“Pancytopenia”

Dana C. Johnson

Mississippi State University College of Veterinary Medicine

Class of 2018

Clinicopathologic Conference

Presented on December 15, 2017

CPC Advisor:

Andrew Mackin, BSc, BVMS, MVS, DVSc, FANZCVSc, DACVIM

Introduction

Pancytopenia is a hematologic disorder described as a deficiency in all blood cell lines, resulting in a non-regenerative anemia, neutropenia, and thrombocytopenia[1]. The disruption in the ability of the bone marrow to produce normal functioning or adequate quantities of multipotential hematopoietic cells has been well established as a cause of pancytopenia[1]. Primary bone marrow disorders resulting in decreased cell production can include marrow aplasia, marrow hypoplasia, marrow necrosis, marrow fibrosis, and neoplasia[1]. Aplastic marrow occurs when all hematopoietic cells are absent and are replaced by other tissues such as fat and stromal cells, whereas a hypoplastic marrow indicates some hematopoietic cells still remain, and only about 75% of cell lines have been replaced[2]. Marrow necrosis is often the result of an ischemic event that has caused loss of the medullary architecture preventing normal cell lines from developing[2]. Decreased blood cell production due to neoplasia can be caused by either abnormal marrow cell proliferation (leukemia) or by infiltration with other cell lines (myelophthisis)[1]. Both of these cause damage to the normal existing marrow and result in fewer normal cells being produced[1]. In myeloproliferative disorders, such as acute myeloid leukemia, bone marrow cells are produced and grow rapidly; however, the maturation process is impaired by disruption of colony stimulating growth factors needed for normal development. Myelodysplastic syndrome (MDS), often referred to as "pre-leukemia", is due to a cell mutation allowing proliferation of an undifferentiated cell line[2]. This leads to ineffective hematopoiesis and an accumulation of poorly differentiated blood cells which then cause damage to surrounding tissue[3]. MDS can progress into acute myeloid leukemia, however defects in growth and differentiation, induction of apoptosis, and production of myelomonocytic precursors often result in peripheral cytopenias[2].

Extramedullary diseases resulting in either peripheral destruction or sequestration of blood cell lines have also been shown to contribute to pancytopenia[2]. Peripheral destruction of blood cell lines often leads to a hypercellular bone marrow as the marrow responds to the increased demand[2]. Disease processes such as severe bacterial sepsis, endotoxemia, infectious disease, disseminated intravascular coagulation, hemophagocytic syndrome or immune-mediated destruction can result in deficiencies of either specific blood cells or multiple blood cell lines[1]. In such diseases, the bone marrow is essentially normal and, if the underlying disease process is addressed, the cytopenias will often resolve.

The cause of pancytopenia can be difficult to diagnose, since many of these diseases have common clinical signs which are often nonspecific. Pallor and bleeding tendencies, such as petechiae, are the most common clinical signs noted, as well as increased lethargy and respiratory rate[2]. It is important to first exclude peripheral destruction of blood as a cause of pancytopenia, since many of these diseases can be identified without a bone marrow biopsy[2]. Differentiation of primary bone marrow disorders typically relies on the evaluation of marrow cytology and/or histopathology, and often requires more advanced diagnostics such as immunostaining and flow cytometry to identify the specific disease process[3]. It is essential to identify which marrow disorder is present since it will dictate treatment protocols and prognosis[4].

History and Presentation

Rascal was a 9 year old male neutered English bulldog that presented to the Mississippi State University College of Veterinary Medicine Small Animal Internal Medicine Service for decreased white blood cells and red blood cells. Rascal was referred by his primary veterinarian for a two-week history of lethargy, decreased appetite, and difficulty walking up stairs. Rascal's initial blood work showed neutropenia, moderate anemia (PCV 27%), and he was heartworm

negative and *Ehrlichia canis* negative. He was treated with prednisone, doxycycline, and iron supplements (Lixotinic) with no improvement seen. Rascal presented with a stiff gait affecting the hind limbs (lameness grade 3/5), and was overweight (BCS 7/9), with normal heart and lung sounds, and a mildly elevated temperature (103.9^{o}F) that returned to normal shortly after arrival. His mucus membranes were a very pale pink color, with a CRT of <2 seconds. All lymph nodes palpated normally. Rascal was slightly painful in the abdomen upon palpation. When bloodwork was repeated, it showed that Rascal's anemia had become more severe (PCV 20%), and his condition had progressed to include deficiencies in all blood cell lines (pancytopenia).

