
Douglas J. Weiss, DVM, PhD, DACVP

Objective—To determine the frequency, potential causes, and clinical and clinicopathologic features of hemophagocytic syndrome in dogs.

Design—Retrospective study.

Animals—24 client-owned dogs.

Procedures—Records for dogs in which diagnostic bone marrow specimens (including an aspiration smear and core biopsy material) were obtained from 1996 to 2005 were reviewed. Inclusion criteria were presence of bicytopenia or pancytopenia in the blood and > 2% hemophagocytic macrophages in the bone marrow aspirate.

Results—Of 617 bone marrow specimens evaluated, evidence of hemophagocytic syndrome was detected in 24 (3.9%). The Tibetan Terrier breed was overrepresented among dogs with hemophagocytic syndrome. Clinical signs associated with hemophagocytic syndrome included fever, icterus, splenomegaly, hepatomegaly, and diarrhea. Hemophagocytic syndrome was associated with immune-mediated, infectious, and neoplastic-myelodysplastic conditions and also occurred as an idiopathic condition. Overall, dogs with infection-associated hemophagocytic syndrome had better 1-month survival rates than dogs with immune-associated and idiopathic hemophagocytic syndrome.

Conclusions and Clinical Relevance—Results indicated that hemophagocytic syndrome may occur more frequently in dogs than has previously been suspected on the basis of the paucity of reported cases. Although most dogs had definable underlying disease conditions, idiopathic hemophagocytic syndrome was also identified. Hemophagocytic syndrome of any cause is potentially life-threatening; however, the prognosis should be adjusted on the basis of the associated disease process and potential for successful treatment. (J Am Vet Med Assoc 2007;230:697–701)

Hemophagocytic syndrome is a benign proliferative disorder of activated macrophages that is associated with multiple cytopenias in the blood.1-6 Cytopenias are thought to result from hemophagocytosis because the bone marrow in affected animals is usually hypercellular.1,2,4 The syndrome has been described in dogs, cats, and humans.1-4 In dogs and humans, hemophagocytic syndromes have been described as developing secondary to infectious, neoplastic, or immune-mediated diseases.1-3,5 In humans, familial and acquired forms of hemophagocytic syndrome have been described.3-3,6 Acquired forms have been referred to as reactive hemophagocytic syndrome or macrophage activation syndrome.3,5 Familial hemophagocytic syndromes have been called primary hemophagocytic lymphohistiocytosis.6,7 Whether primary or secondary, hemophagocytic syndrome is associated with a high mortality rate.3-7 The mortality rate for macrophage activation syndrome in humans varies from 22% to 62%.3

Few reports of hemophagocytic syndrome in dogs have been published. The purposes of the study reported here were to determine the prevalence of hemophagocytic syndrome at a veterinary teaching hospital and to evaluate potential causes and clinical and clinicopathologic features of hemophagocytic syndrome in dogs.

From the Department of Veterinary and Biomedical Sciences, College of Veterinary Medicine, University of Minnesota, Saint Paul, MN 55108.

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>IMHA</td>
<td>Immune-mediated hemolytic anemia</td>
</tr>
<tr>
<td>MDS</td>
<td>Myelodysplastic syndrome</td>
</tr>
<tr>
<td>MDS-EB</td>
<td>MDS with excessive myeloblasts</td>
</tr>
<tr>
<td>MDS-RC</td>
<td>MDS with refractory cytopenias</td>
</tr>
<tr>
<td>IL</td>
<td>Interleukin</td>
</tr>
</tbody>
</table>

