Immune-mediated hemolytic anemia (IMHA) is one of the most common immune-mediated hematologic disorders in dogs and cats:

In dogs, immune-mediated hemolytic anemia:
- Is commonly primary or idiopathic in origin
- Often affects particular breeds, including cocker spaniels, English springer spaniels, collies, poodles, and Irish setters
- Most commonly affects middle-aged female dogs
- Also occurs secondary to triggers, such as infectious, inflammatory, and neoplastic diseases; drugs; and vaccines (Table 1).

In cats, there is no breed predilection for IMHA, and the condition is usually secondary to an underlying cause.

**PATIENT EVALUATION**

**History**
When a patient presents with possible IMHA, history should include a detailed account of any recent medications or vaccines. Historical clues that suggest a possible underlying or triggering disease process should also be investigated (Table 1).

**Clinical Signs**
Clinical signs seen in IMHA patients often include those associated with anemia and tissue hypoxia, including:
- Lethargy
- Weakness
- Tachypnea.

When anemia is severe and acute in onset, patients tend to be the most significantly affected. When red blood cell (RBC) destruction is more chronic, patients may only be mildly affected despite marked anemia.

**PATHOPHYSIOLOGY OF IMHA**
IMHA is a type II immune reaction, where antibody and/or complement formation against RBCs causes accelerated cell destruction and subsequent anemia. The anti-RBC antibodies can be either immunoglobulin G or M (IgG or IgM).

When the body is correctly responding to an immune reaction, antibodies ensure an appropriate immune response while the complement system enhances the ability of antibodies and phagocytic cells to clear pathogens. However, in cases of IMHA:
- High levels of antibodies induce activation of the complement system and formation of the membrane attack complex; RBC destruction tends to be intravascular due to osmotic lysis.
- Macrophages within the spleen, liver, and other organs recognize the Fc portion of antibodies and/or the C3b portion of complement bound to RBCs and prematurely remove cells from circulation and destroy them (extravascular hemolysis).

Classically, the bone marrow mounts an appropriate and strongly regenerative response. Less commonly, antibodies are also directed against marrow RBC precursors, resulting in nonregenerative anemia.
**Physical Examination**

Physical examination may reveal:

- Pale mucous membranes
- Tachycardia
- Bounding pulses
- Less commonly: Splenomegaly, hepatomegaly, lymph node enlargement, and fever.

Other physical examination findings may include:

- Hemoglobinuria, which should resolve once anemia is corrected
- Jaundiced mucous membranes and tissues if there is acute severe hemolysis (Figure 1, page 34)
- Hemoglobinuria ("port wine" urine) in patients with intravascular hemolysis
- Petechiae, ecchymoses, and melena in patients with concurrent immune-mediated thrombocytopenia (Evans' Syndrome)
- Signs that reveal the underlying cause of IMHA.

**DIAGNOSTICS**

Initial diagnostics in an anemic patient should focus on identifying the cause of the anemia. A final diagnosis of IMHA is based on evidence of accelerated RBC destruction, with an underlying immune-mediated pathogenesis.

There is no single test that is definitively diagnostic for IMHA. Instead, evidence from various analyses is used to determine the diagnosis (Table 2). The following signs and results support a diagnosis of primary IMHA:

- Anemia
- Evidence of accelerated RBC lysis, including, but not limited to, hemoglobinemia/hemoglobinuria (intravascular hemolysis) or bilirubinemia/bilirubinuria
- Evidence of an immune-mediated process, such as autoagglutination, a positive Coombs' test, or increased numbers of circulating spherocytes
- Lack of other identifiable causes of anemia.

**Complete Blood Count & Blood Smear Analysis**

In IMHA patients, complete blood count (CBC) with blood smear analysis often reveals anemia and RBC changes, which are suggestive of a regenerative response, such as polychromasia, anisocytosis, and nucleated RBCs.

