

# Immune Mediated Hemolytic Anemia: Can we beat this disease?

WEEKEND WITH THE SPECIALISTS  
APRIL 30, 2011 NEWPORT BEACH, CA

Heather Mineo, DVM, DACVECC Advanced Critical Care and Internal Medicine Tustin, CA

Immune mediated hemolytic anemia (IMHA) remains a common and complicated challenge in the field of veterinary medicine. The degree of patient illness varies widely as do response to treatment and overall prognosis. Progress continues to be made in the treatment of IMHA and new therapeutic modalities are being tested in clinical practice.

IMHA occurs as a both a primary and a secondary condition, resulting in the accelerated destruction of red blood cells (RBCs). The literature suggests that the majority of IMHA cases are primary or idiopathic in origin. Secondary IMHA occurs when a patient is exposed to drugs, medications or vaccinations, or has concurrent or underlying infectious or neoplastic disease, thus leading to antibody attachment to RBCs.

## Immunopathology

RBC destruction is mediated by anti-erythrocyte antibodies, complement, or both. IgG-mediated extravascular hemolysis is the most common type of hemolysis and occurs due to macrophage destruction of RBCs, primarily within the spleen and liver. Alternatively, macrophages may only phagocytize part of the RBC membrane, creating a spherocytes. Large numbers of spherocytes are pathognomonic for IMHA and they are eventually removed from the circulation by the liver and spleen.

IgM-mediated RBC destruction occurs as part of a complement fixation process causing intravascular hemolysis. This leads to weakening of the RBC membrane, water influx into the cell, and cell rupture within the blood vessels (intravascular hemolysis). Hemoglobin escapes into the plasma and is taken up by macrophages or bound to haptoglobin. In IMHA patients with intravascular hemolysis, these hemoglobin scavenging systems are rapidly saturated which leads to the development of hemoglobinemia and hemoglobinuria (port-wine colored urine).

## Diagnosis

### 1) History and Physical Exam

Patient history often includes progressive lethargy and inappetance. Further questioning of the clients reveals information about potential toxin exposure (onions, garlic, zinc, rodenticides, etc.) or envenomation. Initial physical exam findings include pale mucous membranes, tachycardia, tachypnea, weakness or collapse, icterus (gingival, scleral, and cutaneous), heart murmur, intra-abdominal organomegaly, and shock.

### 2) Complete Blood Count

Anemia is confirmed via evaluation of a Complete Blood Count (CBC) and/or Packed Cell Volume (PCV). Erythrocyte parameters vary depending on the chronicity and severity of the anemia. Patients with more chronic anemia will often have characteristics of erythrocyte regeneration: anisocytosis, polychromasia, macrocytosis and elevated reticulocyte counts. In-house blood smears stained with New Methylene Blue will show reticulocytes as slightly larger RBCs containing blue-staining RNA material.

### 3) Saline Auto-agglutination

The binding of anti-erythrocyte antibodies to surface antigens on two different RBCs causes *agglutination*, or clumping, of the cells. This differs from the normal rouleaux formation of RBCs and can be evaluated by mixing one drop of the patient's blood with one drop of normal saline on a microscope slide. The mixture is examined both grossly and under a microscope. Persistent clumping indicates auto-agglutination, whereas rouleaux formation often disappears once the blood is mixed with saline.

#### 4) Chemistry panel

Hyperbilirubinemia will be evident if hemolysis has overwhelmed hepatic clearance. Hypoalbuminemia or especially panhypoproteinemia may be an indicator of blood loss, or may reflect underlying protein-losing disease. Often the BUN to creatinine ratio is elevated due to GI bleeding (either from mucosal sloughing/ulceration due to hypoxia, or from thrombocytopenia or both). Liver enzymes (ALP, ALT, and AST) may be elevated due to hepatic hypoxia.

#### 5) Reticulocyte count

Anemia stimulates increased RBC production by the bone marrow. This hormonal stimulation can take up to 7 days to generate a maximal response. An elevated reticulocyte count at the time of presentation confirms a regenerative anemia, while low or normal counts may indicate lack of adequate time for a regenerative response to be mounted, or a truly non-regenerative anemia.