Criteria for Selection of Cases

Reports of cytologic evaluations of bone marrow specimens submitted to the University of Minnesota Veterinary Medical Center Cytology Service from July 1, 1996, through June 30, 2005, were reviewed. Reports for dogs for which results of a CBC and a bone marrow aspirate and core biopsy were available were reviewed. If the CBC revealed bicytopenia or pancytopenia, the author reviewed the bone marrow aspirate and core biopsy specimens. A 1,000-cell differential cell count was performed on each bone marrow aspirate, and the percentages of macrophages and hemophagocytic macrophages were determined. Criteria for a diagnosis of hemophagocytic syndrome consisted of bicytopenia or pancytopenia in the blood and > 2% hemophagocytic macrophages in bone marrow aspirates, in accordance with described methods.1-4 Anemia was defined as Hct < 36%.9 Neutropenia was defined as a neutrophil count < 3,000 neutrophils/μL.9 Thrombocytopenia was defined as a platelet count < 300,000 platelets/μL in the absence of platelet aggregates.9
Several dogs with > 2% hemophagocytic macrophages in bone marrow were eliminated from the study. Eight dogs were eliminated because they had anemia alone. Of those, 5 had IMHA, 2 were suspected of having IMHA, and 1 had nonregenerative anemia of undetermined cause. Ten dogs with evidence of necrosis in the core biopsy specimen were eliminated because the hemophagocytic macrophages were considered to be part of the normal process of removal of dead cells. Four dogs with malignant histiocytosis (also termed disseminated histiocytic sarcoma) were also eliminated. Hemophagocytic syndrome was differentiated from malignant histiocytosis by the presence of < 30% histiocytic cells in normocellular or hypercellular bone marrow specimens, absence of highly malignant features and multinucleated forms, and flow cytometric and immunophenotypic findings (when available). Flow cytometric forward-angle versus side-angle light scatterplot analysis findings and immunophenotyping were used to aid in discriminating hemophagocytic syndrome from malignant histiocytosis in 11 dogs, according to described methods.

**Procedures**

The following information was obtained from medical records: age, sex, breed, history, clinical signs, results of laboratory testing (including results of cytologic evaluation of bone marrow aspirates and core biopsy specimens), treatments administered, and survival time. Survival was determined on the basis of information in the medical record.

**Statistical analysis**—For age, breed, sex, and clinical signs, 1-group proportions tests were used to compare the population of dogs included in the study with the population of all dogs that had bone marrow examinations at the teaching hospital during the study period but that did not have hemophagocytic syndrome. Median values were compared by use of the rank sum test. Values of P < 0.05 were considered significant.

**Results**

Seven hundred sixty-nine bone marrow reports were reviewed. Of those, 617 unique case files had concurrent CBCs, bone marrow aspiration smears, and core biopsy specimens available for review. Twenty-four dogs (3.9%) met the inclusion criteria, with bicytopenia or pancytopenia and > 2% hemophagocytic macrophages in bone marrow aspirates (Figures 1 and 2). Associated conditions included immune-mediated diseases (n = 9), infectious diseases (5), neoplastic-myalomatous diseases (5), and idiopathic conditions (5; Table 1).

**Signalment and clinical signs**—Breeds included Labrador Retriever (n = 4); Golden Retriever (4); mixed (4); Tibetan Terrier (3); and 1 each of Samoyed, Shih Tzu, Cocker Spaniel, Bichon Frise, Akita, Shar Pei, Newfoundland, Rottweiler, and Great Dane. Compared with the population of dogs with other diagnoses involving the bone marrow during this period, Tibetan Terriers were overrepresented. However, Tibetan Terriers were found to be dispersed among different disease subgroups. One dog had infection-associated hemophagocytic syndrome, 1 had infection-associated hemophagocytic syndrome, and 1 had idiopathic hemophagocytic syndrome. Median age of all dogs with hemophagocytic syndrome was 7.2 years, with a range of 1 to 13 years. The dogs included 1 sexually intact female, 12 spayed females, 4 sexually intact males, and 7 neutered males. There was no significant difference in age or sex between dogs with hemophagocytic syndrome and dogs with other bone marrow diseases. Clinical signs that were significantly different, compared with those in dogs with other bone marrow diseases, included fever (n = 10), icterus (9), splenomegaly (7), hepatomegaly (3), and diarrhea (4).

**Hemophagocytic syndrome associated with immune-mediated disease**—Nine of the 24 dogs with hemophagocytic syndrome had immune-mediated diseases, including IMHA (n = 5), systemic lupus erythematosus (3), and immune-mediated thrombocytopenia (1; Table 1). Physical examination findings included lethargy (n = 9), weight loss (3), icterus (5), pale mucous membranes (4), heart murmur (4), petechiae (2), collapse (1), melena (1), stiff gait (1), splenomegaly (1), and hepatomegaly (1). All dogs were anemic. Seven dogs...
Five of the 24 dogs with hemophagocytic syndrome were identified. Evaluation of bone marrow at that time revealed hemophagocytic syndrome. Eight (33%) dogs were neutropenic (mean neutrophil count for all dogs, 4,820 cells/µL), and all dogs were thrombocytopenic (mean platelet count, 32,000 platelets/µL). One dog with IMHA had large phagocytic monocytes in 3 blood smears collected during a 7-day period. The bone marrow was hypercellular in 7 dogs and normocellular in 2 dogs. The granulocyte-to-erythroid ratio was within reference range in 3 dogs, high in 2 dogs, and low in 4 dogs. Hemophagocytic macrophage numbers varied from 7% to 22% of all nucleated cells. Eight dogs had high numbers of plasma cells in bone marrow. All dogs were treated with prednisone, and 3 dogs with IMHA were also treated with a low-dose regimen of SC-administered heparin. One-month survival data were available for 8 dogs. All dogs died or were euthanized because of deteriorating health within 10 days of diagnosis of hemophagocytic syndrome. Necropsy was performed on 3 dogs, but no additional causes for the hemophagocytic syndrome were identified.