**Reticulocytes**

- An increased absolute reticulocyte count (> 60,000/mcL, dogs; > 50,000/mcL aggregate reticulocytes, cats) or corrected reticulocyte percentage (> 1%, dogs; > 0.5%, cats) documents a regenerative marrow response.<sup>3</sup>
- Since a regenerative response takes approximately 3 to 5 days to mount, acute cases may initially appear poorly regenerative.
- A nonregenerative response may also suggest the presence of antibodies directed against marrow precursors.

**Spherocytes**

Spherocytes may also be seen on blood smears. Spherocytes are small RBCs with a loss of central pallor produced by incomplete destruction of RBCs by macrophages (Figure 2, page 34). Spherocytosis is very suggestive of IMHA.

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**TABLE 1. IMPORTANT DIFFERENTIAL DIAGNOSES FOR HEMOLYTIC ANEMIA**

<table>
<thead>
<tr>
<th>CHEMICAL/TOXIN INJURY</th>
<th>IMMUNE-MEDIATED</th>
</tr>
</thead>
<tbody>
<tr>
<td>Castor bean</td>
<td>Disseminated intravascular coagulation*</td>
</tr>
<tr>
<td>Copper</td>
<td>Glomerulonephritis*</td>
</tr>
<tr>
<td>Garlic/onion</td>
<td>Incompatible transfusions*</td>
</tr>
<tr>
<td>Methylene blue</td>
<td>Neonatal isoerythrolysis*</td>
</tr>
<tr>
<td>Propylene glycol</td>
<td>Primary IMHA*</td>
</tr>
<tr>
<td>Zinc</td>
<td>Systemic lupus erythematosus*</td>
</tr>
</tbody>
</table>

**INFEKTIOUS**

- Anaplasma species*            FeLV*, FIP*, FIV*
- Ancylostoma caninum*          Histoplasmosis*
- Babesia canis*                Leishmaniasis*
- Babesia gibson*               Leptospirosis*
- Cytauxzoon felis*             Mycoplasma haemofelis*'
- Dirofilaria immitis*           Mycoplasma haemofelis*'
- Ehrlichia species*             Mycoplasma haemofelis*'

**INTRINSIC/INHERITED RBC DEFECTS**

- Chondrodysplasia (in malamutes)
- Hereditary osmotic fragility
- Idiopathic Heinz body anemia
- Methemoglobin-reductase deficiency
- Phosphofructokinase deficiency
- Pyruvate kinase deficiency

**MEDICATIONS**

- Acetaminophen*                 Penicillin*
- Cephalosporins*                Phenazopyridine
- Chlorpromazine*                Hydrochloride*
- Dipyrone*                     Procainamide*
- Heparin*                     Sulfonamides*
- Levamisole*                   Topical benzocaine*
- Methimazole/propylthiouracil*  Vitamin K*
- Phenazopyridine hydrochloride*
- Procainamide*              Sulfonamides*
- Topical benzocaine*

**NEOPLASIA**

- Diffuse sarcoma*              Lymphoma*
- Hemangiosarcoma*             Multiple myeloma*
- Lymphocytic leukemia*       Other solid tumors*

**OTHER**

- Bee-sting envenomation*
- Recent vaccination*
- Severe hypophosphatemia
- Snake-bite envenomation

*Denotes conditions that have been suggested to induce immunologic destruction of RBCs

FeLV = feline leukemia virus; FIP = feline infectious peritonitis; FIV = feline immunodeficiency virus; IMHA = immune-mediated hemolytic anemia; RBC = red blood cell
Other Findings
The blood smear should also be carefully evaluated by an experienced clinical pathologist for:
- **Presence of RBC parasites**, such as *Mycoplasma haemofelis* (formerly *Haemobartonella*) and *Babesia*
- **Neutrophilia**, often with a left shift, is commonly seen in IMHA patients
- **Extreme leukocytosis** (“leukemoid response”) occurs in some dogs with IMHA and has been associated with severe tissue injury
- **Thrombocytopenia** will be observed in animals with Evan’s Syndrome.