#### 6) Coomb's Test

This test is based on agglutination of RBCs after the addition of an antibody mixture to the patient's blood. The antibodies in the mixture will bind to Ig or Complement proteins as they are bound to the RBCs. Serial dilutions of the antibody mixture are added to the patient's blood to quantify the degree of agglutination. False positives and false negatives may be frequently observed secondary to other disease processes or even due to previous RBC transfusion.

#### 7) Urinalysis

Urine supernatant appearance may be inspected grossly after centrifugation to assess for **hemoglobinuria**. This is supportive of intravascular hemolysis. The supernatant may also appear icteric due to bilirubinuria. Cystocentesis should be avoided in patients with thrombocytopenia or coagulopathy in order to prevent iatrogenic hemorrhage. Urinalysis may reveal evidence of infection or granular casts to suggest acute tubular necrosis/acute kidney injury.

#### 8) Infectious Titers/PCR Testing

Idexx Panel #2871 Tick/Vector Canine Comprehensive RealPCR Panel with Lab 4Dx:

- Anaplasma spp., Babesia spp., Bartonella spp., Ehrlichia spp., Hepatozoon spp., Leishmania spp., Neorickettsia risticii and Rocky Mountain spotted fever RealPCR tests
- Canine Hemotropic Mycoplasma RealPCR test, which includes Mycoplasma haemocanis and Candidatus Mycoplasma haematoparvum RealPCR tests
- Lab 4Dx Test, which includes ELISAs for heartworm antigen, Ehrlichia canis, Anaplasma phagocytophilum and Lyme C6 antibodies.

Idexx Feline Panel #2827: Tick/Vector Feline RealPCR™ Panel:

- Anaplasma spp., Bartonella spp., Cytauxzoon felis and Ehrlichia spp. RealPCR test
- Feline Hemotropic Mycoplasma RealPCR test, which includes Mycoplasma haemofelis, Candidatus Mycoplasma haemominutum and Candidatus Mycoplasma turicensis RealPCR tests.

#### 9) Coagulation Profile

Since the inflammatory cascade activates the coagulation cascade, changes to PT, PTT and fibrinogen may occur – these can either be increased, decreased or normal. D-dimers are a breakdown product of a previously stable clot, and elevation indicates that there is thrombosis occurring, but does not indicate where.

Thromboelastography can evaluate clot formation and stability.

#### 10) Antinuclear antibody test (ANA)

This is helpful when there is concern for systemic auto-immune in which there are multiple body systems involved. For example, SLE cases may have acute hepatic, renal or integumentary immune-mediated disease along with IMHA or Evans Syndrome.

#### 11) Imaging Studies

- Thoracic radiographs: Mainly performed to rule out neoplasia. May identify concurrent cardiomegaly due to underlying cardiac disease, aspiration pneumonia secondary to vomiting in recumbency, or

occasionally other findings. Note that although relatively common with IMHA (particularly with intravascular hemolysis), pulmonary thromboemboli are too small to be observed on radiographic studies, and a contrast CT scan or ventilation/perfusion CT scan would be needed to definitively diagnose this complication.

- **Abdominal radiographs:** Mainly performed to rule out neoplasia and metallic foreign object ingestion (zinc toxicity). Often identify concurrent benign/secondary hepatosplenomegaly due to the spleen and liver enlarging in response to the increased RBC destruction and production (extramedullary hematopoiesis) occurring with IMHA.
- **Abdominal ultrasound:** Goals and findings often similar to those of radiographs, but can evaluate the parenchymal appearance of the liver and spleen to guide further diagnostic testing such as fine needle aspiration (FNA)/cytology or biopsy if neoplasia or other infiltrative disease is suspected. Occasionally evidence of thromboembolic disease may be observed. Lymphadenopathy may be observed, raising suspicion for infectious or neoplastic disease. FNA/cytology may be required based on findings. Recall that hepatosplenomegaly may be secondary to the IMHA rather than being the underlying cause. Occasionally, abdominal effusion is noted and the fluid can be collected and submitted for analysis. Typically, the effusion is a modified transudate due to vasculitis or due to relative volume overload from compensatory mechanisms initiated due to low effective circulating volume, but it should be confirmed that the effusion is not neoplastic or septic. Gall bladder edema is often observed and considered likely due to tissue hypoxia and/or relative volume overload/edema.
- **Thoracic ultrasound:** Performed to rule out neoplasia or infectious disease. Also used to evaluate for effusions.
- **Echocardiogram:** Used to assess cardiac function and structure in patients presenting with a cardiac murmur or history of cardiac disease. Typically performed in cases where cardiac function must be evaluated due to risk for volume overload with transfusion therapy, to assess for changes consistent with PTE and to assess for heartworm disease.

