



Canine Immune-Mediated Hemolytic Anemia: Pathophysiology, Clinical Signs, and Diagnosis*

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ABSTRACT: Immune-mediated hemolytic anemia (IMHA) is a common type of anemia in dogs and cats that results from a type II hypersensitivity reaction. The disease is most common in middle-aged female dogs, especially American cocker spaniels. The common clinical signs are associated with severe anemia and the resultant inflammatory response. There is no pathognomonic test for IMHA, but the following are suggestive of it: the presence of hemolytic anemia in a young adult or middle-aged dog of a predisposed breed, autoagglutination and/or spherocytosis, positive results from a direct antiglobulin (Coombs') test, elimination of any other underlying cause of anemia, and an appropriate response to immunosuppressive therapy.

Immune-mediated hemolytic anemia (IMHA) is one of the most common types of anemia in small animals. The disease is caused by immune-mediated destruction of red blood cells (RBCs) and results in an accelerated decrease in the total RBC mass. IMHA may occur as an idiopathic (primary) event or secondary to a variety of infectious or neoplastic disorders.^{1,2} The diagnosis is supported by the presence of spherocytes, RBC agglutination, positive results from a Coombs' test, and the absence of a detectable underlying cause of hemolytic anemia.¹ Unfortunately, the overall mortality rate associated with IMHA is high (approximately

50%) despite heightened awareness of the disease and new drug treatment approaches.³⁻⁶

MECHANISM OF DISEASE

The normal life span of the canine RBC is approximately 100 to 120 days.^{3,4,7} Removal of aged RBCs normally occurs within the liver and spleen by the mononuclear phagocyte system (MPS).⁸ This system identifies antibodies directed against senescent membrane antigens and clears them from circulation.⁸

IMHA is a pathologic process that results in premature destruction of RBCs when an immune response directly or indirectly targets RBCs of all ages.⁷ IMHA is considered a predominantly type II hypersensitivity reaction in



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*A companion article on treatment and prognosis begins on page 230.

Causes of Secondary IMHA in Dogs^{1,7,8,10,13,14,17,18,23,a-c}

Infection

- Ehrlichiosis
- Babesiosis
- *Anaplasma phagocytophilum* infection
- *Haemobartonella canis* infection
- Leptospirosis
- Dirofilariasis
- Histoplasmosis

Neoplasia

- Lymphosarcoma
- Hemangiosarcoma
- Lymphocytic leukemia
- Gastric and lung carcinoma
- Diffuse sarcoma

Drugs

- Trimethoprim–sulfonamide
- Penicillins
- Cephalosporins
- Levamisole
- Phenylbutazone
- Dipyrone
- Chlorpromazine

Intrinsic RBC defects

- Phosphofructokinase deficiency
- Pyruvate kinase deficiency
- Hereditary osmotic fragility

Miscellaneous

- Onion
- Garlic
- Zinc
- Bee-sting envenomation
- Vaccination

^aHammer AS, Couto CG, Swanson C, et al: Hemostatic abnormalities in dogs with hemangiosarcoma. *J Vet Intern Med* 5(1):11–14, 1991.

^bMellanby RJ, Holloway A, Chantrey J, et al: Immune-mediated hemolytic anemia associated with a sarcoma in a flat-coated retriever. *J Small Anim Pract* 45(1):21–24, 2004.

^cMackin A: Immune-mediated hemolytic anemia: Pathophysiology and diagnosis. *Proc ACVIM* (suppl):1–4, 2002.

which anti-RBC antibodies, including IgG, IgM, and IgA, attach directly or indirectly to various components of the RBC membrane.^{2,9} Attachment of immunoglobulin to the cell membrane can cause extravascular hemolysis, intravascular hemolysis, and intravascular RBC agglutination. In severe immune reactions, large numbers of antibodies attach to the

RBC membrane, activating the complement cascade. The membrane attack complex produced by activation of the complement cascade causes direct damage to the cell membrane, an influx of extracellular fluid into the RBC, and rupture of the cell while it is still in circulation. This RBC rupture is called *intravascular hemolysis* and results in the release of free hemoglobin into the bloodstream (hemoglobinemia) and subsequent hemoglobinuria. Because IgM is better than IgG at fixing complement, intravascular hemolysis is more likely to occur with IgM-mediated disease.¹⁰ In less severe cases of IMHA with minimal complement-mediated cell-wall damage, antibody (especially IgG) attachment causes removal of affected RBCs by the MPS. This MPS-mediated process occurs outside of the circulation and is, therefore, called *extravascular hemolysis*. Fc receptors on macrophages in the liver and spleen bind to the Fc component of antibody coating the RBC membrane, resulting in RBC phagocytosis and destruction. Because RBC hemoglobin enters the bilirubin metabolic pathway rather than spilling into the circulation, hemoglobinemia and hemoglobinuria do not occur with extravascular hemolysis.

