Aplastic Anemia

Aundrea Fleck

Mississippi State University College of Veterinary Medicine

Class of 2018

Clinicopathologic Conference

Presented on April 13, 2018

CPC Advisor:

Dr. Alyssa M. Sullivant, DVM, MS, DACVIM

Dr. Samantha Muro, DVM, MS

INTRODUCTION

Aplastic anemia in dogs is a life- threatening disease resulting from the bone marrow's inability to replenish all three major cell lines in the peripheral blood supply and presence of hypocellular bone marrow.[1] These cells include erythrocytes, responsible for carrying oxygen to the tissues in the body; leukocytes, responsible for protecting the body from infections; and thrombocytes, responsible for preventing bleeding through primary hemostasis. Aplastic anemia is also more appropriately named aplastic pancytopenia since "pan" meaning "all" of the cell linages are affected, not just the erythroid lineage as the term "anemia." implies[1] The bone marrow spaces that lack these important progenitor cells are also replaced with adipose tissue.[2] Patients that are clinically affected usually are young and show very non-specific signs such as lethargy, weight loss, and possibly vomiting. Bleeding tendencies, such as petechiae, are the most common clinical manifestations and are directly related to thrombocytopenia. Bone marrow aplasia is believed to result from (1) destruction of or genetic defect in stem cells, (2) an altered marrow microenvironment including vascular and stromal components, and/or (3) dysregulation of cell production from abnormal humoral mediators or other cellular products.[1] There are many causes of aplastic anemia, but immune-mediated attack on normal progenitor cells is the most likely cause of most cases of idiopathic aplastic anemia when all other causes are ruled out. Idiopathic aplastic anemia has a poor prognosis as bone marrow repopulation is unpredictable and may take weeks to months to recover, if it all.[3]

HISTORY AND PRESENTATION

Chanel, an approximately 5 month old female intact standard poodle presented to MSU CVM internal medicine department for history of lethargy and pale mucous membranes. She

presented to her regular veterinarian a few days prior to obtain her monthly flea and tick preventative and obtain an evaluation of a rash that was noticed on her abdomen by the groomer. The rash was deemed pyoderma at that time, and Chanel received her oral preventative and went home. The following day she began to be more lethargic and was not acting like herself so she was taken back to her regular veterinarian. During this visit it was noted that Chanel had markedly pale mucous membranes. A fecal exam was performed and no ova were observed. No other diagnostics were performed, and Chanel received an injection of vitamin K and was sent home with oral vitamin K until her referral with our MSU CVM internal medicine department. Owners reported that there are no rat traps or bait at either residence that she resides; however, Chanel was free to roam outside and did visit the neighbor dog quite often.

Upon presentation, Chanel was depressed, but responsive. She had a temperature at 102.0 degrees F and heart rate of 140 beats per minute. She weighed 10.5 kg (22.4 lbs.) with a body condition score of a 2/9. Her mucous membranes were pale to grey, and the capillary refill time was difficult to examine. She was mildly dehydrated. Cardiac auscultation revealed normal heart rate with no murmurs or arrhythmias noted. There were no crackles or wheezes auscultated on either side of the chest. She had moderate dental tartar and halitosis (bad breath), and bleeding was noted on her gingiva around a couple of her teeth. No pain response was observed on abdominal palpation. Petechiae was observed on her gingiva, periocular skin, inner pinnae, and on the ventral abdomen. Some areas of ecchymosis were observed on the ventral abdomen as well. A rectal exam was performed and was unremarkable, revealing normal soft formed stool with no melena observed. Chanel did urinate during examination and no hematuria was observed.

PATHOPHYSIOLOGY

The bone marrow is comprised of cells that undergo complex mechanisms such as proliferation, differentiation, and maturation of specific cell lineages the body requires. All of these mechanisms combined is known as hematopoiesis.

Hematopoiesis begins with a non-specific stem cell which is made during embryonic development. One stem cell will then proliferate to create an identical copy itself. One copy will then differentiate into the appropriate cell type while the second is stored in case there is damage during the process.[6] The first copy will differentiate into a hematopoietic stem cell, which in turn differentiates again into either myeloid or lymphoid cell lineages. These cells undergo further differentiation and maturation and form populations of progenitor cells which are committed to the main marrow cell lines: erythroid, granulocytic and monocytic, megakaryocytic, and lymphocytic. These cells are then released from the bone marrow as mature cells that replenish senescent cells in the peripheral circulation.[6] Therefore, direct destruction or genetic defects of the stem cells can cause an overall decrease of all cell lineages by halting the entire hematopoietic process.

