“The Cocker Who Fell Off Her Rocker”

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Introduction

Immune-mediated hemolytic anemia, or IMHA, is a common cause of hemolysis in dogs and is associated with high morbidity and high mortality. This autoimmune destruction of red blood cells via complement and/or opsonization may be primary (idiopathic), or secondary to certain triggers, resulting in moderate to severe and life-threatening anemia and systemic inflammation.[1] Some patients may initially present cardiovascularly unstable due to a severe, sudden decreased oxygen carrying capacity.[8] Diagnosing primary IMHA involves the exclusion of secondary causes of hemolysis while proving immune-mediated destruction of red blood cells. Treatment typically entails immunosuppressive doses of glucocorticoids with possible addition of other immunosuppressives, as well as thromboprophylaxis and supportive therapies such as blood transfusions. Managing IMHA cases may be difficult due to the pro-inflammatory, hypercoagulable state of the patients that puts them at risk of thromboembolism, in addition to the risk of secondary infections and costs associated with the immunosuppressive medications.[7]

History and Presentation

Kona is an approximately 3-year-old female spayed Cocker Spaniel who presented to Mississippi State University College of Veterinary Medicine Emergency Service on 5/26/20 for lethargy, anorexia, icterus, and anemia. She had a history of declining over the past 3-4 days, as she was getting progressively weaker and more icteric.

On initial presentation, Kona was depressed, quiet, and non-responsive. She was tachycardic at 180 beats per minute, tachypneic at 40 breaths per minute, and febrile with a temperature of 104.0 degrees Fahrenheit. On cardiothoracic auscultation, no crackles, wheezes, murmurs, or arrhythmias were appreciated. However, her ECG did reveal numerous runs of premature ventricular contractions. Her mucous membranes were icteric with a capillary refill time of 3 seconds. She had severe, green

mucoid ocular discharge bilaterally. She was ambulatory, but weak in all four limbs with no evidence of lameness. Her peripheral lymph nodes all palpated smooth, soft, and symmetrical. Her PCV was 11% with yellow-tinged serum present.

Kona's CBC revealed a leukocytosis classified by a neutrophilia with a regenerative left shift and lymphopenia, as well as a marked, macrocytic, hypochromic anemia. A reticulocyte count confirmed the anemia was regenerative, which narrowed down the causes for her anemia to blood loss or hemolysis. Due to the normal protein levels and lack of clinical signs of hemorrhage, blood loss was lower on the differential list. Kona's neurochemistry revealed hypokalemia, elevated BUN, decreased creatinine, elevated liver enzymes (ALT, ALP), hyperbilirubinemia, hyperglobulinemia, hypermagnesemia, and elevated creatine kinase. Hyperbilirubinemia with a regenerative anemia was suggestive of extravascular hemolysis. A blood smear revealed spherocytosis, which is highly indicative of IMHA. Kona's slide agglutination test was positive for abnormal agglutination at both the macroscopic and microscopic level, confirming IMHA.

Pathophysiology

Immune-mediated hemolytic anemia is a primary (idiopathic) or secondary, spontaneous autoimmune response directed against glycoprotein molecules on the surface of the erythrocytes.[1,4] Essentially, the body produces autoreactive antibodies that destroy the red blood cells at an accelerated rate.[1] This destruction can occur via complement-mediated lysis and/or opsonization, thus categorizing it into 2 subtypes—intravascular and extravascular hemolysis, respectively.[1] Intravascular hemolysis is facilitated by IgM through complement fixation, which leads to red blood cell membrane damage and intravascular destruction.[1] Extravascular hemolysis entails phagocytosis mediated lysis by the monocytic and phagocytic cells in the liver and spleen.[1] The macrophages recognize the Fc receptor of IgG or C3b of complement and phagocytize the red blood cell.[1] Both intravascular and extravascular hemolysis

typically result in a regenerative anemia, in which the bone marrow increases erythropoiesis to compensate for the decreased circulating red blood cell mass.[1,6,8] In another subtype of IMHA, the immune-mediated destruction occurs in the bone marrow, lysing the erythrocyte precursor cells.[6,8] This form of IMHA, known as IMHA at the bone marrow level, results in a nonregenerative anemia since the bone marrow is incapable of effective erythropoiesis.[6,8]