IMHA can be divided into two main types, primary and secondary, based on the presence of underlying disease. Primary (idiopathic) IMHA is a classic example of an autoimmune disorder with no identifiable underlying cause and is the predominant form of IMHA.¹ Autoantibodies are produced against the animal's own RBC membrane antigens. Glycophorin, a glycoprotein that spans the plasma membrane, is one of the more common RBC membrane antigens targeted by autoantibodies.¹¹ Normally, autoantibodies are prevented from reacting with host tissues by suppressor T cells. It is believed, however, that IMHA-affected animals have poorly regulated suppressor T-cell function or overstimulated immune systems that allow autoantibodies to attach to normal cells and trigger RBC destruction.^{8,12} Impaired suppressor T-cell function has been demonstrated in some genetically related mice and humans, which may suggest a genetic predisposition to the development of IMHA.⁸

Secondary IMHA is caused by an immunologic response to nonself antigens that have modified or are associated with normal RBC membranes. Secondary IMHA can be caused by a number of underlying processes. Affected RBCs may become infected by pathogens or coated with foreign antigen. Documented or hypothesized causes of secondary IMHA include

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bacterial, viral, rickettsial, parasitic, protozoan, and neoplastic disorders^{1,10,13} (see box on page 218).

A temporal association between recent vaccination and IMHA has been identified.¹⁴ Approximately one-quarter of the patients with IMHA in several studies^{7,14-16} have been found to be vaccinated within 30 days of presentation. No particular vaccine has been implicated as the trigger, and direct causation has not been conclusively established. There are certainly theoretical explanations for a causal link between vaccination and IMHA: vaccination may be a nonspecific trigger that activates macrophages; heightens a low-grade, inflammatory condition; or deregulates the balance of the immune system.^{7,14}

Several drugs (e.g., sulfa antibiotics, penicillins, cephalosporins, levamisole, insulin, acetaminophen, tetracycline, phenylbutazone, dipyrone, quinidine, chlorpromazine) have been suggested to cause IMHA, but there is limited proof^{10,17,18} (see box on page 218). Drug-induced immune-mediated hemolysis involves several

DIFFERENTIAL DIAGNOSIS

Not all causes of hemolysis are immune mediated, and non-immune-mediated hemolytic anemia must be differentiated from IMHA. Relatively common nonimmune causes of hemolytic anemia in small animals include inherited defects, toxin exposure, hypophosphatemia, and microangiopathic hemolytic anemia. In young animals with hemolytic anemia, a hereditary disorder, such as pyruvate kinase deficiency (found in basenjis and other canine breeds), phosphofructokinase deficiency (found in English springer and American cocker spaniels), or hereditary RBC osmotic fragility (found in Alaskan malamutes and miniature schnauzers), should be considered.^{10,20} Hemolytic anemia has been associated with exposure to a number of toxins, including zinc, acetaminophen, onion, garlic, and other oxidant agents, that can lead to Heinz body hemolytic anemia. Zinc intoxication typically occurs from ingestion of US pennies minted after 1983 or zinc oxide diaper ointment or sunscreen. Severe hypophosphatemia

Primary IMHA is a diagnosis of exclusion; therefore, all other causes of hemolytic anemia, including underlying infection, toxin exposure, and neoplasia, must be thoroughly ruled out.

proposed mechanisms of action. The major proposed mechanism for penicillin- and cephalosporin-associated IMHA is adhesion of drug or drug breakdown products to the RBC membrane, with subsequent induction of complement attack or cell removal by the MPS. Sulfonamides, quinidine, insulin, acetaminophen, and tetracycline may induce IgM antibody production. The consequent drug-antibody complex binds to RBC membranes and initiates complement activation, resulting in intravascular hemolysis.¹⁹