## Shock

Shock occurs when oxygen delivery to tissues is less than oxygen consumption. In a hemodynamically-stable patient, oxygen consumption ( $VO_2$ ) occurs at a rate independent of oxygen delivery ( $DO_2$ ). The ratio of oxygen extraction (OER) is low. At the point of critical oxygen delivery, oxygen consumption becomes dependent on oxygen delivery and OER increases. This is where shock begins. Most importantly, shock occurs at the cellular level. Our patients appear “in shock” when perfusion reaches a critical low point and oxygen delivery cannot meet metabolic demands. Cells undergo hypoxic damage, including swelling and lysis, and this contributes to tissue injury and necrosis.

Examination parameters for shock include evaluation of:

- Mentation
- Heart rate
- Extremity temperature
- Pulse quality
- Capillary refill time

Patients in shock are expected to have altered mentation (dull to stuporous), tachycardia (or bradycardia in cats), cool or cold extremities (due to peripheral vasoconstriction), weak, thready, or absent pulses, white or cyanotic mm's, and CRT greater than 2 seconds. These six parameters should be evaluated together in patients whom are suspected to be in shock. Together, they are very sensitive (although not specific) for shock.

Treatment for shock involves improving oxygen delivery. Oxygen delivery depends on multiple factors, including cardiac output, oxygen content of the blood, and peripheral distribution of the blood. Blood oxygen content is a function of hemoglobin concentration, oxygen saturation (SaO<sub>2</sub>), and the partial pressure of arterial oxygen (PaO<sub>2</sub>).

**Oxygen supplementation** increases the amount of dissolved oxygen in the blood. This is a relatively small portion of the overall blood oxygen content, but any increase can be helpful in cases of hypoxemia and/or shock. Hyperbaric oxygen can increase the dissolved O<sub>2</sub> content more effectively, but isn't practical during a hemolytic crisis. It may be an option if the client's religious beliefs preclude blood transfusion.

## Transfusion Therapy

Blood transfusion aims to improve the hemoglobin concentration of the blood. Transfusion product options include Fresh Whole Blood, Stored Red Blood Cells, and Hemoglobin-Based Oxygen Carrying Solutions (HBOCS, Oxyglobin). **The goal is to improve the oxygen content of the blood, thereby improving oxygen delivery to the tissues, rather than completely correcting the PCV/HCT.** Remember, most IMHA patients are normovolemic or hypervolemic, so transfusion volumes are best kept to the minimum required to improve tissue oxygenation.

The transfusion of stored, packed red blood cells is generally preferred in IMHA cases. This allows for the transfusion of much-needed RBCs in a smaller fluid volume, thus reducing the risk of hypervolemia. In patients with Evans syndrome (IMHA and ITP), fresh whole blood (collected within 4-8 hours of transfusion) is preferred since this contains RBCs, coagulation factors, and blood proteins.

## Blood Typing in Cats

All cats should be blood-typed and crossmatched prior to transfusion. Cats have naturally-occurring alloantibodies and a transfusion reaction can be fatal. If a Type B cat receives Type A blood, the reaction may cause severe, acute hemolysis and possibly death (with less than 1 ml of blood in less than 1 hour's time.) Type B blood given to a Type A cat typically causes a less severe, delayed hemolytic reaction. Type AB cats should be able to receive any type of donor blood because, in theory, they do not have antibodies to either Type A or Type B blood. However, if autoagglutination is present in the recipient's blood, blood typing results are likely to be inaccurate.