The bone marrow microenvironment contains osteoblasts and stromal cells such as adipose cells, blood vessel endothelial cells, osteocytes, and fibroblasts. These cells secrete signals, especially cytokines, to alter the bone marrow environment and keep stem cells in an immature state. Bone marrow stroma are key to providing the structural and physiological support for blood cell production.[6] They interact together and promote humoral growth and inhibitory factors necessary to maintain normal hematopoiesis. Certain drugs and toxins disrupt this environment by causing necrosis of all the mitotically active cells which causes transient

injury to progenitor cells.[3] Loss of the early progenitor cells will in turn halt the entire hematopoietic process causing aplastic anemia.

Another cause of aplastic anemia is T-cell mediated immunosuppression. Unlike the majority of cases of aplastic anemia that are caused by direct destruction to the stem cells and destruction to the bone marrow microenvironment, either by drugs, toxins, or infectious agents that may be identifiable, idiopathic, or immune-mediated, aplastic anemia is rare and is suspected to be caused by the T-cell mediated inflammation or destruction immunosuppression of the bone marrow cells, thereby resulting in inhibition of hematopoiesis. This is the suspected cause of idiopathic aplastic anemia in animals. Genetic factors along with certain environmental factors may lead to T-lymphocyte mediated stem cell reactions and cytokine release, ultimately leading to decreased hematopoiesis. Antigens are presented to the T-lymphocytes, triggering them to activate and proliferate.[7] Cytokines such as interferon gamma (IFN-γ) and TNF alpha (TNF-α) are released by the T lymphocytes and inhibit stem cell proliferation by mitotic disruption and apoptosis.[7] IFN-γ and TNF-α up-regulate other T cell cellular receptors as well as the Fas receptor. Activation of Fas receptor by the Fas ligand leads to apoptosis of target cells as well.[2] These events ultimately lead to reduced cell cycling and increased cell death by apoptosis. In support of the hypothesis that T cells have a central role in the pathophysiology of bone marrow destruction, it was shown that transfusion of bone marrow lymphocytes of patients with aplastic anemia can inhibit hematopoiesis when cultured with patient or normal marrow.[7]

DIAGNOSTIC APPROACH/CONSIDERATIONS

Diagnosis of idiopathic aplastic anemia is most often that of exclusion.[1] Clinical signs alone can be vague and unhelpful in identifying a definitive diagnosis. The only clinical signs that may be seen on physical exam are lethargy, pallor of the gingiva, and bleeding tendencies such as petechiae. Obtaining a comprehensive history can provide information that may lead to many differentials, and is beneficial when added to objective diagnostics gathered thereafter. A complete history of current medications, or even all the medication the owner may be taking that could have been dropped and ingested by the pet is essential. A minimum database should be performed with a complete blood count, serum chemistry, and urinalysis. The complete blood count and reticulocyte count can offer a large amount of information regarding the cellularity of the blood assessing overall cell counts and regeneration status, which would, in turn, assess the health of the bone marrow. The main advantage of classifying anemia as regenerative or non-regenerative is that in most cases, it effectively identifies the cause of anemia as being either increased loss or destruction of erythrocytes (regenerative) or impaired production of erythrocytes (non-regenerative).[8] Aplastic anemia will typically reveal a non-regenerative anemia on the complete blood count since the bone marrow has lost the capabilities of hematopoiesis and replenishing the specific erythroid cell lineages within circulation.[8] Other abnormalities observed will be a thrombocytopenia and neutropenia. A serum chemistry profile should also be performed on any anemic patient as it will allow evaluation of kidney, liver, and metabolic functions within the body and potentially aid in identification of an underlying etiology. For example, hyperbilirubinemia may suggest peripheral hemolysis and lead a clinician to investigate peripheral causes of anemia. A slide agglutination test can also be performed to help rule out other forms of anemia such as immune-mediated hemolytic anemia. A blood smear

can also be made to help screen for infectious diseases such as *Babesia canis*, *Ehrlichia canis*, or *Anaplasma* before full infectious panel results are available. A blood smear will also provide a more specific count and morphology of all the cell lineages.

A urinalysis is helpful as some hemolytic anemias can cause signs such as bilirubinuria or hemoglobinuria. When petechiae are present or when anemia, thrombocytopenia, or pancytopenia are present on the complete blood count, it is necessary to run a coagulation profile to evaluate secondary hemostasis. Infectious disease testing, in particular *Rickettsial* and *Babesia* testing, is also recommended in cases of anemia, thrombocytopenia or pancytopenia. Cobalamin (vitamin B12) and folate levels may also be tested in pancytopenia dogs since DNA synthesis requires folate and vitamin B12. Absence of these essential nutrients can result in general malnutrition and anemia, classically characterized as normocytic normochromic, or non-regenerative.[9]

The presence of pancytopenia prompts the decision to obtain a bone marrow sample. Bone marrow aspirates and core biopsies are the standard tests for aplastic anemia. Hypocellular bone marrow aspirates can be misleading as this can be caused by myelofibrosis or from a truly hypocellular marrow.[3] Bone marrow core histopathology will show low to absent cellularity for all cellular lineages, and the spaces that lack these cell lines will be replaced by adipose tissue.[3]

