Immune-Mediated Hemolytic Anemia

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Immune-mediated hemolytic anemia (IMHA) is a life-threatening hematologic disorder that is relatively common in dogs but rare in cats. IMHA is characterized by a type II immune reaction in which red blood cells (RBCs) coated with immunoglobulin, complement, or both are removed from circulation by direct destruction or phagocytosis. Extravascular hemolysis occurs when coated erythrocytes are removed by the mononuclear phagocyte system (MPS) in the liver and spleen. Intravascular hemolysis occurs when erythrocytes are coated with enough immunoglobulin to fix complement.

Primary (idiopathic) IMHA (i.e., autoimmune hemolytic anemia) is a true autoimmune reaction against self-antigens on erythrocytes. Sixty to 75% of dogs have the primary form when no underlying cause can be identified; however, potential causes of secondary IMHA must be ruled out. Secondary IMHA is associated with the presence of a foreign antigen that stimulates an immune response resulting in erythrocyte destruction without a true autoantibody. The most common triggers are systemic infections, drugs, and neoplasia (Table 1). Secondary IMHA is more common in cats.

DIAGNOSTIC CRITERIA

Historical Information
- Investigate potential etiologies of secondary IMHA (Table 1).

Gender Predisposition
- Female dogs and male cats are overrepresented.

Age Predisposition
- Middle-aged dogs (mean age ~6.5 years; range, 13 weeks to 13 years).
- Young to middle-aged cats (mean ~3 years; range, 0.5 to 9 years).

Breed Predisposition
- Any breed may be affected.
- Overrepresented dog breeds include cocker spaniels (~1/3 of all cases), English springer spaniels, collies, poodles, Old English sheepdogs, dachshunds, and Irish setters.
- No cat breed predisposition identified.

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**Owner Observations**
The onset of IMHA can be acute/fulminant or subacute/chronic. Clinical signs are related to RBC destruction, inflammation, and anemia.

- Lethargy, depression, and anorexia are the most common clinical signs.
- Vomiting, diarrhea.
- Polyuria, polydipsia.
- Exercise intolerance/weakness.
- Sudden collapse, syncope.
- Pigmenturia.
- Respiratory distress.

**Other Historical Considerations/Predispositions**
- Possible seasonal incidence in dogs (spring/summer).
- In one retrospective study of IMHA, 25% of dogs with IMHA had been vaccinated within 30 days of diagnosis, but direct causation was not established. No such association is documented in cats.

**Physical Examination Findings**
- Mucous membrane pallor; icterus.
- Weakness.
- Hepatosplenomegaly; lymphadenomegaly.
- Tachycardia ± prominent pulses; systolic heart murmur or gallop.
- Tachypnea, respiratory distress.
- Pyrexia (dogs); hypothermia (cats).
- Abdominal pain.
- Petechiae/ecchymoses.

**Laboratory Findings**

**Complete Blood Count**

- Erythrocyte changes:
  - Anemia (PCV <25%–30%; as low as 6% [reference range: 35%–55%]).
  - Spherocytosis (~89%–95% of dogs; difficult to identify in cats due to erythrocyte size).
  - Regenerative response: Polychromasia, anisocytosis, macrocytosis, nucleated RBCs, reticulocytosis (absolute reticulocyte count >60 × 10³/µL, corrected reticulocyte percentage >1% in an anemic patient).
  - Regeneration not evident in peracute cases.
  - 50% of dogs have nonregenerative anemia. Cats are more likely to have nonregenerative anemia at presentation.

- Leukocyte changes:
  - Marked leukocytosis or leukopenia; leukopenia occurs from immune-mediated neutropenia, sepsis, or decreased production.
  - Cats often have normal leukograms.

- Thrombocyte changes:
  - Thrombocytopenia (~50%–70% of dogs; much less common in cats).
  - Concurrent IMHA and thrombocytopenia is known as **Evans syndrome**.