Diagnostics for disorders that result in pancytopenia should initially include a search for conditions such as drug, estrogen or toxin exposure, testing for immune-mediated diseases such as systemic lupus erythematosus (SLE), and serology, virus detection, and bacterial culture for detection of infectious agents. Once those potential conditions have been eliminated, then a bone marrow aspiration and core biopsy examination is indicated[5].

Evaluation of the marrow will help determine which of the primary bone marrow disorders previously mentioned is present. Conditions that cause hypocellular bone marrow include aplastic pancytopenia, myelofibrosis, and marrow necrosis[5]. Conditions associated with normocellular or hypercellular bone marrow include leukemia (defined as >30% blast cells) and hemophagocytic syndrome. Dysmyelopoiesis also has a normal to hypercellular marrow and is differentiated into myelodysplastic syndrome with excess myeloblasts (MDS-EB), myelodysplastic syndrome with refractory cytopenia (MDS-RC), and secondary dysmyelopoiesis[5].

In leukemic disorders, the use of morphological evaluation has had limitations in identifying the specific lineage of the poorly differentiated cells. The similarities between lymphoid blast

cells and myeloid blast cells make it difficult to determine the leukemia cell line[6]. Human medicine has added the use of cytochemical staining and immunophenotyping by flow cytometry to improve diagnosis. The recent development of monoclonal antibodies for specific leukocytes in domestic animals has now made immunophenotyping via flow cytometry an available option in veterinary medicine[4]. Immunophenotyping can also be used to distinguish acute leukemia from chronic leukemia by identifying the CD34 glycoprotein on the surface of the progenitor cells. This glycoprotein is seen on the immature cells found in acute leukemia, and is absent on the more differentiated cells of the chronic leukemia form[6].

Pathophysiology

Normal bone marrow should consist of 80-90% mature cells of varying types. As an animal ages, marrow cellularity decreases, and cells are replaced by adipose tissues[6]. Blast cells make up only a small percentage of the bone marrow, since one blast cell develops into many mature cells[6]. When blast cells increase to over 30% of the marrow population, it is indicative of an acute leukemia[6]. It was previously thought that acute myeloid leukemia occurred less frequently than acute lymphoid leukemia. However, this assumption was based on cell morphology alone. The recent implementation of cytochemical stains and flow cytometry immunophenotyping has determined that almost 50% of cases previously diagnosed as acute lymphoid leukemia would now be reclassified as acute myeloid leukemia[6].

Acute myeloid leukemia (AML) can be seen at any age, and includes proliferations of granulocyte, monocyte, erythroid, and megakaryocyte precursors[4]. It is classified as having more than 20-30% of blast cells in the marrow population. It is an aggressive, rapidly progressing disease that often occurs suddenly and manifests with a non-regenerative anemia, neutropenia, and thrombocytopenia. When the proliferation of the malignant cells is seen in the

bone marrow, but have not yet spilled into the peripheral circulation, the condition is referred to as "aleukemic leukemia"[3]. When the proliferation of the malignant cells can be seen in the peripheral blood, it is considered a true leukemia.

Acute myeloid leukemia in animals is classified by the World Health Organization classification system of AML-NOS (not otherwise specified), which are generally sub-classified according to the old French-American-British (FAB) criteria[7]. The FAB criteria depends on morphologic features of differentiation and expression of differentiation markers by the tumor cells. Acute monoblastic leukemia (AML-M5a) is the most common subtype of AML in the dog, and acute erythroblastic leukemia (AML-M6) is the most common subtype seen in the cat. Acute myeloid leukemia is more common in the cat than the dog, and was often caused by the feline leukemia virus (FeLV) until the use of the FeLV vaccine become common. Since then there has been a significant decrease the frequency of these feline leukemias[7].

