Correlation between leukocytosis and necropsy findings in dogs with immune-mediated hemolytic anemia: 34 cases (1994–1999)

Patricia M. McManus, VMD, PhD, DACVP, and Linden E. Craig, DVM, PhD, DACVP

Objective—To determine whether severity of leukocytosis correlates with severity of postmortem lesions in dogs with immune-mediated hemolytic anemia (IMHA).

Design—Retrospective study.

Animals—34 dogs with IMHA that had CBC performed within 48 hours prior to death and complete necropsy examinations.

Procedure—Dogs were independently assigned to 4 leukocyte groups (within reference range; mild leukocytosis, moderate leukocytosis, marked leukocytosis) and 3 lesion severity groups (mild lesions, moderate lesions, severe lesions).

Results—Moderate to marked leukocytosis correlated with moderate to severe postmortem lesions. Ischemic necrosis within liver, kidney, heart, lung, and spleen attributable to thromboembolic disease or anemic hypoxia were the most common important lesions found at necropsy. None of the dogs with mild lesions had moderate or marked leukocytosis. Four of 14 severely affected dogs had WBC counts within reference range, but all 4 had neutrophilic left shifts. Three of these 4 dogs had toxic change in neutrophils.

Conclusion and Clinical Relevance—Moderate to marked leukocytosis correlated with moderate to severe postmortem lesions. Ischemic necrosis within liver, kidney, heart, lung, and spleen attributable to thromboembolic disease or anemic hypoxia were the most common important lesions found at necropsy. None of the dogs with mild lesions had moderate or marked leukocytosis. Four of 14 severely affected dogs had WBC counts within reference range, but all 4 had neutrophilic left shifts. Three of these 4 dogs had toxic change in neutrophils.

Mild to moderate leukocytosis is a common abnormality in dogs with immune-mediated hemolytic anemia (IMHA), but the mechanism for this leukocytosis has never been determined. Generally, the leukocytosis is thought to be representative of the immune-mediated basis for the hemolysis rather than attributable to inflammation, infection, tissue necrosis, or other observable lesions. Because of this, many clinicians downplay the importance of leukocytosis in these patients. We theorized that leukogram changes are more likely a reflection of specific and identifiable lesions such as necrosis and inflammation; the purpose of the study reported here was to determine whether severity of leukocytosis correlates with severity of postmortem lesions in dogs with IMHA.

Criteria for Selection of Cases

Medical records of dogs with IMHA that had a CBC performed within 48 hours prior to death and a complete necropsy examination at the Veterinary Hospital of the University of Pennsylvania (VHUP) were reviewed. A diagnosis of IMHA was based on clinical signs and laboratory evidence of anemia and agglutination, erythrocyte spherocytosis, or both. Records of dogs with an equivocal diagnosis and those with concurrent diseases such as neoplasia and diabetes mellitus were not included.

Procedures

An anatomic pathologist (LEC) evaluated tissues collected at necropsy for severity of inflammation, tissue necrosis, or both; dogs were allocated without knowledge of leukocyte counts into 3 groups: mild lesions, moderate lesions, and severe lesions. Mild lesions included thrombi with no evidence of necrosis (Fig 1A), corticosteroid-induced hepatopathy, and cholestasis. Moderate and severe lesions included centrilobular hepatic necrosis (Fig 1B) and infarcts in spleen (Fig 1C), kidney, lung, or heart. Those lesions with < 25% of parenchymal tissue destruction attributable to necrosis (in the examined tissue sections) were graded as moderate, and those with ≥ 25% were graded as severe.

A clinical pathologist (PMM) allocated dogs into 4 leukocyte groups, without knowledge of necropsy results, as follows: within reference range (6,200 to 17,500 cells/µl); mild leukocytosis (17,600 to < 28,000 cells/µl); moderate leukocytosis (28,000 to 40,000 cells/µl); and marked leukocytosis (> 40,000 cells/µl). None of the dogs were leukopenic.