Anti-RBC Antibodies
**Spontaneous Autoagglutination**
High levels of anti-RBC antibodies sometimes result in their attachment to more than one cell, causing spontaneous RBC agglutination. Agglutination may be appreciated as red speckles when blood is placed in an EDTA tube (Figure 3) or onto a microscope slide.

**Slide Agglutination Test**
The slide agglutination test can be easily performed in practice, and is used to differentiate true autoagglutination from rouleaux formation (nonimmune RBC adhesion).
- A single drop of EDTA-anticoagulated blood is placed onto a microscope slide and mixed with saline (1–2 drops in dogs; 3–4 drops in cats due to their greater propensity to develop rouleaux).
- The slide is rocked back and forth; then evaluated for the formation of macroagglutination (obvious agglutination to the naked eye) (Figure 4).
- A coverslip can then be placed on the mixture, and the slide evaluated under a microscope for microagglutination (4 or more RBCs in a cluster) (Figure 5, page 36).
- True agglutination appears as “clusters of grapes” while rouleaux appear as “stacks of coins” (Figure 6, page 36).
- Rouleaux can further be differentiated from autoagglutination by adding additional saline and by RBC washing techniques; extra saline often disperses rouleaux but will not disperse true autoagglutination.

Since autoagglutination is only seen with high antibody levels, a negative slide agglutination test does not rule out IMHA.

**Direct Coombs’ Test**
The direct Coombs’ test is also known as the direct antiglobulin test (DAT) and identifies antibodies or complement adhered to RBCs. IMHA patients that do not demonstrate autoagglutination may still test positive on the Coombs’ test. However, diagnostic sensitivity of the Coombs’ test ranges from 60% to 89%, so a negative test does not exclude
DIAGNOSIS OF IMMUNE-MEDIATED HEMOLYTIC ANEMIA

Since false positives can also occur, the Coombs’ test should be carefully interpreted in each individual patient.

Additional Diagnostics

Serum Biochemical Profile

Common serum biochemistry changes in IMHA patients include hyperbilirubinemia and increased liver enzymes.

Hyperbilirubinemia

• With accelerated RBC destruction, increased bilirubin production by macrophages can overwhelm hepatic processing capacity, resulting in hyperbilirubinemia. However, it may also be due to concurrent hepatobiliary disease.

• Normal bilirubin levels are often seen in mild or chronic cases of IMHA because a healthy liver can still handle the extra bilirubin.

Increased Liver Enzymes

• Liver enzyme elevation, especially alanine aminotransferase, may be present due to hypoxic liver damage.

• Azotemia may sometimes occur due to either prerenal causes (dehydration) or renal causes (hemoglobin-induced renal damage).

Coagulation Tests

Coagulation tests, such as the 1-stage prothrombin time (PT) or activated partial thromboplastin time.
time (aPTT), are indicated to assess for hemostatic disorders, such as:
• Disseminated intravascular coagulation (DIC)
• Thromboembolic disease.

D-dimer concentration or antithrombin activity may be needed to characterize DIC or thrombotic diseases. Both conditions can be common and serious complications of IMHA.

Bone Marrow Evaluation
Marrow evaluation is indicated if there is a persistent (beyond 3–5 days) nonregenerative anemia or pancytopenia is present on blood analysis.

Infectious Disease
Testing for infectious causes of hemolysis, such as *Mycoplasma haemofelis*, *Babesia canis*, or *Babesia gibsoni* should be considered in individual patients based on signalment, clinical signs, and geographic location.

Urinalysis
Urinalysis often reveals bilirubinuria or, with intravascular hemolysis, hemoglobinuria.

Imaging
Diagnostic imaging is indicated to identify underlying causes of IMHA, such as neoplasia.
• Thoracic radiographs, abdominal radiographs, and abdominal ultrasound should be considered.
• Aspirates and histologic biopsies should be performed on any masses/abnormal-appearing organs found on imaging.
• Abdominal radiographs are also indicated to exclude zinc foreign bodies, since zinc toxicosis can mimic IMHA.

References