Myelodysplastic syndrome (MDS) in humans is used to describe the hematopoetic dysfunction often seen before acute myeloid leukemia develops, and is often referred to as a pre-neoplastic syndrome for leukemia or "pre-leukemia"[3]. The bone marrow is usually hypercellular with abnormalities seen mostly in the erythroid precursor cells, with more subtle abnormalities in the megakaryocyte and granulocyte precursors[8]. This syndrome is extremely rare in dogs and manifests itself clinically as a non-regenerative anemia, thrombocytopenia, and neutropenia, with macrocytic erythrocytes, metarubricytosis, and reticulocytosis[3]. The morphological changes indicate that the marrow is responding to the increased demand for blood cell production. During the course of pre-leukemia, precursor cell maturation becomes progressively impaired and leads to the severe blockage of the maturational process characteristic of acute myeloid leukemia[8]. With acute myeloproliferative disorders, such as MDS, blast cells are usually found in both

circulating peripheral blood and bone marrow. However, even if the blast cells are absent from the peripheral blood, evidence of bone marrow disease such as non-regenerative anemia or thrombocytopenia may indicate that the condition has already progressed to leukemia[3]. MDS also uses the French, American, British (FAB) Classification to categorize the neoplasia. According to the FAB classification system, the criteria used for a condition to be considered a myelodysplastic syndrome are very similar to the criteria for acute myeloid leukemia[2]. The marrow must be hypercellular, have over 30% blast cell population, and contain dysplastic features in one or more cell lines[2]. Dysplastic features include cytoplasm hypogranulation, nuclear abnormalities, and pelgeroid neutrophils[9]. The most common category of pancytopenia is MDS-EB (excessive blast cells) featuring high blast counts, multiple cytopenias, short survival times, and fast progression to acute myeloid leukemia[10].

Diagnostic Approach/Considerations

Rascal had thoracic radiographs performed and showed age and breed-related changes, as well as a small ovoid nodule in the right cranial lung lobe that appeared to be either a cyst or abscess. Abdominal radiographs showed an enlarged spleen, so fine needle splenic aspirates were obtained. The spleen contained a mixed population of neutrophils, macrophages, erythroid precursors, and myeloid precursors, with a few megakaryocytes seen. This indicated that extra medullary hematopoiesis was occurring within the spleen, and that the source of Rascal's pancytopenia could be within his bone marrow. Rascal required a transfusion of one unit of packed blood cells before general anesthesia for bone marrow collection could be safely performed. The transfusion increased his red blood cell count to 25%, and he experienced a mild arrhythmia with occasional multifocal ventricular premature contractions during the procedure that were attributed to his anemia. Rascal was hospitalized overnight and treated with

doxycycline and enrofloxacin. The red blood cell count decreased to 23% overnight, causing concern that a disease causing peripheral destruction of red blood cells may have been present, despite the fact that current blood work results did not appear to support this. Rascal was discharged the following day for continued monitoring at home while awaiting bone marrow results. His owner was given instructions to continue his antibiotics (enrofloxacin, doxycycline) and to avoid unnecessary exposure to pathogens while he had a compromised immune system.