- Autoagglutination:
  - Erythrocytes coated with high titers of antibody and complement spontaneously agglutinate. Macroscopic agglutination is visible when blood with a low hematocrit is placed in an EDTA tube or on a glass slide. Microscopic agglutination is evident as small clumps of erythrocytes on a stained blood smear or in a saline wet mount.
  - Differentiate from rouleaux formation.
  - Negates the need for a direct (Coomb’s) antiglobulin test.
  - Not diagnostic for IMHA in cats.

**Slide Agglutination Test**
- Differentiates agglutination from rouleaux formation.
- Place one drop of EDTA-anticoagulated blood on a glass slide with 1 to 2 drops of saline. Mix gently. Evaluate for macro- and microscopic agglutination. If microscopic agglutination is present, add a second drop of saline to the slide. If the RBCs disperse, the agglutination was associated with rouleaux. If the agglutination remains, it is autoagglutination. Some authors believe saline washing is required to determine true autoagglutination.
- Positive in ~40% to 89% of dogs with IMHA.

**Direct Antiglobulin Test or Direct Coombs’ Test**
- Identifies antierthrocyte antibodies or complement on the erythrocyte.
- False positives and false negatives occur; therefore, the result must be interpreted in light of the individual patient.

**Serum Biochemistry**
- Hyperbilirubinemia (present in >66% of dogs).
- Hepatic transaminases (especially ALT) elevated as a consequence of hepatocyte hypoxia. ALP elevated due to cholestasis from MPS hyperplasia or hepatic extramedullary hematopoiesis.
- Azotemia: Prerenal (dehydration); or renal (pigment-induced nephropathy, renal ischemia, disseminated intravascular coagulation [DIC], or sepsis).
- Hyperglobulinemia.

**Coagulation Testing**
- Prolonged prothrombin time and partial thromboplastin time (PTT), increased fibrin degradation productions or D-dimers, and thrombocytopenia are consistent with DIC.

**Acid–Base Evaluation**
- Metabolic acidosis may be present.

**Arterial Blood Gas Evaluation**
- Profound hypoxemia with normocapnia is consistent with pulmonary thromboembolism (PTE). Elevated alveolar-arterial oxygen gradient (A-a gradient >25; calculated on room air) and decreased PaO2-FiO2 ratio (<400).

**Urinalysis**
- Bilirubinuria (most common); hemoglobinuria.
- Bacteriuria and pyuria warrant culture and susceptibility testing.
Infectious Disease Screening

- Erythrocyte parasites (Table 1) may be visualized on a stained blood smear. $–$$
- Testing for appropriate infectious diseases (Table 1).

Other Diagnostic Findings

- Bone marrow cytology: $ 
  — Indicated with persistent nonregenerative anemia (> 4 to 5 days), if other cell lines are depressed (e.g., pancytopenia), and to rule out neoplasia. 
  — The most common finding is erythroid or generalized hyperplasia. Erythropagocytosis, erythroid hypoplasia, or erythroid maturation arrest are seen occasionally. Chronic IMHA may progress to secondary myelofibrosis. 
- Thoracic radiography to rule out underlying infection or neoplasia, and with respiratory distress or hypoxemia to evaluate for evidence of PTE. $ 
- Abdominal imaging: $–$$ 
  — Radiographs rule out heavy metal ingestion. 
  — Ultrasonography detects intraabdominal neoplasis or other pathology. 
- Lymph node aspirate or biopsy, hepatic aspirate or biopsy, or splenic aspirates may be indicated.

Summary of Diagnostic Criteria

- The hemogram classically indicates moderate-to-severe, regenerative anemia, neutrophilic leukocytosis, and thrombocytopenia.