There are criteria used when diagnosing immune mediated aplastic anemia from a core bone marrow biopsy and they are as follows: (1)Pancytopenia has been present for greater than two weeks, persists after treatment of endotoxemia/sepsis, (2) no known exposure to aplastic anemia inducing agents in the four weeks prior to the development of pancytopenia, (3) exclusion of renal disease, (4) no evidence of retained testicular masses, or persistent estrus, (5) negative titers for infectious diseases including *Ehrlichia*, *Babesia*, *Rickettsia*, and *Leishmania*,

and (6) bone marrow core biopsy sample reveals replacement of bone marrow with adipose tissue, with hemic tissue occupying only 0% to 25% of bone marrow space.[2,6] Once all of the previously stated criteria are met, only then can the pancytopenia be characterized as immune mediated aplastic anemia.

DIFFERENTIAL DIAGNOSIS

As previously stated the diagnosis of immune-mediated aplastic anemia is usually that of exclusion. There are a number of possible causes of aplastic anemia that includes specific drugs such as chloramphenicol, griseofulvin, sulfonamides, chemotherapeutic agents, albendazole, fenbendazole, and certain toxins.[4] Other considerations are given to infectious diseases, including *Ehrlichia canis*, parvovirus, canine distemper virus, and other tick-borne diseases.[1] Canine monocytic ehrlichiosis accounted for 42% of 119 dogs in one study.[5] Other differentials that need to be ruled out include hormonal estrogen influences from exogenous sources or from sertoli cell tumors, Leydig (interstitial) cell tumors, and granulosa cell tumors.[4] In Chanel's case, a specific cause was not identified, raising suspicion that she was dealing with an immune-mediated aplastic anemia in which the body produced antibodies towards her own bone marrow cells. There is no known reason why this occurs; however, it has been noted in younger dogs and is associated with a poor prognosis.

TREATMENT AND MANAGEMENT

There is no specific treatment to cure idiopathic aplastic anemia. Supportive therapy should be provided, and it takes weeks to months for the bone marrow to repair itself. Appropriately matched (compatible) blood transfusions or packed red cell transfusions should be

provided as needed while the patient is recovering. Some diagnostic tests take several days to obtain results so it is important to begin therapy immediately. Supportive therapy includes strict reverse isolation as these patients do not have enough white blood cells to prevent possible infections. Additionally, broad spectrum antibiotic coverage should be administered such as Unasyn at 30mg/kg intravenously every 8 hours, and Baytril at 15mg/kg intravenously every 24 hours. Doxycycline at 5mg/kg orally every 12 hours is usually administered to cover for rickettsial diseases while awaiting test results.[2] Intravenous fluid therapy should also be administered to correct any abnormalities with hydration status. Due to the non-regenerative, and typically severe nature of the anemia, whole blood, packed RBC, and /or platelet transfusion may be necessary throughout the entire treatment process. The immune-mediated aspect of this disease also warrants immunosuppressive therapy with medications such as prednisone, cyclosporine, azathioprine, mycophenolate mofetil, or combination of two together.[10] The fact that these medical treatments block T-cell function, and that positive patient response to therapy is seen strongly suggests that immuno-suppression accounts for their success.[2]

Human recombinant erythropoietin and granulocyte colony-stimulating factor can also be used until the bone marrow can recover, but is very expensive and not commonly used in veterinary medicine.[1] In people, bone marrow transplantation is commonly performed using suitable donors such as family members, but there is less availability and there is limited research in veterinary medicine.[1]

The prognosis for aplastic anemia can vary due each underlying cause. Acute aplastic anemia often can be reversible in about 2 to 3 weeks if the causative agent is removed, such as with drug toxicities, but even some drugs can have much longer lasting detrimental effects.[11] For example, in untreated cases, the prognosis of dogs with estrogen- induced myelotoxicity is

always considered to be unfavorable even with early diagnosis.[12] Idiopathic and chronic aplastic anemia tend not to be as responsive to therapy and recovery times can take up to several months. Younger dogs less than 3 years of age may be more likely to respond to immunosuppressive therapy and recover spontaneously, but prognosis remains poor to guarded.[11]