Total neutrophil counts, including mature and immature cells, were determined for all dogs, but not stratified into categories according to severity or relationship to postmortem lesions. This approach was taken because slides made prior to 1996 were not stored and therefore could not be reviewed. Many different technicians had performed the differential analysis of smears. Given the variability in skill level at performing these reviews and subjectivity in assessment of monocytes, immature neutrophils, and toxic change, the decision was made to rely on the instrument-generated total leukocyte counts, which have a lower coefficient of variation.

From the Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA 19104.

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Eighty-six (80.6%) dogs had PCV transfusion, were known for 31 dogs. Twenty-five of 31 (80.6%) dogs had PCV ≤ 20% at referral (reference range, 42 to 60%). Significant differences were not detected among mean PCV values of dogs with mild lesions (mean ± SD, 16.5 ± 8.3%), moderate lesions (14.7 ± 6.3%), and severe lesions (16 ± 4.9%).

Red blood cell indices were not valid in 17 of the 34 dogs because of agglutination of the samples. Furthermore, hemolysis, often accompanying agglutination, commonly affected the MCHC and MCH, preventing valid evaluation of these indices. In the 17 dogs with valid MCV values, mean MCV was 86.0 ± 9.0 fl (range, 72.4 to 106 fl). Three dogs had MCV within the reference range of 66 to 77.1 fl, whereas 13 dogs had macrocytosis, with values ranging from 78 to 106 fl. Mean MCHC for these dogs was 31.7 ± 3.8 g/dl (range, 25.7 to 38.7; reference range, 32.4 to 36.3 g/dl). Spuriously increased MCHC values were common in grossly hemolyzed specimens.

An attempt to classify anemias as regenerative versus nonregenerative was hampered by the variability of information regarding RBC indices and reticulocyte counts for each dog. For 4 dogs, reticulocyte counts were not determined. One of these dogs had 2+ polychromasia, but subjective comments regarding this variable were not recorded for the other 3 dogs. Two of these 3 dogs had macrocytosis, whereas 1 dog had marked agglutination that invalidated the RBC count and MCV values.

Reticulocyte counts were determined for 30 dogs; however, reticulocyte counts were determined at various times relative to onset of anemia. Agglutination prevented calculation of absolute reticulocyte counts for 13 of these dogs. Corrected reticulocyte counts, based on the ratio of the observed to expected PCV multiplied by the reticulocyte percentage, indicated a regenerative response in 12 of these 15 dogs. Of the 15 dogs with calculated absolute reticulocyte counts, 13 had regenerative anemia as judged by reticulocyte count > 65,000 cells/µl. Mean reticulocyte count for these 13 dogs was 247,000 ± 208,000 cells/µl (range, 71,000 to 803,000 cells/µl). Thus, 5 dogs were tentatively classified as having nonregenerative anemia by quantifying reticulocytes by use of corrected or absolute values. Verification of nonregenerative anemia requires repeat analysis 3 to 4 days after onset of anemia to allow for delayed marrow release of RBC. The nonregenerative status for 3 of the 5 dogs was not verified, typically because these dogs were euthanatized within 1 to 2 days of referral.

Cocker Spaniels were overrepresented (n = 6 [17.6%] dogs), compared with the proportion of Cocker Spaniels (3.0%) within the general population at VHUP during the same period. Eleven (32.4%) dogs were mixed-breed dogs. Purchased dogs included Labrador Retriever (n = 3), Golden Retriever (2), Shih Tzu (2), Lhasa Apso (2), Bichon Frise (2), Toy Poodle (1), Standard Poodle (1), Yorkshire Terrier (1), Irish Wolfhound (1), Beagle (1), and German Shepherd Dog (1). Age ranged from 2 to 12 years (mean, 6.9 ± 2.9 years). There were 17 females (15 spayed) and 17 males (14 castrated).

