMHA between human and veterinary patients is that splenectomy is much more commonly performed in humans (16)". Splenectomy is a potential option in patients that do not respond to immunosuppressive therapy or unwanted adverse effects are exhibited. Performing a splenectomy can improve the survival of red blood cells that have structural or metabolic abnormalities impairing the ability of the cell membranes to deform when passing through vessels and capillaries. In this study, IMHA dogs that had a splenectomy performed, showed a rapid increase in PCV and a reduction in the need for transfusions (16). "A commonly cited risk associated with splenectomy in dogs is Mycoplasma canis infection (16)". An infection with *Mycoplasma canis* will cause destruction of red blood cells.

Case outcome

Bonnie was scheduled to return to MSU CVM Animal Health Center on 1/29/18 for a recheck of her bloodwork. She presented on 1/26/18 for vomiting, diarrhea and shivering. Bonnie's owner called the emergency service around 7:00 that morning because the night before, Bonnie had two episodes of vomiting, one episode of diarrhea that may have consisted of melena after her rDVM visit and bouts of shivering. She appeared to be painful to her owner as she would crawl up in a ball and when getting back up, had an arched back. Mrs. Alexander was advised to bring Bonnie it for repeat bloodwork and an abdominal ultrasound. Upon presentation, Bonnie was bright, alert and responsive. She currently weighed 6.6 kg (previously was at 7.9 kg). Her body condition score was a 5/9. Her temperature was 102.2 degrees Fahrenheit, heart rate of 160 beats per minute and she was panting. No murmurs or wheezes or crackles were heard upon auscultation. She seemed to be somewhat nervous which could cause increased vital parameter numbers. Her mucous membranes were pink and she had a capillary refill time of less than two seconds. Pain was elicited on palpation of her back. Her abdomen was

non painful. A rectal revealed mild melena. The rest of her physical examination was within normal limits.

Her CBC revealed a PCV of 30%. Her chemistry panel revealed a mild increase in BUN (30 mg/dl) compared to previous bloodwork, a persistently increased ALP (261 U/L), and a moderate increase in total bilirubin (1.1 mg/dl). A canine pancreatic lipase immunoreactivity test was performed to test for pancreatitis due to her clinical signs and was within normal limits. Her abdominal ultrasound revealed a small amount of anechoic free fluid throughout the abdomen outlining the small bowel, an ovoid, 3.6 mm thick hypoechoic nodule in the caudal aspect of the liver, and bilaterally enlarged adrenal glands. A newly identified smoothly marginated, ovoid (largest at 4.7 mm thick) anechoic, structure was present in the renal cortices bilaterally. A newly identified irregularly marginated, amorphous (up to 2.2 cm thick), hypoechoic mass adjacent to a loop of small bowel containing a few linear hyperechoic regions was found. Persistent nodules adhered to portions of the small bowel were now mildly enlarged (up to 1.56 cm in thickness). A single mass within her cranial abdomen in the area of the pancreas was found. The mass near the pancreas and a nodule were aspirated and cytology revealed macrophagic and neutrophilic inflammation.

Due to the abdominal masses increasing in size and Bonnie's clinical signs showing no improvement, it was recommended that Bonnie return on 1/29/18 for an abdominal laparotomy for biopsies of the abdominal masses. She was instructed to continue the cyclosporine at 6.25 mg/kg now at 25 milligrams every morning and one 10 milligram capsule very night, ondansetron at 1 mg/kg (24 milligrams every 24 hours), and to continue gabapentin and mirtazapine as previously prescribed.

Bonnie presented on 1/29/18 for a recheck of her bloodwork and a surgery consult. Over the weekend, Bonnie continued to have inappetance and had an episode of diarrhea on the morning of 1/29 with melena. Upon presentation, Bonnie was alert but depressed. Her temperature was 101.1 degrees Fahrenheit, heart rate was 168 beats per minute, and respiration rate was 44 breaths per minute. Her mucous membranes were pale and her capillary refill time was less than 2 seconds. Bloodwork revealed persistent hypochromic anemia with a hematocrit of 29% and a red blood cell count of 4.11 M/ul, mild hypoproteinemia (140.9 mmol/L), mild hypokalemia (3.62 mmom/L), mild hypochloridemia (102.9 mmom/L), moderate hyperglycemia (253 mg/dl), moderately increased BUN (40 mg/dl), persistently moderately increased ALP (242 U/L), and mildly increased total bilirubin (1.2 mg/dl). Her platelets decreased from a count of 256 K/ul on Friday to 80 K/ul on 1/29. Her coagulation times were within normal limits. Thoracic radiographs indicated metastatic neoplasia to the chest. After discovering these findings and discussing them with Bonnie's owner, it was elected to not pursue surgery and to send Bonnie home on palliative care. She was sent home with instructions to continue her other medications as previously prescribed and administer one 25 milligram capsule of cyclosporine twice daily.