Immune-mediated hemolysis may also be caused by alloantibodies (antibodies produced by one individual that react with antigens in another member of the same species) directed against RBC membrane components. Classic causes of alloimmune hemolytic anemia in small animals include blood group incompatibility transfusion reactions and neonatal isoerythrolysis. The transfusion of blood positive for dog erythrocyte antigen (DEA) 1.1 to a bitch with blood negative for DEA 1.1 can sensitize its immune system, resulting in neonatal isoerythrolysis if it is bred with a male with blood positive for DEA 1.1.^{1,8}

(which is commonly associated with initial treatment of diabetic ketoacidosis or aggressive nutritional support of malnourished patients) has reportedly caused acute hemolytic anemia. Microangiopathic hemolytic anemia is a form of hemolytic anemia in which RBCs are physically damaged while in circulation. RBCs may become sheared, fragmented, or injured as they pass through tumors, fibrin clots, or damaged vessels and are subsequently pulled out of circulation by the MPS system. Common causes of microangiopathic hemolytic anemia include heartworm disease, vascular or gastrointestinal (GI) neoplasia, vasculitis, heart valve disease, cardiovascular implants, intravenous catheters, splenic diseases or torsion, liver disease, and disseminated intravascular coagulation (DIC).^{10,21,22}

Sometimes, it can be difficult to determine whether hemolytic anemia is immune or nonimmune mediated. For example, a recent case study²³ reported suspected IMHA secondary to bee-sting envenomation in two dogs. However, because bee venom contains hemolysins, it may be difficult to distinguish antibody-mediated destruction from direct toxin-induced hemolysis.²³ Sim-

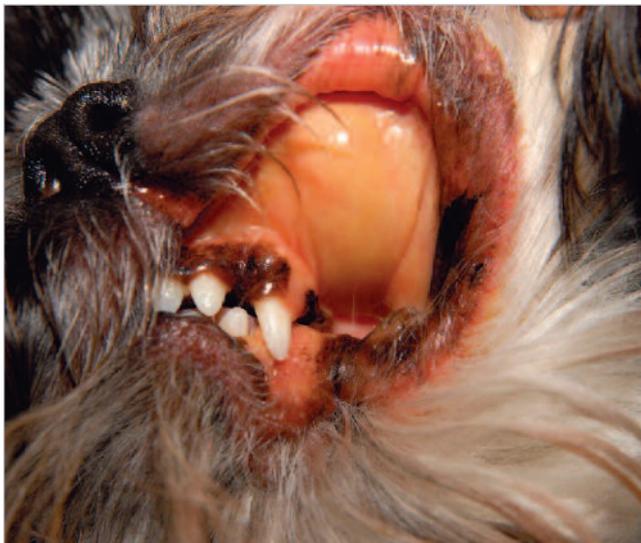


Figure 1. Icteric mucous membranes.

ilarly, in patients with parasitic diseases that affect RBCs such as hemobartonellosis or babesiosis, it may be difficult to determine whether hemolysis is caused by direct parasite-associated RBC damage or by secondary IMHA.

SIGNALMENT

IMHA is much more common in dogs than in cats. Primary IMHA can occur in any canine breed, but cocker spaniels, English springer spaniels, poodles, Old English sheepdogs, Irish setters, and collies are overrepresented.^{4,5,9,16,20,24,25} American cocker spaniels reportedly represent approximately one-third of all dogs with IMHA.¹⁰ One study²⁴ found an increased incidence of IMHA in bichons frises, miniature pinschers, rough-coated collies, and Finnish spitz breeds, while two studies^{15,20} found an increased incidence in miniature schnauzers. Variations in breed predilection found in different case studies may indicate geographic overrepresentation of certain breeds. Most studies^{3-5,15,20,25} have found an increased incidence of IMHA in female dogs. The mean age of onset is approximately 6 years, but IMHA can develop at 1 to 13 years of age.^{4,5,9,15,20,25} Two studies^{5,15} noted an increased incidence of disease in spring and summer, with 40% of cases occurring in May and June. Other studies^{4,26} have shown no similar seasonal prevalence. The possibility of higher rates of IMHA during the warmer months may suggest an undiagnosed infectious cause, including tick-borne disorders.



Figure 2. Icteric soft palate.