Table 1—Summary of hematologic findings, bone marrow characteristics, and 1-month survival data by associated disease category in 24 dogs with hemophagocytic syndrome.

<table>
<thead>
<tr>
<th>Disease category</th>
<th>No. of dogs</th>
<th>Anemia</th>
<th>Neutropenia</th>
<th>Thrombocytopenia</th>
<th>Hemophagocytic macrophages in bone marrow (mean ± SD)</th>
<th>Survival at 1 month</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immune-associated</td>
<td>9</td>
<td>9</td>
<td>3</td>
<td>9</td>
<td>9 ± 2</td>
<td>3/5</td>
</tr>
<tr>
<td>Infection-associated</td>
<td>5</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5 ± 2</td>
<td>3/5</td>
</tr>
<tr>
<td>Neoplasia-myelodysplasia-associated</td>
<td>3</td>
<td></td>
<td>2</td>
<td>4</td>
<td>7 ± 2</td>
<td>2/5</td>
</tr>
<tr>
<td>Idiopathic</td>
<td>5</td>
<td>5</td>
<td>3</td>
<td>5</td>
<td>10 ± 4</td>
<td>1/5</td>
</tr>
</tbody>
</table>

Hemophagocytic syndrome associated with infectious disease—Five of the 24 dogs with hemophagocytic syndrome had associated infectious diseases (Table 1). These conditions included septic pyometra (n = 1), septic pleuritis (1), monocytic ehrlichiosis (1), blastomycosis (1), and Lyme disease (1). Physical examination findings included anorexia (n = 4), lethargy (3), fever (3), and diarrhea (2). The diagnosis of blastomycosis was established by identification of organisms in lung aspirates. The diagnosis of monocytic ehrlichiosis was made on the basis of a serum *Ehrlichia canis* antibody titer of 1:163,840. The diagnosis of Lyme disease was made on the basis of findings of polyarthritis, a serum antibody titer of 1:640, negative results of serologic tests for other tick-borne diseases, negative results of tests for immune-mediated diseases, and recovery after treatment with antimicrobials. The diagnosis of septic pyometra was made on the basis of histopathologic findings after surgery of the uterus and culture of *Escherichia coli* from the uterine wall. Two dogs were anemic, 3 were neutropenic, and 4 were thrombocytopenic. Bone marrow was hypercellular in 4 dogs and hypocellular in 1 dog. The granulocyte-to-erythroid ratio was in reference range in 4 dogs and high in 1 dog. The frequency of hemophagocytic macrophages ranged from 3% to 8%. Two dogs had large numbers of plasma cells in the bone marrow aspirate. One-month survival information was available for all dogs. The dog with pyometra recovered after surgical removal of the uterus. The dog with Lyme disease recovered clinically and hematologically after treatment with antimicrobials. The dog with septic pleuritis was euthanized at the owner's request. The dog with monocytic ehrlichiosis was alive 1 month after initial evaluation, but its clinical and hematologic condition continued to decline. The dog with blastomycosis was euthanized at the request of the owner.

Hemophagocytic syndrome associated with neoplasia-myelodysplasia—Hemophagocytic syndrome was associated with malignant lymphoma in 2 dogs, MDS-EB in 2 dogs, and MDS-RC in 1 dog (Table 1). Physical examination findings included anorexia (n = 4), lethargy (3), fever (3), large peripheral lymph nodes (2), splenomegaly (2), hepatomegaly (2), and diarrhea (1). The diagnosis of malignant lymphoma was established by aspiration of peripheral lymph nodes. The diagnoses of MDS-RC and MDS-EB were established by evaluation of bone marrow aspiration smears and core biopsy specimens. Three dogs were anemic, 2 dogs were neutropenic, and 4 dogs were thrombocytopenic. Bone marrow was hypercellular in 2 dogs, normocellular in 2 dogs, and hypocellular in 1 dog. The granulocyte-to-erythroid ratio was considered normal in 2 dogs and was high in 3 dogs. Hemophagocytic macrophages comprised from 4% to 9% of the cell population. One-month survival data were available for all dogs. One of 2 dogs with malignant lymphoma was alive after 1 month. The clinical condition of the 2 dogs with MDS-EB declined rapidly and they were euthanized 1 and 4 days after diagnosis of hemophagocytic syndrome. The dog with MDS-RC survived >3 months after initial evaluation. That dog's clinical signs, anemia, and neutropenia continued to worsen during this time, and the dog was euthanized 97 days after initial evaluation.