## Blood Typing in Dogs

Most dogs do not have naturally occurring alloantibodies and blood typing is therefore less important. However, dogs do have blood types, the most important being Dog Erythrocyte Antigen (DEA) 1.1. 33-45% of dogs are DEA 1.1 positive. Antibodies to the DEA 1.1 antigen can form within 4 days after an incompatible transfusion or pregnancy. Subsequent transfusions may result in acute, severe hemolysis. Therefore, the universally accepted canine donor is DEA 1.1 negative. In-house card testing for dog blood type is reliable and inexpensive. As with cats, autoagglutination invalidates the result of the card typing test.

## Crossmatching

We routinely crossmatch all blood to be transfused. This involves both gross and microscopic evaluation for reaction between the donor and recipient blood samples. The donor RBCs are evaluated for the presence of bacterial contamination and crenation (loss of membrane integrity due to 2, 3 DPG depletion during storage). The recipient red cells and plasma are evaluated for evidence of autoagglutination and spherocytosis. Together, the donor cells and recipient plasma (Major crossmatch) are evaluated for clumping or other

evidence of reaction, as are the donor plasma and recipient cells (Minor crossmatch). Severe clumping is noted with incompatible feline blood products (incorrect typing).

### **HBOCS**

If compatible canine or feline blood products are not available for transfusion, Oxyglobin® can be given as a substitute. The dose is 30mL/kg (dog) or 10mL/kg (cat) and it should be administered at a rate no greater than 10mL/kg/hour to prevent hypervolemia and congestive heart failure.

### **Administration Guidelines**

- 1) Consider pre-treatment with diphenhydramine
- 2) Recommend pre-treatment with steroids and/or intravenous cyclosporine in IMHA patients.
- 3) Use 170 micron integrated filter or 18 micro syringe filter for leukocyte removal
- 4) Administer entire volume within 4 hours
- 5) Use caution to prevent hypervolemia if giving other IV fluids during transfusion
- 6) Monitor closely:
  - a. Start at ½ desired rate for 30 minutes
  - b. Monitor HR and rectal temperature every 15 minutes
  - c. Monitor for urticaria, angioedema, restlessness and vomiting or ptyalism
  - d. If reaction occurs, stop transfusion and wait 30 minutes. Administer diphenhydramine if not previously given.
  - e. Restart at ½ previous rate if reasonable and continue monitoring for persistent/recurrent reaction

### **Immunosuppressive Therapy**

The targets of immunosuppressive therapy in IMHA are: 1) to suppress RBC phagocytosis and removal by the reticuloendothelial system (RES), to inhibit complement-mediated RBC lysis, and to reduce antibody production by lymphocytes. The optimal immunosuppressive drug will have a rapid onset of effect, will be available in oral and Injectable formulations, will be highly efficacious, will have minimal side effects, and will be readily available and easily affordable.

### **Glucocorticoids**

These remain the mainstay of treatment, especially in the initial induction period. Adverse effects include polyuria, polydipsia, polyphagia, pica, panting, restlessness, fluid and electrolyte abnormalities, muscle loss, insulin resistance, exacerbation of hypertension or cardiac disease, calciuresis, and gastrointestinal ulceration among others.

Induction doses for prednisone (divided BID):

- Cats and Toy Breed Dogs up to 4-6mg/kg/day
- Small Breed Dogs 3 to 4 mg/kg/day
- Medium-Sized Dogs 2-3mg/kg/day
- Large Breed Dogs do not exceed 2mg/kg/day or 30mg/M<sup>2</sup>/day (rarely exceed 80-120mg/day total dose)

Dexamethasone: equivalent dose is 1/7 of comparable prednisone dose.

Methylprednisolone: equivalent dose is 4/5 of comparable prednisone dose.

The induction dose of glucocorticoids should be continued for at least 10-14 days. Taper by 25% every 2 to 4 weeks once PCV is normal and stable or increasing and there is no evidence of ongoing hemolysis. Once dose is tapered to 0.25 to 0.5mg/kg/day, reduce frequency to alternate days. Once dose reaches 0.25mg/kg on

alternate days for 4 to 6 weeks, discontinue. Recheck CBC 2-4 weeks once glucocorticoid therapy is completed and quarterly for one year.