HISTORY AND CLINICAL SIGNS

The patient history and clinical signs are crucial in diagnosing IMHA. Clinical signs of tissue hypoxia caused by both acute and severe anemia typically predominate, although signs associated with excess hemolytic breakdown products (bilirubin or free hemoglobin) and a generalized inflammatory and immune-mediated process may also be observed. If the onset of anemia is slow, the clinical signs may be minimal until anemia is severe.⁸ Tachypnea, tachycardia, and increased cardiac output can initially compensate for anemia but eventually cause decompensation if anemia progresses. The historical findings often include collapse, weakness, exercise intolerance, lethargy, anorexia, tachypnea, dyspnea, vomiting, and diarrhea and occasionally include polyuria and polydipsia. In some anemic patients, the signs are minimal at rest but worsen during stress or exercise.

Physical examination typically reveals pale mucous membranes, tachypnea, splenomegaly, hepatomegaly, icterus, pigmenturia (hemoglobinuria or bilirubinuria), fever, and lymphadenopathy.²⁵ Jaundice is a common and easily observed physical examination abnormality. Jaundice is usually first noted on the mucous membranes when the serum bilirubin level exceeds 2 to 3 mg/dl and affects the skin later in the disease when bilirubin concentrations are higher²⁷ (Figures 1 and 2). Cardiovascular changes, including tachycardia, S₃ gallop, and systolic murmur, are common in anemic patients. A grade II or III of VI systolic hemic murmur is frequently detected in animals with a packed cell volume (PCV) of less than 15% to 20% and is caused by anemia-associated blood turbulence.

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Approximately 50% to 70% of dogs with IMHA have concurrent thrombocytopenia known as *Evans syndrome*.^{5,16,20,26} Petechiae, ecchymoses, and melena may be present if thrombocytopenia is severe. Immune-mediated platelet destruction and platelet consumption due to DIC should be considered as differentials in cases of concurrent hemolytic anemia and thrombocytopenia.

Several uncommon or rare conditions in which antibodies against RBCs are active only at reduced body temperatures have been reported. In one form, cold weather causes agglutination of RBCs in cool parts of the body, impairing blood flow and resulting in anemia and ischemic skin lesions of the extremities. In another form, cold weather causes intravascular hemolysis, resulting in anemia and hemoglobinuria.

In patients with IMHA, systemic signs consistent with other antigen–antibody complex diseases, including polyarthritis and glomerulonephritis, may uncommonly be identified. Systemic lupus erythematosus affects multiple organs and involves both types II and III immune injury. Systemic lupus erythematosus is caused by circulating autoantibodies against nuclear components, including DNA, RNA, and nuclear proteins. Immune injury may be directed against RBCs, platelets, and other organ systems, such as the skin, joints, and kidneys.

DIAGNOSIS

Distinguishing between primary and secondary IMHA is crucial for effective treatment. The primary disease generally requires aggressive immunosuppressive therapy. Secondary IMHA, however, rarely responds well to treatment unless the underlying cause is eliminated and may even worsen with immunosuppressive therapy. Most cases of IMHA in dogs are thought to be primary and are, therefore, treated as such. The preponderance of primary disease probably reflects an inability to identify an underlying cause rather than a true high incidence of autoimmune hemolysis. Failure to identify an inciting cause may contribute to the overall poor prognosis reported with canine IMHA cases.

No single finding is pathognomonic for primary IMHA, but the following have been suggested as adequate criteria for a diagnosis^{1,4,20,24}:

- Anemia with a hematocrit less than 25% to 30%
- Evidence of hemolysis characterized by hemoglobinemia or hemoglobinuria
- Evidence of antibodies directed against RBCs, with

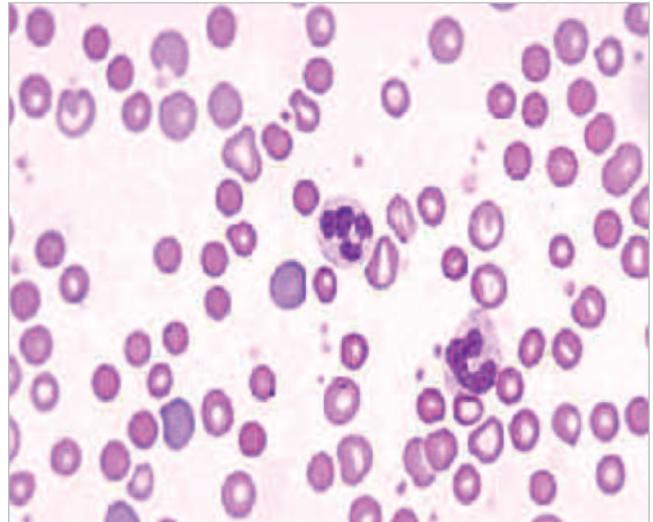


Figure 3. Blood smear showing polychromasia, anisocytosis, and spherocytosis.