Cytology of the bone marrow revealed a mild to moderate level of cellularity with minimal amounts of associated fat, as well as numerous megakaryocytes at various stages of maturation. Erythroid and myeloid precursor cells were also seen in mildly increased numbers. The myeloid:erythroid ratio (M:E ratio) is approximately 1:3, suggests either low myeloid or high erythroid precursors (normal 1:1 to 2:1). However, given the cellularity, this was believed to be from a disproportionate increase in both the myeloid and erythroid lineages, with the erythroid proliferation appearing much more profound than the myeloid. No infectious agents or overt evidence of neoplasia were observed. Less than 20% of cells observed were blast cells, which did not meet the criteria for leukemia, and it was hypothesized that the marrow was showing evidence of responding to an unknown injury. Analysis of the bone marrow core biopsy revealed a different very picture than the cytology, with the marrow being very hypercellular (approx. 95% cellularity), with over 70% of the cells consisting of blast cells. Core biopsy findings fit the criteria for neoplasia. The core marrow sample did not show evidence of mature cell lines, and specialized testing such as flow cytometry would have been required to help determine the lineage of the neoplastic cells. Rascal's owner, however, elected not to pursue further diagnostics.

Treatment and Management

Acute myeloid leukemia is rapidly fatal if left untreated[10]. In a small study of 16 dogs with confirmed AML, the mean survival time was 20 days after diagnosis. Many of the dogs were euthanized due to the rapid progression of clinical signs[10]. Treatment options for AML are limited, with the majority of therapies used to provide supportive care. Aggressive use of antibiotics is used to prevent infection, and transfusion of blood products is used to supplement the low supply of existing blood cells[3]. AMLs have a poor response to chemotherapy, and with a lack of published information regarding the response of the subtypes of AML, there is no consistency on which protocols are effective[11]. Cytosine arabinoside in combination with doxorubicin or cyclophosphamide, and vincristine and prednisone are the most common agents used in the treatment of AML[11]. The COAP (cyclophosphamide, vincristine, cytosine arabinoside and prednisone) protocol can be used as maintenance therapy once remission is achieved[11].

There is also no standard treatment protocol for myelodysplastic syndrome. As with humans, the goals of therapy in animals are to control symptoms, improve quality of life and decrease progression into AML[12]. In humans, supportive care with blood products and erythropoietin are the mainstays of therapy[12]. Chemotherapy has also been shown to decrease blood transfusion requirements and slow the progression of MDS to AML[12].

MDS in cats are typically associated with FeLV infection, and typically have a 2-3 year duration of survival. Survival in dogs with MDS depends on the subclassification of the disease[13]. Dogs with MDS-RC (refractory cytopenia) and MDS-Er (erythroid component) seem to respond to erythropoietin and prednisone administration, and have prolonged survival. Dogs with MDS-EB (excessive blasts) respond poorly to present treatments, and survival is short[13].

In one study of 16 cats with MDS, overall survival ranged from 2-18 months. Cats with lower blast cell counts had a longer survival, 5 months versus 2 months[14]. In a study of 12 dogs with MDS, those with refractory anemia responded to human recombinant erythropoietin and had a longer survival time than dogs with other forms of MDS; however mean survival time was only 13-14 days[14]. Chemotherapy protocols used in humans have not proven to improve survival times in animals and are often used only when there is a high risk of converting into AML[14]. Bone marrow transplants are the only potential cure for MDS, however transplants are currently in the experimental phase for veterinary medicine[14].

Case Outcome

Rascal had his red blood cell count rechecked two days after discharge, which showed his red blood cell count to have increased (PCV 28%). However, when bloodwork was repeated six days after the transfusion, his red blood cell count was back down to 20%, and his white blood cells and platelets were severely decreased, indicating that the bone marrow was not responding to treatment. Further diagnostics such as flow cytometry and chemotherapeutic options were discussed but, due to Rascal's poor prognosis, his owner elected for palliative care at home for his remaining days. A high dose of prednisone (50mg, PO, q24h) was added to Rascal's medical regimen. Prednisone can be used alone or in combination with other drugs to treat conditions relating to cancer such as anemia, drug hypersensitivity, hypercalcemia, and thrombocytopenia[15]. While it is not completely understood how prednisone works in the treatment of cancer, it is theorized that in certain cancers of blood cells, prednisone might cause cancerous white blood cells to die[16]. Unfortunately, Rascal's condition progressed quickly, and he passed away seventeen days after presentation to the Mississippi State University College of Veterinary Medicine.

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