### TABLE 1. Causes of Secondary IMHA in Dogs and Cats

<table>
<thead>
<tr>
<th>Type of Cause</th>
<th>Dogs</th>
<th>Cats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infectious</td>
<td>• Anaplasma phagocytophilum</td>
<td>• Ehrlichia canis</td>
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<tr>
<td></td>
<td>• Anaplectostrina caninum</td>
<td>• Leishmaniasis</td>
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<tr>
<td></td>
<td>• Babesia canis (especially in greyhounds)</td>
<td>• Leptospirosis</td>
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<tr>
<td></td>
<td>• Babesia gibsoni (especially in American pit bull terriers or dogs bitten by pit bulls)</td>
<td>• Mycoplasma haemocanis</td>
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<tr>
<td></td>
<td>• Dirofilaria immitis</td>
<td>• Various acute and chronic infections (bacterial endocarditis, abscess, pyometra, diskospondylitis)</td>
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<td>Toxin or drug related</td>
<td>• Acetaminophen</td>
<td>• Methimazole</td>
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<tr>
<td></td>
<td>• Castor beans</td>
<td>• Propylthioracil (an antithyroid)</td>
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<tr>
<td></td>
<td>• Cephalosporins</td>
<td>• Cephalosporins</td>
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<tr>
<td></td>
<td>• Copper toxicity</td>
<td>• Copper toxicity</td>
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<tr>
<td></td>
<td>• Dipyrone</td>
<td>• Dipyrone</td>
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<td></td>
<td>• Methylen blue</td>
<td>• Methylen blue</td>
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<td></td>
<td>• Onion toxicity</td>
<td>• Onion toxicity</td>
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<td></td>
<td>• Penicillins</td>
<td>• Penicillins</td>
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<td></td>
<td>• Phenazopyridine hydrochloride</td>
<td>• Phenazopyridine hydrochloride</td>
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<td>• Phenothiazines</td>
<td>• Phenothiazines</td>
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<td></td>
<td>• Procainamide</td>
<td>• Procainamide</td>
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<td>• Quinidine</td>
<td>• Quinidine</td>
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<td></td>
<td>• Sulfonamides</td>
<td>• Sulfonamides</td>
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<td></td>
<td>• Topical benzocaine</td>
<td>• Topical benzocaine</td>
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<tr>
<td></td>
<td>• Vaccines</td>
<td>• Vaccines</td>
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<tr>
<td></td>
<td>• Zinc toxicity</td>
<td>• Zinc toxicity</td>
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<tr>
<td>Neoplastic</td>
<td>• Hemolympathic neplasia resulting in microangiopathic anemia</td>
<td>• Methimazole</td>
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<tr>
<td></td>
<td>(hemangiosarcoma, leukemia, lymphoma/lymphosarcoma, multiple myeloma/plasmacytoma)</td>
<td>• Propylthioracil (an antithyroid)</td>
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<tr>
<td></td>
<td>• Solid tumors</td>
<td>• Cytomegalyosis</td>
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<tr>
<td>Immune disorders</td>
<td>• Hypothyroidism</td>
<td>• Cytomegalyosis</td>
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<tr>
<td></td>
<td>• Primary and secondary immunodefiencies</td>
<td>• Cytomegalyosis</td>
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<tr>
<td></td>
<td>• Systemic lupus erythematosus</td>
<td>• Cytomegalyosis</td>
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<tr>
<td>Metabolic</td>
<td>• Hypophosphatemia (severe)</td>
<td>• Cytomegalyosis</td>
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<tr>
<td>Inherited/intrinsic RBC</td>
<td>• Chondrodysplasia/anemia (in malamutes)</td>
<td>• Cytomegalyosis</td>
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<tr>
<td>defects</td>
<td>• Methemoglobin reductase deficiency</td>
<td>• Cytomegalyosis</td>
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<tr>
<td></td>
<td>• Nonspherocytic hemolytic anemia</td>
<td>• Cytomegalyosis</td>
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<tr>
<td></td>
<td>• Phosphofructokinase deficiency</td>
<td>• Cytomegalyosis</td>
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<tr>
<td></td>
<td>• Pyruvate kinase deficiency</td>
<td>• Cytomegalyosis</td>
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**Infectious Disease Screening $–$$**
- Erythrocyte parasites (Table 1) may be visualized on a stained blood smear.
- Testing for appropriate infectious diseases (Table 1).
Thromboprophylaxis/Anticoagulant Therapy

• Definitive diagnosis is based on the presence of hemolytic anemia and one or more of the following: spherocytosis, autoagglutination, or positive direct Coomb’s test. Secondary causes of IMHA should be ruled out.