autoagglutination, spherocytosis, or positive results from a direct antiglobulin (Coombs') test

- Elimination of other underlying causes of anemia
- An appropriate response to immunosuppressive therapy

Blood Smear and Complete Blood Count

The most simple and beneficial test in diagnosing IMHA is a complete blood count, including a reticulocyte count. The classic patient with IMHA has mild to severe, highly regenerative anemia.⁵ One study²⁷ found that 88% of dogs admitted for IMHA had severe anemia with a PCV of less than 20%. Blood smear analysis should be conducted to identify signs of regeneration, including reticulocytosis, polychromasia, anisocytosis, and nucleated RBCs^{8,28} (Figure 3). Reticulocytes are immature RBCs that have extruded their nucleus but still contain polyribosomes, ribosomes, and mitochondria. Their number increases in the peripheral blood in response to blood loss, hemolytic diseases, or remission of other types of anemia.¹ Reticulocytes can be visualized using the vital stain new methylene blue, which induces ribosomes and other organelles to clump into visible granules. A reticulocyte count can be obtained by counting the number of reticulocytes in 1,000 cells and then multiplying the resultant percentage by the total RBC count to yield the number of reticulocytes per microliter of blood. An absolute reticulocyte count of greater than 60,000/ μ l is indicative of RBC regeneration in dogs. Alternatively, the percentage of reticulocytes per 1,000



Figure 4. Positive results from a slide agglutination test.

cells can be used to determine a corrected reticulocyte percentage via the following equation:

$$\text{Corrected reticulocyte percentage} = \frac{\text{Patient's PCV} \times \text{Reticulocyte percentage}}{45\% \text{ (Dogs)}}$$

Healthy, nonanemic dogs have 0.5% to 1% reticulocytes in their peripheral blood.¹ A corrected reticulocyte percentage greater than 1% in an anemic patient is a sign of regeneration. Interestingly, approximately one-third of all patients with IMHA present with poorly regenerative anemia. Many of these patients have an acute onset and, therefore, lack sufficient time to mount an adequate regenerative response, whereas other patients have antibodies directed against marrow erythroid precursors.^{2,10}

Spherocytes are small, round, intensely stained RBCs that lack central pallor and are created by MPS phagocytosis of a portion of antibody-coated RBC membrane. Although the presence of spherocytes is not considered to be pathognomonic for IMHA, marked spherocytosis is certainly highly suggestive of the diagnosis. Spherocytosis has been identified in 89% to 95% of dogs with IMHA.^{15,16,20}

A positive saline agglutination test result is common in animals with IMHA and has been reported in approximately 40% to 89% of dogs with IMHA.^{4,15,16,20} Autoagglutination (antibody-mediated RBC clumping) is believed to be induced by five-armed IgM but can also be caused by large quantities of cell membrane-associated IgG. A simple slide agglutination test can be conducted by mixing one drop of anticoagulated whole blood with one drop of physiologic saline on a microscope slide. If enough antibody molecules are present on the RBCs, agglutination may be visualized both grossly and microscopically (Figure 4). Persistent agglutination can be differentiated from nonspecific agglutination or rouleaux (nonimmune RBC “stacking”) by dispersing the cells with saline: persistent agglutination does not disperse with saline. Some clinicians recommend a vigorous RBC saline washing process to ensure that RBC agglutination is genuine and not artifactual. Persistent agglutination following addition of saline is thought to represent severe IMHA and may be associated with high mortality rates.³

Patients with anti-RBC antibody levels that are too low to cause agglutination can often be identified by the Coombs' or antiglobulin test.^{10,29} There are two types of Coombs' test: the direct and indirect antiglobulin tests. The indirect Coombs' test detects antibodies to RBCs in the serum, whereas the direct Coombs' test detects antibodies attached to RBCs. Patients with IMHA should be tested via the direct Coombs' test because the antibodies of most concern are attached to RBCs. The sensitivity of the direct Coombs' test in patients with IMHA has ranged from as low as 60% in some studies to as high as 89% in others.^{4,5,16,20} These findings demonstrate that a negative Coombs' test result does not conclusively exclude a diagnosis of IMHA because this test may produce negative results in more than one-third of dogs with IMHA. Coombs' testing is often considered unnecessary in patients with documented persistent agglutination after saline washing.