The etiology of primary IMHA is unknown. While certain breed predispositions have been established, a specific gene associated with IMHA has not been found.[6] Predisposed breeds include cocker spaniels, springer spaniels, miniature schnauzers, Old English sheepdogs, and poodles.[1,6] IMHA is definitively diagnosed by documenting red blood cell agglutination, which is caused by antibodies coating the surface of the red blood cells.[1] It is imperative to differentiate primary IMHA from secondary IMHA, as the treatments vary significantly. Therefore, part of the diagnostic plan includes ruling out secondary causes of IMHA. Secondary IMHA can occur due to toxins, neoplasia, drugs, infections, tick borne diseases, and other inflammatory diseases.[3]

Clinical signs of IMHA are typically centered around signs of anemia and hypoxia. Patients will present with a history of lethargy, weakness, icterus, vomiting, diarrhea, and/or anorexia.[1] On physical exam, the signs of anemia and hypoxia may include pale mucous membranes, hemic murmur, tachycardia, tachypnea, and bounding pulses.[1] Other physical exam findings may include splenomegaly, hepatomegaly, lymphadenopathy, and fever.[1] With acute IMHA, more severe clinical signs are typically observed due to the body not having time to accommodate. With chronic IMHA, the body can compensate for the slowly progressing anemia, allowing the patient to only demonstrate minimal clinical signs.

Diagnostic Approach

Minimum databases are often performed as a starting point in diagnosing IMHA and a multitude of other diseases. CBC may reveal a macrocytic anemia with a reticulocytosis and thrombocytopenia.[1,6] Furthermore, a leukemoid response, as seen on Kona's CBC, is common with IMHA patients due to the increased bone marrow stimulation.[6] IMHA is often strongly regenerative unless the disease is acute or involves immune-mediated destruction of the erythroid precursors.[6] If it is within just 3 to 5 days of onset, a regenerative response will not be seen because it takes the bone marrow this long to produce red blood cells, specifically reticulocytes. If the disease is intravascular, hemoglobinemia is typically seen in the plasma; however, extravascular hemolysis will yield an icteric plasma. Furthermore, extravascular hemolysis can lead to excretion of bilirubin in the urine resulting in bilirubinuria and increased urobilinogen.[6] Intravascular hemolysis can lead to hemoglobinuria, which may potentially damage the kidneys.[6] Serum chemistry may reflect hyperbilirubinemia as well as inflammatory changes to acute phase proteins and a pseudohypophosphatemia.[6]

Although hemolysis is supported by the minimum database, definitively diagnosing IMHA occurs by demonstrating the presence of immune-mediated destruction. This can be done by performing a blood smear and slide agglutination test. A slide agglutination test evaluates for auto-agglutination by testing for antibody or complement on the surface of red blood cells.[3,6] To increase specificity, it is recommended to wash the erythrocytes 3 times in a 1:4 ratio with saline to rule out artificial agglutination and rouleaux.[3] Unfortunately, not all IMHA's demonstrate a positive slide agglutination so further diagnostics are recommended to identify potential false negatives. Spherocytosis is highly suggestive of IMHA.[3,6] Other blood smear findings that can be seen include nucleated red blood cells, Howell-jolly body, Heinz bodies, ghost cells, eccentrocytes, schistocytes, acanthocytes, and keratocytes.[6]