CASE OUTCOME

Chanel's complete blood count revealed a severe pancytopenia. Serum chemistry revealed a mild hypokalemia, mildly elevated BUN, mild hyperphosphatemia, and mild hypomagnesemia. Coagulation profile was unremarkable and a baseline cortisol was elevated (normal). Thoracic and abdominal radiographs, as well as abdominal ultrasound, were unremarkable. Reticulocyte count was markedly decreased therefore her anemia was classified as non-regenerative. Chanel was blood-typed as DEA 1.1 negative. On the day of presentation, due to clinical signs related to severe anemia, a blood transfusion was performed with 240mLs of DEA 1.1 positive whole blood, and a mild reaction of blepharedema and facial pruritus were noted. Subsequently, dexamethasone SP and diphenhydramine injections were administered subcutaneously, and those signs resolved. Chanel was hospitalized at Mississippi State University College of Veterinary Medicine in the intensive care unit under strict "reverse" isolation given the severity of her pancytopenia. A bone marrow aspirate and core biopsy were performed under sedation with dexmedetomidine and hydromorphone intravenously. Bone marrow aspirates were non-diagnostic, but suspicious for aplastic anemia. The bone core biopsy results revealed a lack of hematopoietic cells and an increase in immature red blood cells. Given that other diagnostics nor her history provided any clues to a specific cause of her pancytopenia, these findings are consistent with an immune-mediated aplastic anemia. On Day 5 of

hospitalization, in response to a progressively decreasing PCV and clinical signs of anemia, a second whole blood transfusion was administered with 240 mL of DEA 1.1 positive blood after cross-matching determined compatibility. No signs of hypersensitivity were noted during the second transfusion. Tick-borne disease titers were sent out and the results were still pending at time of the second transfusion. On the evening of Day 7 of hospitalization Chanel was transitioned to oral medications. Intravenous fluids and gastroprotectants were discontinued. Given the poor to guarded prognosis for bone marrow recovery and the length of time it would likely take, the owners elected to take Chanel home to monitor closely with instructions to return to the veterinary school at the first sign of clinical anemia, infection, or acute hemorrhage. Chanel was discharged on Baytril 11mg/kg orally every 24 hours, Clavamox 13.75 mg/kg orally every 12 hours, and prednisone 2mg/kg orally every 24 hours, to be continued until her bone marrow showed signs of recovery, as well as a two week course of doxycycline 5mg/kg orally every 12 hours (while awaiting test results). A few days later Chanel's clinical status deteriorated and the owners elected to humanely euthanize her at their primary veterinarian. The final results of her tick-borne diseases titers were negative for all possible pathogens, and a post mortem examination was not performed.

REFERENCES

1. Brazzell J, Weiss D. A retrospective study of aplastic pancytopenia in the dog: 9 cases (1996 – 2003). Veterinary Clinical Pathology 2006; 35:413-417.

2. Weiss, Douglas. New insights into the physiology and treatment of acquired myelodysplastic syndromes and aplastic pancytopenia. The Veterinary Clinics Small Animal Practice 2003:33;1317-1334.

3. Ettinger S, Feldman E, Cote E. Bone Marrow Disorders. In: Textbook of Veterinary Internal Medicine Expert Consult. 8th ed. Philadelphia: Saunders Elsevier, 2017

4. Gary A, Kerl M, Wiedmeyer C, Turnquist S, Cohn L. Bone Marrow Hypoplasia Associated With Fenbendazole Administration in a Dog. Journal of the American Animal Hospital Association 2004;40:224-229.

5. Frezoulis P, Angelidou E, Karnezi D, Oikonomidis I, Kritsepi-Konstantinou D, Kasabalis D, Mylonakis M. Canine Pancytopenia in a Mediterranean region: a retrospective study of 119 cases (2005-2013). Journal of Small Animal Practice 2017:58;395-402.

6. Gonzaga VF, Wenceslau CV, Lisboa GS, Frare EO, Kerkis I. Mesenchymal Stem Cell Benefits Observed in Bone Marrow Failure and Acquired Aplastic Anemia. *Stem Cells International*. 2017;2017:8076529.

7. Young NS, Calado RT, Scheinberg P. Current concepts in the pathophysiology and treatment of aplastic anemia. *Blood*. 2006;108(8):2509-2519.

8. Grimes C, Fry, M. Nonregenerative Anemia: Mechanisms of Decreased or Ineffective Erythropoiesis. Veterinary Pathology 2015: 52; 298-311.

9. Kearns S, Ewing P. Causes of Canine and Feline Pancytopenia. Internal Medicine Compendium 2006:28.

10. Yuki M, Sugimoto N, Otsuka H, Tanahashi S, Katoh M, Hirano T, Nishi N, Suzuki K. Recovery of a dog from aplastic anaemia after treatment with mycophenolate mofetil. Australian Veterinary Journal 2007:85;495-497.

11. Cote, Etienne. Aplastic Anemia. In: Clinical Veterinary Advisor Dogs and Cats. 3rd ed. St. Louis: Elsevier, 2015;62-63.

12. Sontas HB, Dokuzeylu B, Turna O, Ekici H. Estrogen-induced myelotoxicity in dogs: A review. *The Canadian Veterinary Journal*. 2009;50(10):1054-1058.