Leukocytosis with a neutrophilic left shift (leukemoid response) is another common complete blood count finding in dogs with IMHA. Leukocytosis is thought to be caused by a combination of increased marrow release during the strong regenerative erythroid response, cytokine-stimulated myeloid hyperplasia, neutrophil demargination, and decreased migration into poorly perfused necrotic tissues.³⁰ A recent necropsy-based study³⁰ suggested that leukocytosis should alert clinicians to potential tissue necrosis secondary to anemic hypoxia.

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Blood smears should be reviewed by an experienced clinical pathologist to help identify classic indicators of IMHA, including spherocytosis and agglutination, as well as to identify possible RBC parasites, such as *Haemobartonella*, *Ehrlichia*, and *Babesia*.

Other Blood Testing

Routine serum biochemistry and clotting times are useful in evaluating the presence of hemolysis, DIC, and underlying diseases. Common blood chemistry abnormalities include hyperbilirubinemia and elevated levels of liver enzymes, especially alanine transaminase. An elevated bilirubin level is common in patients with IMHA and can result from either hemolytic or hepatobiliary causes. Severe and acute hemolysis can lead to prehepatic accumulation of bilirubin, resulting from excessive tissue breakdown of RBC hemoglobin. Hyperbilirubinemia may not be present in mild or chronic cases of IMHA if the bilirubin produced by RBC breakdown does not overwhelm hepatic bilirubin metabolic pathways. Hepatocellular damage secondary to

tion. Additional blood testing for *Dirofilaria immitis* and fecal examination for *Ancylostoma caninum* are warranted.

Diagnostic Imaging

Diagnostic imaging in patients with hemolytic anemia may detect underlying diseases that mimic or trigger IMHA. Abdominal radiographs should be obtained to evaluate spleen and liver size as well as to detect metallic (zinc) GI foreign bodies and mass lesions. Abdominal ultrasonography and thoracic radiography may also be indicated, especially in older animals, to exclude underlying neoplasia. Because animals with anemia often have concurrent murmurs, chest radiographs may be useful in differentiating patients with primary heart disease from those with anemia-induced hemic murmurs.

Animals that present with severe tachypnea should be evaluated for pulmonary thromboembolism, a common complication of the hypercoagulable state associated with IMHA.^{20,27,31} The diagnosis of pulmonary throm-

Patients with IMHA should be tested via the direct Coombs' test, which detects antibodies attached to RBCs.

hypoxemic damage, thromboembolism, and ischemia in patients with IMHA can also contribute to hyperbilirubinemia and mild to marked elevations in alanine transaminase levels. IMHA predisposes affected animals to hypercoagulability and the development of DIC, and clotting times should be evaluated in all patients suspected of having IMHA.²⁰ Patients with decreased platelet counts due to Evans syndrome can be difficult to differentiate from patients with thrombocytopenia due to early DIC, but clotting times may help differentiate the two conditions. Thrombocytopenia combined with prolonged prothrombin and partial thromboplastin times and positive D-dimer or fibrin degradation products suggests the presence of clot dissolution, as might occur with DIC and pulmonary thromboembolism.