A Coombs' test was not performed with Kona; however, it is a direct antiglobulin test that is commonly used when diagnosing IMHA if slide agglutination is negative.[6] This test uses species-specific antibodies to detect Ig and/or C3 bound to erythrocytes in a patient blood sample.[1] The antibody

binding reaction is temperature dependent, so the test is run at both 4C and 37C, utilizing both polyvalent and monovalent antibodies to reduce false negatives.[6] An alternative to the Coombs' test, flow cytometry can detect immunoglobulins bound to the red blood cells.[6] It is important to acquire samples for these tests prior to initiating immunosuppressive treatment and administering blood transfusions, as these can hinder the sensitivity and specificity.[3]

To definitively diagnose primary IMHA, all secondary causes must be ruled out. This begins with a thorough history. Certain drugs can cause hemolysis including sulfonamides, methimazole, cephalosporins, and penicillins.[1,3,6] Toxins that can cause hemolysis include onions, garlic, hydrogen peroxide, zinc, propylene glycol, and acetaminophen.[1,3,6] Studies have shown a correlation between vaccine administration within the last 30 days, especially modified-live vaccines, and hemolytic anemia.[2,3] Tick-borne diseases such as babesiosis, ehrlichiosis, and anaplasmosis can cause hemolysis; therefore, tick serology and/or PCR panels are recommended.[3] Furthermore, since neoplasia can cause hemolysis, thoracic and abdominal imaging, including ultrasound, are recommended.[2,3]

Treatment and Management

Once all secondary causes have been ruled out, immunosuppressive treatment can be initiated for management of primary immune-mediated hemolytic anemia. Glucocorticoids are utilized as the first line of treatment. This medication alters gene transcription, downregulates Fc receptor expression, decreases antigen processing, and suppresses T-cell function.[8] Oral prednisone is administered at immunosuppressive doses of 2-3mg/kg/day for dogs less than 25kg and 40-60mg/m2 for dogs greater than 25kg.[7] The doses differ with large breed dogs to minimize clinical signs associated with glucocorticoids.[7] These include polyuria, polydipsia, polyphagia, panting, lethargy, weakness, weight gain, gastrointestinal ulceration, and secondary infection.[2,7,8] Since dexamethasone is seven times more

potent than prednisone, the dose is decreased to 0.3-0.5mg/kg/day but can be administered IV.[2,7] It is important to note that it will take 3-7 days for glucocorticoids to take effect.[7]

Additional immunosuppressants may be started in dogs based on the severity of the disease, response to glucocorticoids, and/or side effects of the glucocorticoids.[5,7] Measurements of severity of the disease may include hyperbilirubinemia, icterus, and increased BUN concentration. [5,7] Furthermore, ACVIM guidelines state that indications to start a second immunosuppressant are a decrease in PCV greater than 5% within 24 hours while on steroids or the need for repeated transfusions. [5,7] Immunosuppressants utilized with IMHA patients include azathioprine, cyclosporine, mycophenolate, and less commonly, leflunomide. The glucocorticoids and immunosuppressives are typically administered for a minimum of 3 to 6 months, so it is important to inform the owner about the chronicity of managing this disease.[7]

Azathioprine disrupts purine synthesis required for DNA and RNA replication.[2,5,7,8] The initial dose is 2mg/kg or 50mg/m2 every 24 hours, but it takes up to 2 weeks or longer for full effect. [2,5,7,8] Side effects include gastrointestinal signs, hepatotoxicity, myelosuppression, and pancreatitis. [2,5,7,8] Therefore, bloodwork is imperative to monitor liver enzymes and bone marrow cell production. Cyclosporine is a calcineurin inhibitor which decreases T-cell proliferation. [2,5,7,8] It is recommended to start the dosing at 5mg/kg every 12 hours to reduce the risk of side effects, which include gastrointestinal signs, gingival hyperplasia, and rarely, hepatotoxicity. [2,5,7,8] The availability of therapeutic drug monitoring with cyclosporine is highly beneficial, especially with a pharmacodynamic assay which measures T-cell activation as well as IL-2 and interferon gamma expression.[2] Mycophenolate prevents guanine nucleotide synthesis resulting in decreased T-cell and B-cell proliferation. [2,5,7,8] This drug is dosed at 8 to 12 mg/kg every 12 hours and monitored via CBC panels to look for signs of myelosuppression. [2,5,7,8] Monitoring for gastrointestinal signs is also important, as diarrhea is a common side effect. Leflunomide is a relatively new immunosuppressant to veterinary medicine and inhibits the production