### **Cyclosporine A**

This is a medication originally used to prevent organ transplant rejection in people. It targets T lymphocytes and inhibits cytokine production. In general, cyclosporine A is used in combination with glucocorticoids. This combination is used as first line therapy for dogs with IMHA at ACCIM. The modified capsules (microemulsified) are best for optimal bioavailability. Human brands include Neoral, GenGraf, and generic microemulsified. Veterinary brands include Atopica<sup>®</sup>. Adverse effects include gastrointestinal upset, gingival hyperplasia, and opportunistic infections.

- Induction dose for cyclosporine A: 5mg/kg BID x 5 days.
- Maintenance dose: 5mg/kg daily, adjusted as needed based on clinical response and serum drug levels.

The injectable formulation is indicated in patients with peracute IMHA and/or autoagglutination. The medication should be diluted with normal saline 0.5-1mg/ml and given intravenously over 2 to 4 hours. There is a low risk of adverse reaction (vomiting, facial pruritus) due to the castor oil vehicle in the injectable formulation.

### **Azathioprine (Imuran<sup>®</sup>)**

This is a competitive purine agonist which reduces lymphocyte proliferation and antibody production as well as reducing macrophage phagocytic activity. It has a delayed onset of activity (2-4 weeks) and therefore is not useful as an induction agent. It is only used in dogs. Adverse effects include myelosuppression, gastrointestinal upset, and hepatotoxicity. Recommended dose: 1.5-2mg/kg/day for 7-14 days, then every other day.

### **Mycophenolate Mofetil (CellCept<sup>®</sup>)**

Its mechanism of action is similar to azathioprine, and it is a more selective suppressor of lymphocytes. It is used in combination with Prednisone and Cyclosporine for the treatment of IMHA in dogs and cats. Adverse effects include possible gastrointestinal irritation or ulceration, although it is less myelosuppressive than azathioprine and the development of pancreatitis is less common. It is relatively expensive. Recommended dose: 10-20mg/kg BID (dogs) and 10mg/kg BID (cats).

### **Leflunomide (Arava<sup>®</sup>)**

This medication is intended for treating rheumatoid arthritis in people. It acts to inhibit pyrimidine synthesis and selectively inhibits lymphocytes over other cell types. Adverse effects are generally mild and until recently, its biggest drawback was its expense. Currently, clients can purchase 30 10mg tablets of the generic formulation for \$37. The previous cost was more than \$1000. Recommended dose: 4mg/kg/day.

### **Cyclophosphamide (Cytoxan<sup>®</sup>)**

Previously used more commonly, cyclophosphamide has fallen out of favor for the treatment of IMHA. This is due to a number of factors including a high rate of side effects (gastroenteritis, hemorrhagic cystitis) and decreased survival times associated with treatment.

### **Liposome-Encapsulated Clodronate**

This investigational drug is a liposome-encapsulated bisphosphonate. Phagocytosis of the liposomes by macrophages in the liver and spleen leads to rapid apoptosis of the macrophages. This results in a rapid reduction in red cell opsonization and allows time for additional drugs to act against other arms of the immune system. The effects of clodronate last approximately one week and a pilot study at CSU showed improved survival times in dogs treated at low doses in combination with prednisone, azathioprine and heparin.

## Adjunctive Therapies

### Gastrointestinal treatments

- Anti-emetics: metoclopramide, maropitant, dolasetron, ondansetron, chlorpromazine, prochlorperazine
- Metronidazole for diarrhea
- GI protectants: administered to all patients until glucocorticoids are tapered to alternate day schedule
  - Sucralfate, omeprazole, famotidine, ranitidine (avoid cimetidine due to effects against cytochrome P450 pathway)

### Antithrombotic Therapy

The pro-inflammatory state of our IMHA patients can induce inappropriate coagulation and these patients are typically hypercoagulable, although a hypocoagulable or DIC state may also be present. Thromboelastography (TEG) can help to further characterize the coagulation state of the patient, although this is not readily available.

Recommended doses:

- Micro low-dose aspirin: 0.5mg/kg PO BID for 7-10 days
- Sodium heparin 100U/kg SQ once then 50U/kg SQ TID for 7-10 days
- Low Molecular Weight Heparins

### Nutrition

Early enteric nutrition has been shown to improve survival in human patients. Gastrointestinal mucosal healing and repopulation can occur within 2-3 days of re-feeding. Placement of a nasogastric tube (in non-coagulopathic patients) can facilitate enteric nutritional support. Microenteral nutrition (Vivonex®, Clinicare®, etc.) can be fed via the tube at 0.1ml/kg/hour initially, then increased to 2ml/kg/hr after 24 hours, doubled to 4ml/kg/hour on Day 2, and supplemented with bolus or solid feedings by Day 3. A low fat diet should be considered due to the increased risk of pancreatitis.