Idiopathic hemophagocytic syndrome—Five dogs had hemophagocytic syndromes in which a specific associated disease process was not identified (Table 1). Physical examination findings included lethargy (n = 5), fever (4), icterus (4), diarrhea (1), splenomegaly (4), and hepatomegaly (2). Various tests were performed to rule out infectious, immune-mediated, and neoplastic diseases. All dogs were anemic and thrombocytopenic, and 3 dogs were neutropenic. The anemia was nonregenerative.
generative in all dogs. Two dogs had monocytosis, with monocyte counts from 3,000 to 6,000 cells/µL. No morphologic alterations were observed in these cells. The bone marrow was hypercellular in 4 dogs and hypocellular in 1 dog. The granulocyte-to-erythroid ratio was low in 3 dogs and within reference range in 2 dogs. Mild dyserythropoiesis was detected in 1 dog. Plasma cell numbers were high in 2 dogs. Results of splenic aspirates were available for 2 dogs; both contained many benign-appearing hemophagocytic macrophages. Treatments administered consisted of various combinations of prednisone, cyclophosphamide, and antimicrobials. One-month survival data were available for 4 dogs. One of those dogs died 4 days after initial evaluation, 2 were euthanized 8 and 15 days after initial evaluation because of rapidly declining clinical condition, and 1 was alive 33 days after initial evaluation but was reported to be lethargic and anorectic and the owners were considering euthanasia. Necropsy was not performed on any dogs with idiopathic hemophagocytic syndrome.

Discussion

Hemophagocytic syndrome is difficult to diagnose either clinically or cytologically. Because the condition frequently occurs secondary to other diseases and clinical signs resemble those associated with bacterial sepsis and the systemic inflammatory response syndrome, the condition may be underdiagnosed. Furthermore, cytopenias may be attributed to excessive demand for neutrophils in tissues, consumption of platelets secondary to disseminated intravascular coagulation, or intravascular fragmentation of erythrocytes. Therefore, it is important to determine in which dogs bone marrow evaluation is needed for detection of hemophagocytic syndrome. In humans, serum ferritin concentration has been used as a screening test for detection of hemophagocytic syndrome and malignant histiocytosis. In 1 study, 91% of adult patients with hemophagocytic syndrome had serum ferritin concentrations > 10,000 µg/L. Serum ferritin concentration has not been evaluated as a diagnostic test in dogs with hemophagocytic syndrome, but has been evaluated in dogs with malignant histiocytosis. Moderate hyperferritinemia was detected in dogs with hemolympathic necrosis and hepatic disease. Severe hyperferritinemia was detected in dogs with malignant histiocytosis and immune-mediated hemolytic disease. Therefore, although serum ferritin concentration may be a useful marker for canine malignant histiocytosis, and by inference hemophagocytic syndrome, it may also be high in other disease conditions.

Pathologic conditions that may be confused with hemophagocytic syndrome include malignant histiocytosis, necrosis, granulomatous inflammation, and monocytic and myelomonocytic leukemias. Malignant histiocytosis (also termed disseminated histiocytic sarcoma) is a malignant proliferation of myeloid dendritic cells. However, cytoclastic features alone may be insufficient to differentiate malignant histiocytosis from hemophagocytic syndrome in some instances. Features of malignant histiocytosis that are useful in differentiating it from hemophagocytic syndrome include highly anaplastic features, large cell size, multinucleate giant cells, and > 30% histiocytic cells in cellular bone marrow aspirates. Additional tests to differentiate hemophagocytic macrophages from malignant dendritic cells include flow cytometric scatterplots and immunophenotyping. Monocytic and myelomonocytic leukemia are characterized by hypercellular bone marrow with > 30% blast cells.