Diagnostic Differentials
• Causes of secondary IMHA should be ruled out by appropriate testing. Non–immune-mediated causes of hemolytic anemia should be ruled out via signalment and history, serum biochemistry, and diagnostic imaging.

TREATMENT RECOMMENDATIONS

Initial Treatment Glucocorticoids $
• Mainstay of therapy for IMHA.
• Immunosuppressive prednisone is recommended. Doses in dogs range from 1–2 mg/kg PO q12h to 1 mg/kg/day or 30 mg/m². Higher doses have not been associated with higher control rates in dogs. Higher doses are more uniformly recommended in cats; anecdotally, most cats require at least 2 mg/kg/day and some up to 4 mg/kg/day.
• Dexamethasone may be substituted when oral dosing is not tolerated. Dose is determined by calculating the desired prednisone dose and dividing by a factor of 7.5 (dexamethasone is 7.5 times more potent than prednisone). Do not exceed a maximum of 0.4 mg/kg IV q24h.
• A response to immunosuppressive corticosteroids, as indicated by a rise in hematocrit, generally takes 3 to 7 days.
• Glucocorticoid weaning may begin after 4 weeks of therapy or when the patient shows a rising PCV for at least 5 to 7 days, based upon clinician preference. To wean, the dose can be reduced by 25% of the initial dose every 2 to 6 weeks (generally every 4 weeks) as long as remission is maintained.
• Alternate-day therapy may be initiated when the dose is reduced to 0.5 mg/kg/day.
• If there is a relapse, as evidenced by autoagglutination or anemia, the dose should be increased. In severe relapses, the initial immunosuppressive dose protocol may be reinstituted.
• Lifelong therapy may be indicated in certain individuals.
• Adverse effects include polyuria, polydipsia, polyphagia, tachypnea/panting, muscle wasting, hypertension, poor wound healing, and increased susceptibility to infection. These effects are pronounced with high doses for prolonged periods.

Thromboprophylaxis/Anticoagulant Therapy
• Current antithrombotic therapy for IMHA is empiric, with few prospective studies to identify safe, effective drug regimens. The following therapies have been used:
  — Ultra–low-dose (0.5 mg/kg/day PO) aspirin: Associated with improved short- and long-term survival compared with heparin when used in combination with prednisone and azathioprine. Effects on development of thromboembolic complications have not been reported. $
  — Unfractionated heparin: Reported doses vary considerably, including 150 U/kg SC q8h, 250 U/kg IV/SC q6h, and as a CRI (e.g., 12–15 U/kg/hr, 10–25 U/kg/hr). Monitor PTT before each SC dose or q12h with a CRI. Dosing is titrated to achieve PTT prolongation of 1.5–2× normal. When no longer needed, taper slowly. Can be given concurrently with ultra–low-dose aspirin. $
  — Low-molecular-weight heparin: More predictable pharmacokinetics; monitoring is not required, and bleeding complications are lower than with unfractionated heparin. High cost and the need for frequent dosing limit use. $$
  • Enoxaparin: 0.8 mg/kg SC q6h in dogs.
  • Dalteparin: 150 U/kg SC q12h in dogs.
• Because antithrombin is decreased in many dogs with IMHA, heparin may not provide effective anticoagulation.