The 2 most common postmortem abnormalities, which were detected evenly among the 3 lesion severity groups, were lesions consistent with coagulopathy (macrothrombi, microthrombi, widespread fibrin...
Counts of 31,600 dogs with moderate and severe lesions had mean WBC 8,200 to 23,800 cells/µl. Nine dogs had marked leukocytosis, with counts each in the mild and moderate leukocytosis groups. Range (6,200 to 17,500 cells/µl) for all dogs was 86.8 ± 20.8%. Dogs with severe lesions that had WBC counts within the reference interval and degenerative neutrophilic left shifts. It was considered inappropriate to include these dogs because this is a situation in which consumption of neutrophils at the site of inflammation exceeds production by the marrow, resulting in a WBC count within reference range and an ineffective myeloid hyperplasia; WBC count within reference range is therefore misleading. After deletion of these 4 dogs, mean WBC count of dogs with severe lesions (31,600 ± 12,800 cells/µl) was significantly (P = 0.041) different from mean WBC count of dogs with severe lesions (42,500 ± 16,700 cells/µl). Furthermore, results of 1-way ANOVA (P = 0.003) and linear trend contrast (P < 0.001) analyses of the WBC data for the 3 lesion severity groups supported the hypothesis that there was an association between high WBC count with lesion severity.

Mean percentage neutrophils (mature and immature) for all dogs was 86.8 ± 8.9% (range, 54 to 99%). Ninety-four percent of all dogs had > 74% neutrophils; 2 dogs had 54 and 63% neutrophils, respectively. High total WBC counts were not attributable to high lymphocyte, eosinophil, or basophil counts in any dogs.

Thirty-two dogs had total bilirubin concentration evaluated within 48 hours prior to death (range, 0.4 to 77.6 mg/dl). Five dogs had bilirubin concentration within reference range (0.3 to 0.9 mg/dl), whereas 8 dogs had bilirubin concentration between 1 and < 10 mg/dl, 9 dogs had bilirubin concentration between 10 and 20 mg/dl, and 10 dogs had bilirubin concentration > 20 mg/dl (Fig 3). For dogs with mild microscopic deposition, hemorrhage; n = 25 [73.5%]) and cholestasis (27 [79.4%]).

Lesions in 6 of the 34 dogs were classified as mild and included cholestasis (n = 4), thrombi in the liver, lung, or both, without necrosis (4), hemosiderosis (4), extramedullary hematopoiesis (3), corticosteroid-induced hepatopathy (1), hepatic lipidosis (1), mild pneumonia (1), mild chronic active hepatitis (1), mild splenic necrosis (< 2% of parenchyma [1]), and mild centrilobular hepatic necrosis (< 2% of parenchyma [1]).

Twenty-eight of 34 (82.4%) dogs had notable necrosis in 1 or more organs (moderate or severe lesions, n = 14; severe lesions, 14). Organs most commonly affected included liver, spleen, and kidneys. Neutrophilic infiltrates were commonly observed within regions of necrosis, at margins between healthy tissue and infarcts, or both. Nineteen of the 28 (67.9%) dogs had notable liver abnormalities; 18 dogs had moderate to severe centrilobular hepatic necrosis, and 1 had chronic active hepatitis with fibrosis and bile duct hyperplasia. Sixteen (57.1%) dogs had splenic necrosis that was either multifocal or regionally extensive. Ten (35.7%) dogs had renal tubular necrosis. Ten (35.7%) dogs had lesions in the heart; 6 dogs had myocardiac necrosis, and 4 had myocarditis. Seven (25%) dogs had notable lung lesions; 1 dog had thrombus-associated pulmonary infarction, and 6 had interstitial pneumonia. Six dogs had thrombi in pulmonary vessels but no associated necrosis. Other lesions included hemosiderosis (n = 17), extramedullary hematopoiesis (14), corticosteroid-induced hepatopathy (5), pancreatic necrosis (2), duodenal crypt necrosis (1), falciform fat necrosis (1), polioencephalomalacia (1), chronic perforating duodenal ulcer with chronic localized peri-tonitis (1), and urinary bladder necrosis (1).

Leukocyte counts ranged from 6,800 to 66,100 cells/µl. With the exception of 3 dogs that were not treated, WBC counts represented posttreatment values. Nine dogs had total WBC counts within reference range (6,200 to 17,500 cells/µl). There were 8 dogs each in the mild and moderate leukocytosis groups. Nine dogs had marked leukocytosis, with counts > 40,000 cells/µl. Means for each of the lesion severity groups were calculated. Dogs with mild lesions had WBC values (mean, 15,900 ± 6,600 cells/µl; range, 8,200 to 23,800 cells/µl) within the reference range. Dogs with moderate and severe lesions had mean WBC counts of 31,600 ± 12,800 (range, 6,800 to 60,900 cells/µl) and 35,000 ± 18,600 cells/µl (range, 13,400 to 66,100 cells/µl), respectively; these values were not significantly different, although they were significantly (P = 0.042) greater than mean WBC count of the dogs with mild lesions.