Bone marrow necrosis and granulomatous inflammation are accompanied by accumulation of phagocytic macrophages in bone marrow. Cytologic features of necrosis have been described in bone marrow aspiration smears; however, these features are subtle. Therefore, evaluation of a core biopsy specimen is needed to eliminate necrosis as a possible cause of hemophagocytic macrophage proliferation. It has been hypothesized that hemophagocytic macrophages are part of the process of removing dying cells and necrotic debris, as is seen in many tissues after a necrotic event. Granulomatous inflammation in dog bone marrow has been associated with disseminated fungal infection. However, cytophagia was not a prominent feature of the condition.

In the present study, hemophagocytic syndrome was associated with immune-mediated, infectious, neoplastic-myelodysplastic, and idiopathic disorders. Immune-mediated diseases have not been recognized as a disease category frequently associated with hemophagocytic syndrome in dogs, although they are an important cause of hemophagocytic syndrome in humans. It could be argued that hemophagocytosis is the result of antibody coating of blood cells and not the result of macrophage activation. However, several lines of evidence suggest that dogs in the present study had a true hemophagocytic syndrome. First, macrophage proliferation was detected in all dogs. Healthy dogs have 0.4 ± 0.2% macrophages in bone marrow, whereas dogs with immune-associated hemophagocytic syndrome had 15 ± 3% macrophages. Second, multiple cytopenias and phagocytosis of leukocytes, platelets, and erythrocytes were observed in dogs with IMHA. Third, 1 dog with IMHA initially had regenerative anemia and subsequently developed pancytopenia with nonregenerative anemia at the time hemophagocytic syndrome was detected. This indicates that the hemophagocytic macrophages may have been responsible for the nonregenerative anemia and the onset of leukopenia and thrombocytopenia. Fourth, 1 dog had circulating activated monocytes as has previously been described in dogs with hemophagocytic syndrome. Finally, all 5 dogs with IMHA and hemophagocytic syndrome died or were euthanized because of worsening clinical condition. This indicates poor survival, compared with a survival rate > 50% in dogs with IMHA.
ated hemophagocytic syndrome. However, monocytic ehrlichiosis is consistently associated with the presence of antiplatelet antibodies. Hemophagocytic syndrome has also been associated with acute leukemia; malignant lymphoma; and, to a lesser extent, other malignancies in dogs and humans. To the author’s knowledge, myelodysplastic syndromes have not been previously recognized as important disease conditions associated with hemophagocytic syndrome.

Five dogs in the present study had hemophagocytic syndromes, and no associated disease condition was identified. Four of those dogs had a similar history and clinical appearance, consisting of peracute onset of anorexia and weakness, fever, icterus, and splenomegaly. The fever could have developed secondary to the hemophagocytic syndrome or may have resulted from a primary infection. The similarity of clinical appearance in these dogs suggests a common etiology such as a subclinical viral infection.

The pathogenesis of hemophagocytic syndrome remains elusive. Among human patients, hemophagocytic syndrome is divided into 2 subcategories of primary hemophagocytic lymphohistiocytosis and macrophage activation syndrome. Primary hemophagocytic lymphohistiocytosis is a group of autosomal recessive immune disorders in which approximately one third of affected individuals have a mutation in the gene encoding perforin. Perforin is a protein that mediates the cytotoxic activity of natural killer cells and cytotoxic T cells. This suppresses natural killer activity and results in lack of control of cellular immune responses. Oversecretion of Th1-type cytokines, including interferon-γ, IL-12, and IL-18, may cause activation and proliferation of macrophages. Resultant secretion of proinflammatory cytokines, including tumor necrosis factor-α, IL-1, and IL-6, further amplifies the inflammatory process. Although natural killer and cytotoxic T cells have been incriminated in macrophage activation syndrome as well, the mechanism is poorly understood.

Hemophagocytic syndrome was detected in 3.9% of diagnostic bone marrow specimens evaluated at a veterinary teaching hospital. The Tibetan Terrier breed was overrepresented. Clinical signs associated with hemophagocytic syndrome included fever, icterus, splenomegaly, hepatomegaly, and diarrhea. Hemophagocytic syndrome was associated with immune-mediated, infectious, and neoplastic-myelodysplastic disorders and also occurred as an idiopathic condition. Overall, dogs with infection-associated hemophagocytic syndrome appeared to have better 1-month survival rates than dogs with immune-associated and idiopathic hemophagocytic syndrome. Therefore, although hemophagocytic syndrome is a serious disease condition, the prognosis should be made on the basis of the associated disease process and the potential for successfully treating the underlying disease.

References