Antibiotics
• Not routinely used.
• Avoid sulfa drugs, penicillins, and cephalosporins because of their propensity to precipitate immune-mediated reactions.
• Doxycycline (5–10 mg/kg IV or PO q12–24h) is indicated in tick-endemic regions pending serology or to treat susceptible infections. $
• Blood cultures should be obtained if sepsis is suspected.
• The results of culture and susceptibility will dictate antibiotic choice.

Alternative/Optional Treatments/Therapy
Second-Line Immunosuppressive Agents
Additional immunosuppressive drugs should be considered in patients with autoagglutination, intravascular hemolysis, nonregenerative anemia, or transfusion dependence. Some clinicians treat all IMHA with aggressive combination immunosuppressive therapy at the outset of therapy. If not started initially, adjunct immunosuppressives should be initiated if glucocorticoids fail to induce remission, cannot maintain remission unless given at persistently high doses, or cause unacceptable adverse effects. The most common secondary immunosuppressives are azathioprine and cyclosporine. Prednisone remains the first-line immunosuppressive therapy and other drugs are additive. If response occurs, combination immunosuppressive therapy may permit more rapid glucocorticoid dose reduction.

Azathioprine $
• This is the second-line immunosuppressive agent of choice in several veterinary studies, although any benefit over other second-line agents has not been uniformly documented. May confer a survival advantage when used in combination with prednisone.
• Slow onset of action (7–14 days) precludes use as sole therapy during initial treatment, but it is ideal for long-term management.
Blood Transfusion

• Administer at 2 mg/kg PO q24h for 5 to 14 days or until prednisone is tapered to alternate-day therapy, with azathioprine then being given every other day.
• Can be discontinued if remission is maintained after 4 weeks of alternate-day therapy, or alternate-day therapy can be continued and prednisone discontinued. Side effects include gastrointestinal upset, bone marrow suppression, poor hair growth, hepatotoxicity (uncommon), and pancreatitis (rare).
• Azathioprine should not be used in cats.

**Cyclosporine A $5—$$$**

• Brand-name cyclosporine products include an original formulation and a microemulsion formulation. Recommended dose varies by product. Generic cyclosporine preparations are not recommended due to variable absorption.
• GI drug absorption is variable, necessitating monitoring of serum drug concentrations to ensure therapeutic dosing. Monitoring should be done 48 hr after starting therapy and every 2 to 4 weeks until clinical remission is achieved. Recommended target trough whole blood concentrations are published elsewhere.
• Concurrent administration of vitamin E may improve absorption.
• Discontinue when remission has been achieved and maintained for at least 2 weeks, or continue after discontinuation of prednisone therapy in patients with adverse glucocorticoid effects.
• Adverse effects include vomiting, diarrhea, and anorexia, which can be severe. Use is generally reserved for patients with severe, unresponsive disease.

**Supportive Treatment**

Supportive care involves the maintenance of organ perfusion, hydration, and acid–base balance while waiting for a response to immunosuppressive therapy and avoidance of PTE risk factors. To minimize the risk of nosocomial infection and sepsis, strict attention to aseptic technique is vital.

**Blood Transfusion $—$$$**

• There is no exact PCV that indicates transfusion need; however, severe anemia (PCV of <12% to 15%) usually necessitates transfusion. Clinical signs indicating the need for a transfusion include severe weakness, mental obtundation, respiratory distress, tachycardia, and arrhythmia.
• Most (~70%—90%) IMHA dogs require transfusion. Dose can be approximated using the following equation: Volume of blood for transfusion = 90 × body weight (kg) × (desired change in PCV/PCV of donor blood). Target PCV is usually subnormal (e.g., <25%—30%) to ensure stimulus for regeneration.
• The presence of RBC autoantibodies is thought to shorten the survival of transfused erythrocytes. Nonetheless, if there is evidence of tissue hypoxia and transfusion is necessary to maintain life, it is indicated.
• All cats must be typed and transfused with blood from a compatible donor; dogs should receive blood from DEA 1.1- and 1.2-negative donors.
• A cross-match must be performed if a second transfusion is performed >5 days after the first.
• The presence of autoagglutination can make accurate typing and cross-matching extremely difficult or impossible.
• Component therapy is preferred.