of pyrimidines. Benefits of this medication include its once-daily dosing at 2mg/kg, as well as the ability to measure blood concentrations.[2,7,8] However, it is important to note that unlike cyclosporine, this is a pharmacokinetic form of therapeutic drug monitoring since it only measures the concentration of the drug and not its effect. In addition to blood concentration levels, bloodwork is recommended as well to monitor for side effects such as thrombocytopenia, hypercholesterolemia, and anemia.[2,8]

Regardless of the immunosuppressive used, an appropriate weaning protocol must be in place to prevent relapse or adverse effects. Prior to weaning, the disease must be under control with a PCV greater than 30% for at least two weeks duration, an improved bilirubin concentration, and no evidence of agglutination or spherocytosis.[7] Once the disease is stable, the glucocorticoid dose, or whichever immunosuppressant is causing the most severe side effects, can be tapered by 20-25% every two to three weeks if no signs of relapse are observed.[7]

Thromboprophylaxis is highly recommended when managing IMHA due to the risk of thromboembolism. An exception to this is when the patient is thrombocytopenic with a platelet count of less than 30,000 since those patients are at an increased risk of spontaneous hemorrhage.[5] Studies have shown that anticoagulants, such as low molecular weight heparins (dalteparin, enoxaparin, or rivaroxaban), are preferred over antiplatelet medications.[5,7] However, antiplatelet medications are often used in conjunction with the anticoagulants. Clopidogrel has been proven to be more efficacious than aspirin with less risk of gastrointestinal side effects, including bleeding and microulcerations.[5,7]

Upon initial presentation, a transfusion may be indicated to achieve cardiovascular stability and increase blood oxygen content. Indications to transfuse are based on the speed of onset, PCV at the time of presentation, and severity of clinical signs.[5,7] Chronic changes often present with less severe clinical signs because the body can accommodate to the slowly decreasing PCV. However, if the PCV is less than 12%, a transfusion is often recommended regardless of clinical signs due to the limited ability

to transport oxygen to tissues.[5] Packed red blood cells are preferred over other blood products due to its higher oxygen carrying capacity.[5,7] Fresh whole blood can be used as an alternative but increases the risk of intravascular volume overload. Ideally, it should be less than 7 to 10 days old to decrease the mortality risk associated with older products.[5,7] Dogs do not have pre-formed antibodies against DEA1.1; therefore, they are unlikely to have a reaction during their first transfusion.[1,2] Nevertheless, clinical signs to monitor for during the transfusion include vomiting, tachypnea, tachycardia, febrile, sepsis, and acute lung injury.[2]

Additional supportive therapy may include IV fluids, oxygen therapy, gastroprotectants, and, in the case of secondary IMHA, sometimes antibiotics for hemotropic/vector borne pathogens. Intravenous fluids are controversial due to the presence of an IV catheter while the patient is in a proinflammatory and hypercoagulable state; however, a primary indication is for intravascular hemolysis patients at risk of renal damage from hemoglobinemia and hemoglobinuria.[2]

Other treatments utilized in refractory cases include intravenous immunoglobulin, splenectomy, and lithium carbonate.[2,5,7] These are salvage medications and procedures that should only be considered when the patient is not responding to the immunosuppressives and/or is suffering relapses despite appropriate drug therapy. Future treatments may entail liposomal clodronate, hyperbaric oxygen therapy, and therapeutic plasma exchange.[5]