Distribution of dogs with mild, moderate, or severe lesions among the 4 groups classified by leukocyte count was determined (Fig 2). Dogs with severe lesions had a bimodal distribution with peaks in groups with the lowest and highest WBC ranges. Six of 9 dogs with marked leukocytosis had severe lesions. All 4 of the dogs with severe lesions and WBC counts within reference range had neutrophilic left shifts, and 3 of these dogs had toxic changes in neutrophils. Dogs with moderate lesions were most numerous in the group with moderate leukocytosis. Among dogs with leukocyte counts within reference range, 4 dogs had mild postmortem lesions; none of these dogs had neutrophilic left shifts or toxic change reported in the medical records. Dogs with mild lesions also did not have moderate or marked leukocytosis.

When all data were included, mean WBC counts of dogs with moderate lesions were not significantly different from those of dogs with severe lesions; however, different findings resulted from deletion of the 4 dogs with severe lesions that had WBC counts within the reference interval. Dogs with severe lesions among groups with various total leukocyte counts.
lesions, mean bilirubin concentration was 2.7 ± 4.3 mg/dl (range, 5 to 11.3 mg/dl). For dogs with moderate lesions, mean bilirubin concentration was 26.2 ± 24.4 mg/dl (range, 3 to 77.6 mg/dl). For dogs with severe lesions, mean bilirubin concentration was 15.2 ± 13.1 mg/dl (range, 0.7 to 48.9 mg/dl). Mean bilirubin concentration in dogs with mild lesions was significantly (P = 0.037) less than that of dogs with moderate or severe lesions; however, a significant difference between dogs with moderate versus severe lesions was not detected. The 2 highest values (77.6 and 67.7 mg/dl) were reported in dogs with moderate lesions.

Total bilirubin concentrations were evaluated with regard to severity of hepatic necrosis or hepatitis. None of the 5 dogs with total bilirubin concentrations within reference range had evidence of hepatic necrosis or hepatitis; 3 of these dogs had corticosteroid-induced hepatopathy. Seventeen of the 27 (63%) dogs with hyperbilirubinemia (≥ 0.9 mg/dl) had evidence of some degree of hepatic necrosis or inflammation. Five of the 8 dogs with bilirubin concentration between 1 and < 10 mg/dl had evidence of centrilobular necrosis (2 severe, 2 moderate, 1 mild). One dog had chronic active hepatitis with fibrosis and biliary duct hyperplasia. Two dogs had no evidence of hepatic necrosis or hepatitis, but 1 of these had corticosteroid-induced hepatopathy. Six of the 9 dogs with bilirubin concentration of 10 to 20 mg/dl had evidence of centrilobular necrosis (5 severe, 1 moderate). Three dogs had no evidence of hepatic necrosis, hepatitis, or corticosteroid-induced hepatopathy. Six of the 10 dogs with bilirubin concentration > 20 mg/dl had evidence of centrilobular necrosis (2 severe, 3 moderate, 1 mild). Three dogs had cholestasis but no hepatic necrosis, hepatitis, or corticosteroid-induced hepatopathy; these 3 dogs had bilirubin concentrations of 45.2, 77.6, and 48.9 mg/dl, respectively. One dog with > 20 mg of bilirubin/dl had an autolyzed liver that could not be evaluated for necrosis.

Total bilirubin concentration ≥ 10 mg/dl is used as an index for a poor prognosis. Thirteen (38.2%) dogs in the study reported here had total bilirubin concentration < 10 mg/dl (5 of 6 dogs with mild lesions, 4 of 13 with moderate lesions, and 4 of 13 with severe lesions); a significant correlation between WBC count and total bilirubin concentration was not detected (correlation coefficient = 0.14).