### Pentoxifylline (Trental®)

To be used in cases with confirmed or suspected concurrent vasculitis. It has been shown to improve rheology and causes RBCs to become more deformable, thus improving microvascular circulation. However, there are no reports of improved survival with treatment. Recommended dose: 10mg/kg PO TID (dog/cat).

### Ursodiol (Actigall®)

This is a choloretic agent which helps to treat intrahepatic cholestasis by improving bile production and flow. It may also improve biliary clearance. Recommended dose: 10mg/kg/day PO (dog/cat).

### Antibiotics

All of our IMHA and ITP patients are started on Doxycycline empirically, pending Tick Titer/PCR results. Alternatively, this therapy is continued for 21 days if testing is declined by the owner. Broad-spectrum antibiotic therapy (Clavamox®) is indicated if gastrointestinal ulceration is present to reduce the risk for bacterial translocation.

### Hepatoprotectants

S-adenosylmethionine (Sam-e, Denosyl®) have not been shown to be of benefit, but should be considered on an individual basis, especially where hepatic hypoxia is suspected to be severe and/or reperfusion injury is likely. These nutritional supplements help with hepatic detoxification and prevent lipid peroxidation.

### **Human Intravenous Immunoglobulin (hIVIG) (Gammagard®)**

This formulation of concentrated human anti-IgG immunoglobulins prevents macrophages from binding to anti-RBC antibodies, thus reducing RBC removal by white blood cells and inhibiting Ig-mediated hemolysis. However, it does not interfere with complement-mediated hemolysis and so may not be completely effective in IMHA patients. A single infusion has a relatively low risk of side effects, including hypervolemia and hypersensitivity reactions. There is no proven benefit of administration to IMHA patients and no known improvement in survival. It is relatively expensive (\$120-140/g, available as a 5g bottle). The goal of therapy is to allow time for the immunosuppressant medications to be effective. Recommended dose: 0.5-1.5g/kg IV over 4 hours.

### **Therapeutic Plasmapheresis**

This is a dialytic therapy in which the patient's plasma is removed from circulation and discarded, then replaced with fresh plasma. The goal is to remove all circulating anti-erythrocyte antibodies. Disadvantages include the need for placement of a potentially thrombogenic, large jugular catheter, prolonged treatment time (several hours) and expense (approximately \$6000 per session). A study performed at ACCIM showed a reduction in plasma IgG and IgM levels following therapy, but no study has shown significant improvement in survival.

### **Splenectomy**

This surgical procedure comes into and falls out of favor periodically. The spleen is the major RES organ responsible for RBC breakdown. Studies show potential benefit if standard therapy and hIVIG treatment fail. Owners must be cautioned about potential iatrogenic infections and poor tissue healing due to concurrent immunosuppressive and glucocorticoid therapies.

### **Complications of IMHA**

IMHA patients are in a pro-inflammatory state, predisposing them to numerous complications, including thromboembolic disease and pancreatitis. Prolonged, severe anemia can lead to tissue hypoxia, resulting in hepatic dysfunction, gastrointestinal bacterial translocation, renal tubular necrosis as well as cerebral ischemia and edema. Therapies with glucocorticoid and immunosuppressive medications increase the risk for potential gastrointestinal ulceration, opportunistic infections and cardiovascular compromise. These complications often increase morbidity and mortality rates, as well as prolong hospital stays and owner expense. The adverse effects and significant cost of some therapeutic modalities can lead to poor owner compliance which may result in a poor response to treatment and overall decreased survival time.

### **Prognosis**

Studies report survival rates of 50-75% during the first 14 days. This increases to 90% in dogs that survive to Day 14. Negative prognostic indicators include concurrent thrombocytopenia, increased BUN, and a normal clotting profile as evaluated with TEG. Relapse occurs in up to 20% of dogs even after initial remission.