Serologic and/or polymerase chain reaction testing for blood-borne infectious agents (e.g., *Ehrlichia*, *Bartonella*, *Babesia*, *Leptospira*, *Mycoplasma haemocanis*, and *Anaplasma phagocytophilum*) that may trigger or mimic IMHA is indicated in patients with hemolytic anemia, particularly considering that RBC parasitemia is often not readily detectable by routine blood smear examina-

tion. boembolism can be extremely difficult because this condition causes no pathognomonic radiographic changes.³² One study³¹ of nine dogs with pulmonary thromboembolism found the following radiographic changes: cardiomegaly in six dogs, prominent interstitial and alveolar patterns in four, pulmonary vascular changes in four, pleural effusion in two, and localized hyperlucent areas in one. Another study²⁷ of seven dogs found more consistent radiographic changes, with a marked pulmonary interstitial pattern in all seven patients and slight pleural effusion in three. Two of the most diagnostic but inconsistent radiographic findings reported in humans with pulmonary thromboembolism are Westermarck's sign and Hampton's hump. Westermarck's sign consists of dilated pulmonary arteries proximal to the thrombus, whereas Hampton's hump is a late finding that represents infarction of the pulmonary parenchyma and appears as a wedge-shaped infiltrate with the apex directed toward the hilus.³³ It is important to remember that pulmonary thromboembolism should be strongly suspected in dyspneic patients with IMHA, even those with normal thoracic radiographs.

Bone Marrow

Bone marrow aspiration cytology and histopathology of marrow core biopsy are especially important to perform in patients with nonregenerative anemia or additional cytopenias, such as thrombocytopenia or leukopenia. In patients with IMHA, bone marrow analysis typically reveals hyperplasia of the erythroid series. However, marrow analysis in patients in which the immune response concurrently affects marrow RBC precursors may reveal decreased erythropoiesis or maturation arrest affecting the erythroid series. Chronic IMHA may progress to bone marrow damage and secondary myelofibrosis.^{34,35}

Primary Versus Secondary Disease

Unfortunately, the detection of strong indicators of IMHA, such as spherocytosis, persistent autoagglutination, or a positive Coombs' test result, does not distinguish primary from secondary IMHA. Definitive exclusion of an infectious or neoplastic disease that triggers secondary IMHA can be attained only by aggressive diagnostic pursuit of underlying conditions via imaging and testing for infectious agents. Such testing is especially indicated in patients that fail to respond to conventional immunosuppressive therapy as well as in patients in which the signalment suggests that primary IMHA is unlikely, such as cats, geriatric animals, and breeds predisposed to blood-borne parasites (e.g., greyhounds [*Babesia canis* infection], American pit bull terriers [*Babesia gibsoni* infection]).

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ARTICLE #2 CE TEST

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1. Which clinical sign identifies intravascular hemolysis in patients with IMHA?

- a. splenomegaly
- b. hemoglobinuria
- c. pale mucous membranes
- d. an increased bilirubin level

2. Which is not a suggested cause of IMHA?

- a. administration of a sulfa antibiotic
- b. GI ulceration
- c. vaccination
- d. neoplasia

3. Which breed reportedly has the highest incidence of IMHA?

- a. poodle
- b. collie
- c. American cocker spaniel
- d. English springer spaniel

4. Anemia secondary to zinc intoxication can occur from ingestion of US pennies minted after

- a. 1995.
- b. 1990.
- c. 1989.
- d. 1983.

5. Evans syndrome is concurrent IMHA and

- a. immune-mediated thrombocytopenia.
- b. hemoglobinuria.
- c. icterus.
- d. DIC.

6. Which change is not indicative of a regenerative response?

- a. a corrected reticulocyte percentage of 4%
- b. a reticulocyte count of 200,000/ μ l
- c. macrocytosis
- d. the presence of spherocytes

7. Which is not a possible cause of an increased white blood cell count in a dog with IMHA?

- a. increased marrow release during a strong regenerative response
- b. neutrophil margination
- c. cytokine-stimulated myeloid hyperplasia
- d. decreased migration into poorly perfused necrotic tissue

8. Which is the correct method of conducting a slide agglutination test in dogs?

- a. Mix one drop of anticoagulated blood with one drop of saline.
- b. Mix one drop of fresh blood with one drop of saline.
- c. Mix four drops of anticoagulated blood with one drop of saline.
- d. Mix one drop of anticoagulated blood with five drops of saline.

9. Which statement regarding the Coombs' test is correct?

- a. The direct test detects antibodies to RBCs in the serum.
- b. The indirect test detects antibodies attached to RBCs.
- c. The indirect test detects antibodies to RBCs in the serum.
- d. Neither the indirect nor direct test detects antibodies attached to RBCs.

10. Which is not suggestive of DIC?

- a. a prolonged prothrombin time
- b. a prolonged partial thromboplastin time
- c. a decreased platelet count
- d. a decrease in fibrin degradation products