Conclusion

In conclusion, even if strong indicators of primary immune mediated hemolytic anemia are present, it is imperative to definitely distinguish primary versus secondary IMHA. Aggressive diagnostics must be pursued to definitively obtain an exclusion of an infectious or neoplastic disease triggering secondary IMHA. Further testing is indicated in patients that fail to respond to standard immunosuppressive therapy or in patients that do not match the signalment of primary IMHA such as felines, geriatrics or breeds predisposed to blood borne parasitism (3).

References

1. Goggs, R., S.G. Dennis, A. Di Bella, et al. Predicting Outcome in dogs with Primary Immune-Mediated Hemolytic Anemia: Results of a Multicenter Case Registry. J Vet Intern Med 2015; 29: 1603-1610.
2. Day, Michael J. Canine Immune-Mediated Hemolytic Anemia. NAVC Clinician's Brief, 2012.
3. Balch, Andrea and Andrew Mackin. Canine Immune Mediated Hemolytic Anemia: Pathophysiology, Clinical Signs, and Diagnosis. Compendium Vet. April 2007.
4. Kidd, Linda and Nigel Mackman. Prothrombotic mechanisms and anticoagulant therapy in dogs with immune-mediate hemolytic anemia. Journal of Veterinary Emergency and Critical Care 2013; 23(1).
5. Carr, Anthony, David Panciera and Linda Kidd. Prognostic Factors for Mortality and Thromboembolism in Canine Immune-Mediated Hemolytic Anemia: A Retrospective Study of 72 dogs. J Vet Intern Med 2002; 16:504-509.
6. Grundy, Sophie and Claudia Barton. Influence of drug treatment on survival of dogs with immune-mediated hemolytic anemia: 88 cases (1989-1999). JAVMA 2001; Vol. 218, No. 4.
7. Wang, A, J.R. Smith and K.E. Creevy. Treatment of canine idiopathic immune-mediated haemolytic anaemia with mycophenolate mofetil and glucocorticoids: 30 cases (2008-2011). Journal of Small Animal Practice 2013; 54, 399-404.
8. Swann, J.W. and B.J. Skelly. Evaluation of immunosuppressive regimens for immune-mediated haemolytic anaemia: a retrospective study of 42 dogs. Journal of Small Animal Practice 2011; 52: 353-358.

9. Piek, Christine, Willem Evert van Spil, Greet Junius, et al. Lack of evidence of a beneficial effect of azathioprine in dogs treated with prednisolone for idiopathic immune-mediated hemolytic anemia: a retrospective cohort study. BioMed Central. 2011.
10. Mellett, A.M., R.K. Nakamura and D. Bianco. A Prospective Study of Clopidogrel Therapy in Dogs with Primary Immune-Mediated Hemolytic Anemia. J Vet Intern Med 2011; 25-71-75.
11. Burgess, Kristine, Antony Moore, William Rand, et al. Treatment of Immune-Mediated Hemolytic Anemia in Dogs with Cyclophosphamide. J Vet Intern Med 2000; 14:456-462.
12. Fenty, Renee K., Armelle M. deLaforcade, et al. Identification of hypercoagulability in dogs with primary immune mediated hemolytic anemia by means of thromboelastography. JAVMA, 2011; Vol 238, No.4.
13. Whelan, Megan F., Therese E. O'Toole, Daniel I. Chan, et al. Use of human immunoglobulin in addition to glucocorticoids for initial treatment of dogs with immune-mediated hemolytic anemia. Veterinary Emerg Crit Care, 2009; 19(2): 158-164.
14. Holahan, Melissa, Andrew J. Brown and Kenneth K. Drobatz. The association of blood lactate concentration with outcome in dogs with idiopathic immune-mediated hemolytic anemia: 173 cases (2003–2006). Veterinary Emergency and Critical Care Society, 2010; 20 (4): 413-420.
15. Johnson, Anthony. Pulmonary Thromboembolism. Clinician's Brief. Oct. 2014.
16. Horgan, Jason Elliott, Brian Keith Roberts, et. al. Splenectomy as an adjunctive treatment for dogs with immune mediated hemolytic anemia: ten cases (2003-2006). Journal of Veterinary Emergency and Critical Care, 2009. 19: 254-261.

17. Weiss, D.J. Bone Marrow Pathology in Dogs and Cats with Non-Regenerative Immune-Mediated Haemolytic Anaemia and Pure Red Cell Aplasia. J. Comp. Path. 2008. Vol. 138, 46-53.