Treatment was not attempted in 3 dogs. At necropsy, 1 of these dogs had hepatic sinusoidal thrombi without necrosis; this lesion was considered mild. The other 2 dogs had moderate lesions. Twenty-nine of the 31 treated dogs received blood transfusions. Other treatments included administration of immunosuppressive drugs (27 dogs), doxycycline (17), and oxytetracycline (2). Immunosuppression was most often attempted by administration of corticosteroids (eg, prednisone and dexamethasone); however, a few dogs also received azathioprine, cyclophosphamide, cyclosporine, and vincristine sulfate.

### Discussion

To the authors’ knowledge, the mechanism for IMHA-associated leukocytosis has never been explored. Often the leukocytosis is simply referred to as evidence of the immune-mediated nature of the disease or possibly secondary to use of corticosteroids. Results of the study reported here indicate a relationship between IMHA-associated leukocytosis and demonstrable lesions such as necrosis. We hypothesize that tissue necrosis, secondary to anemic hypoxia or thromboembolic disease, is the primary stimulus for the IMHA-associated leukocytosis evident in dogs of our study and others. Necrosis has been documented as a cause for leukocytosis. In a recent retrospective study of dogs, tissue necrosis is cited as the cause for extreme leukocytosis (≥ 50,000 cells/µl) in 12 of 118 dogs. In humans, leukocytosis is used as an indicator of coronary thrombosis and infarction to distinguish this condition from coronary insufficiency not accompanied by tissue necrosis.

One of the more common and important necrotizing lesions in dogs of our study was centrilobular hepatic necrosis. To our knowledge, reports have not been published that describe the prevalence of this lesion in dogs with IMHA, although this lesion is regarded as typically associated with hypoxia that is secondary to a precipitous decrease in Hct or to heart failure-induced hypoperfusion. Although anemic hypoxia is the probable underlying mechanism for IMHA-associated centrilobular necrosis, we did not detect a correlation between severity of anemia at referral and severity of postmortem lesions. In fact, severe anemia was common to all 3 lesion severity groups, with no significant differences among them. We assume that extended duration of anemic hypoxia aggravates the degree of injury, but we did not examine this variable. Factors that may have affected the duration of anemia include early euthanasia, poor response to treatment, and inability to adequately transfuse the patient because of blood-typing incompatibilities. In humans, the ability to compensate and adequately deliver oxygen to all tissues by adjustments in cardiovascular function is surpassed when Hct decreases to

![Diagram](https://example.com/diagram.png)
granulocytosis is not sustained. Furthermore, in dogs, marrow does not contribute to the leukocytosis, and granulocytosis is primarily attributable to demargination of WBC from the marrow. Most dogs with IMHA have Hct < 22.5%. Compensatory changes include an increase in cardiac output, redistribution of blood flow to favor myocardium and brain at the expense of kidney, liver, spleen, and intestine, and an increase in the whole-body oxygen extraction ratio. It has also been reported that the physiologic response to hemodilution in dogs is similar to that in humans. Given that the reference range of Hct for humans and dogs is similar, perhaps the threshold value of 22.5% also applies to dogs. Most dogs with IMHA have Hct ≥ 20% at referral; therefore, it seems likely that these dogs will not be able to compensate and will enter into a state of severe anemic hypoxia resulting in tissue death (eg, hepatic necrosis). Multifocal necrosis observed in other organs (eg, kidney, heart, brain, and spleen), with the exception of necrosis secondary to thromboembolic disease, may also be attributable to anemic hypoxia.

The other important necrotizing lesion observed in our study and others' was infarction of 1 or more organs secondary to thromboembolic disease. Pulmonary infarction is rare because of the dual blood supply to the lungs; therefore, despite the presence of thrombi in pulmonary vessels of 7 dogs, including 1 dog with mild microscopic lesions, only 1 dog had severe pulmonary infarction. Conversely, splenic, renal, and cardiac infarctions secondary to thromboembolic disease were fairly common. The hypercoagulable state that causes thromboembolic disease observed in dogs with IMHA has been postulated to be secondary to use of corticosteroids, which may reduce concentrations of plasminogen activators.