Patients should be monitored closely for signs of transfusion reaction.

**Intravenous Fluid Therapy $**

• Indicated for hypovolemia, replacement of dehydration, replacement of ongoing losses, and provision of maintenance fluid requirements with adipsia.
• IV catheter placement has been identified as a risk factor for PTE; however, dehydration and poor perfusion must be treated. Placement of a central venous catheter should be avoided.

**Oxygen Therapy $**

Of little benefit in most anemic patients because RBC oxygen saturation is maximized; transfusion therapy is more beneficial to increase oxygen-carrying capacity. It may be helpful in animals with hypoxemia from PTE.

**Gastrointestinal Protectants/Antacids**

Consider in patients with GI signs. Antacids may increase the risk of sepsis from translocation of GI flora. Choices include prostaglandin analogues, H$_2$ blockers, proton pump inhibitors, and sucralfate.

**Patient Monitoring**

• In-hospital monitoring: Heart rate and rhythm, respiratory rate and effort, fluid balance, blood pressure, urine output, and PCV/total protein.
• Follow-up monitoring:
  — Weekly rechecks until the animal and hematologic values show consistent improvement.
  — Owners should recheck if lethargy, anorexia, and discolored urine occur.
  — Dogs maintained on long-term immunosuppressive therapy should have a urine culture every 3 months to identify subclinical urinary tract infections.

**Complications**

Complications of IMHA are severe and life-threatening and include:
• PTE: The most common cause of death in dogs with IMHA.
  — Classic risk factors for development of PTE include blood stasis (may be exacerbated by cage confinement and indwelling IV catheters), hypercoagulability, and vascular endothelial injury. Other reported risk factors include hyperbilirubinemia, hypoalbuminemia, and severe thrombocytopenia.
— Clinical signs include acute respiratory difficulty, orthopnea, profound anorexia, and sudden death.
• Refractory anemia is common and often leads to natural death or euthanasia.
• Hemorrhage from the disease itself or its treatment.
• Bacterial or fungal infections and associated sepsis.

Home Management
• Exercise restriction is recommended to reduce risk for PTE.
• Advise clients of high mortality rate and possibility of sudden death.
• Compliance with medication administration and frequent, scheduled rechecks is vital.

Milestones/Recovery Time Frames
• A response to corticosteroids, as indicated by a rise in hematocrit, generally takes 3 to 7 days.
• Relapse can occur after therapy has been stopped or while the patient is still receiving immunosuppressant drugs.

Treatment Contraindications
• NSAIDs (with the exception of ultra–low-dose aspirin) should not be used concurrently in patients receiving corticosteroids via any route.

PROGNOSIS

The prognosis for dogs with IMHA is guarded. The overall mortality rate for canine IMHA varies in the literature from 26% to 70%. Anecdotally, a 50:50 survival rate is commonly used for owner counseling. The mortality rate is highest in the first 2 weeks after diagnosis, primarily because of thromboembolic events. Dogs may die acutely from PTE even if anemia has resolved. The reported mortality rate in cats is 24%.

Favorable Criteria
• No positive prognostic indicators at the time of diagnosis have been reported.
• For dogs surviving >14 days after diagnosis, an increase in leukocyte count has been associated with decreased risk of death.

Unfavorable Criteria

Many associations have been made between specific clinical or laboratory data and outcome in canine IMHA; however, few are consistently reproducible. Documented poor prognostic indicators include:
• Persistent agglutination despite immunosuppressive therapy.
• Thrombocytopenia/petechiae.
• Coagulopathy.
• Hyperbilirubinemia; hypoalbuminemia.
• Leukocytosis and/or the presence of a left shift.
• Elevation in ALT and/or ALP.
• Tachypnea.
• Azotemia.
• IV catheterization.
• Use of cyclophosphamide.

RECOMMENDED READING