The mortality rate for IMHA has improved to 30-40% in recent studies due to the speed of diagnosis and availability of treatments.[8] Consequences of IMHA include thromboembolism, bilirubin encephalopathy, relapse, secondary infection from immunosuppression, distal tubular acidosis, and hemoglobin-related acute kidney injury.[2,8] The presence of any of these sequelae are associated with increased morbidity and mortality. Furthermore, suggested poor prognostic indicators include serum bilirubin and urea concentrations.[4,7]

Case Outcome

Due to the markedly decreased PCV on presentation at 11% and Kona demonstrating clinical signs associated with anemia, a blood transfusion with DEA 1.1 negative blood was initiated after performing a slide agglutination test, clinicopathologist-reviewed blood smear, and collecting blood samples for infectious disease testing. Abdominal radiographs were performed to rule out secondary causes, such as zinc toxicity, and evaluate for neoplasia; these radiographs were unremarkable. An abdominal ultrasound with cytology of the liver revealed severe cholestasis, suspected mild mixed inflammation, and significant amounts of extramedullary hematopoiesis. Culture of the bile grew *Clostridium* sp.; therefore, metronidazole was added to the treatment regimen. Kona was started on doxycycline as a preemptive measure for tick borne disease while her tick panel was pending; however, results demonstrated she was negative for all tick-borne diseases. It was determined to start her on prednisone and cyclosporine based on the severity of her disease. Throughout the night, she vomited intermittently and had diarrhea, so she was started on maropitant and pantoprazole. She was administered 2 injections of diphenhydramine in case it was a transfusion reaction. Furthermore, clopidogrel and enoxaparin were added to her treatment regimen for thromboprophylaxis.

Her PCV post-transfusion increased to 34%. However, it rapidly decreased to 15% again within the next 36 hours, and she was still demonstrating signs of anemia including weakness, tachycardia, and tachypnea. Another blood transfusion with DEA 1.1 negative blood was administered, which improved her PCV to 24%. She continued to be tachypneic and dyspneic despite her transfusion, so thoracic radiographs were performed to evaluate for aspiration pneumonia due to her recent history of vomiting. Despite her unremarkable radiographs and a normal blood gas analysis, she was closely monitored for worsening respiratory signs due to suspected pulmonary thromboembolism. Another arterial blood gas performed the next day revealed an increased A-a gradient, indicating she was not

oxygenating adequately. Therefore, she was moved into an oxygen cage, which reduced her respiratory rate and effort significantly.

Her progressively decreased mentation arose concern for hepatic encephalopathy, so she was started on oral lactulose with no improvement. Her baseline ammonia was within normal limits and her neurological exam was normal, so a meeting with Mr. Stevens was arranged in a successful attempt to improve her mentation. On 5/29/20, Kona developed petechiation coalescing to ecchymoses on her ventrum. Her clopidogrel was discontinued temporarily to improve platelet function, but she was maintained on enoxaparin. After discontinuing the clopidogrel, the petechiation and ecchymoses resolved.

Over the next few days, Kona's PCV, CBC, and neurochemistry abnormalities were improving and remained within their respective reference ranges. Another slide agglutination test was performed which was negative for macroagglutination and significantly improved on microagglutination. She was transitioned to oral medications and was able to handle them readily. She was weaned out of her oxygen cage and was able to saturate well at room air.

Due to her improved state, she was discharged to Mr. Stevens for at-home supportive care. She was sent home on metronidazole, Tylenol 4, prednisone, cyclosporine, mirtazapine, omeprazole, Cerenia, Clavamox, clopidogrel, and rivaroxaban for her IMHA. While in hospital, she was also diagnosed with yeast otitis externa, bilateral corneal ulcers, a desmetocoele, and severe KCS. This prompted the addition of mometamax, ofloxacin, Refresh eye drops, and cyclosporine ophthalmic drops. Kona has done tremendously well at all rechecks and has maintained her PCV over 30%, even while tapering her prednisone.

References

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