The mechanism responsible for leukocytosis that develops secondary to necrosis is not specifically known, but neutrophilia potentially reflects changes in granulocyte kinetics at 3 levels: marrow, blood, and tissues. These changes include increases in marrow proliferative rate, marrow release, and circulating neutrophil pool (concurrent with a decrease in the margined neutrophil pool), and a decrease in the rate of migration from blood into tissues. Sustained leukocytosis requires marrow myeloid hyperplasia, which is stimulated by a variety of cytokines released secondary to infection, necrosis, and neoplasia. Corticosteroid administration may influence leukocyte concentrations; however, results of our study did not suggest that use of corticosteroids markedly affected WBC concentrations in the dogs with IMHA.

Results of a retrospective study of leukemoid responses in dogs indicates that high WBC counts are not detected in dogs treated with high-dose corticosteroids for dermatologic disease. Results of a recent study using labeled neutrophils suggest that glucocorticoid-induced granulocytosis is primarily attributable to demargination from the circulating pool (61%), with minor contributions from heightened marrow release (10%) and delayed egress from circulation (29%). The mitotic pool in marrow does not contribute to the leukocytosis, and granulocytosis is not sustained. Furthermore, in dogs, margined neutrophils are thought to account for approximately half of the total intravascular neutrophil pool. Any increase attributable to demargination would be limited to a doubling of the WBC count.

Complement factor 3a, and possibly a cleavage product from the third complement component, are thought to also stimulate release of neutrophils from marrow. Given the role complement plays in immune-mediated hemolysis, generation of these molecules may be enhancing the effect of corticosteroids; however, there is a limit to the narrow storage pool capacity. Also, like corticosteroids, complement factors do not affect the mitotic pool.

A corticosteroid-independent decrease in the rate of migration of WBC into tissues may also contribute to leukocytosis. Decreased egression causes marked leukocytosis in leukocyte adhesion deficiencies, which is attributable to an inability to adhere to endothelium. Decreased egression also develops during resolution of inflammation when tissue needs diminish (“rebound” leukocytosis). Perhaps, in dogs with IMHA, poor perfusion of necrotic tissues inhibits migration. We have observed that neutrophilic infiltrates may be confined to regions at the margins of infarcts, whereas the necrotic region may be relatively devoid of neutrophils. The large expanse of necrotic tissue, however, will still result in release of factors that influence myeloid hyperplasia and heighten leukocyte release from marrow. The combination of cytokine-stimulated myeloid hyperplasia, increased marrow release, neutrophil demargination, and decreased migration into poorly perfused severely necrotic tissues may be the collective mechanism for high WBC counts seen in dogs with IMHA. This possibility raises questions regarding the cytokines that stimulate the myeloid hyperplasia. Tumor necrosis factor α, colony stimulating factors, and interleukin 1 have all been implicated in leukocytosis.

Results of our study support the reported observation that high bilirubin concentration is a poor indicator of prognosis. In our study, dogs with mild lesions had significantly lower bilirubin concentrations than dogs with moderate and severe lesions. Hyperbilirubinemia, secondary to hemolytic anemia, is attributable to increased delivery of bilirubin to the liver, which is not matched by comparable clearance. High bilirubin concentration may reflect increased loss of liver function secondary to ischemic injury; however, extremely high bilirubin concentration was not a consistent predictor of hepatic lesions. Three dogs with bilirubin concentrations > 45 mg/dl did not have any evidence of hepatic necrosis or hepatitis. In addition, marked hyperbilirubinemia was not a consistent finding, as all dogs in our study had a poor prognosis and 38% had bilirubin concentrations < 10 mg/dl. Furthermore, approximately 30% of dogs with moderate or severe lesions had bilirubin concentrations < 10 mg/dl. These findings suggest that bilirubin is a less reliable predictor of postmortem lesions, compared with WBC count.

Moderate to marked leukocytosis, neutrophilic left shift, and toxic change in dogs with IMHA should alert clinicians to the potential for moderate to severe tissue necrosis, which could complicate treatment and worsen prognosis. Lesions appear to be secondary to anemic hypoxia, thromboembolic disease, or both; therefore, treatment objectives should focus on improving blood oxygen-carrying capacity and monitoring for thromboembolic disease.
References


