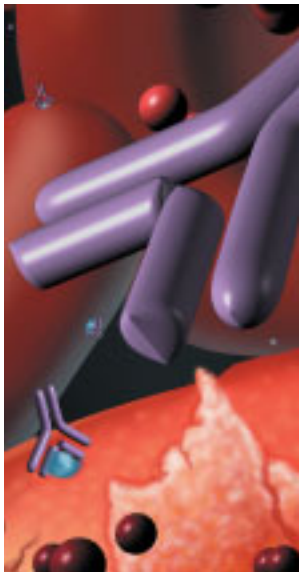

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Immune-mediated hemolytic anemia: Treatment

Choosing a treatment regimen for dogs with immune-mediated hemolytic anemia (IMHA) is a frustrating task. The lack of prospective treatment efficacy studies, the poor prognosis associated with the disease, and the high cost of treatment and supportive care all contribute to this frustration. In addition, complications such as pulmonary thromboembolism and disseminated intravascular coagulation (DIC) are relatively common, and their occurrence is hard to predict. Due to the lack of prospective data, recommendations in this article are based primarily on clinical experience and are not necessarily shared by all clinicians.

The goals of IMHA treatment should include the prevention of red blood cell (RBC) hemolysis, alleviation of tissue hypoxia, prevention of thromboembolism, and supportive care. In addition, it is critical to educate clients about the adverse effects of drug therapy, duration of therapy, potential disease complications, cost of treatment, and prognosis.

Preventing hemolysis

High corticosteroid doses are the first line of treatment for arresting RBC hemolysis in IMHA patients. Prednisone (1 to 2 mg/kg every 12 hours) is the corticosteroid of choice in dogs that can tolerate oral medication (see case study, page 10). Dexamethasone (0.1 to 0.3 mg/kg intravenously or intramuscularly every 12 hours) may be substituted for prednisone in animals that cannot tolerate oral drugs. Researchers believe that glucocorticoids prevent RBC hemolysis by blocking phagocytosis of antibody-coated RBCs by mononuclear cells (*Table 1*). Most dogs that respond to prednisone show some improvement within the first week of treatment.[1]

Corticosteroid therapy also decreases the concentration of antibodies directed against RBCs, but because this effect may not appear for several weeks, the full benefit of corticosteroid treatment is not evident until two to four weeks after treatment begins.[2] The first indication of this response is stabilization of the hematocrit, followed by a slow increase to normal values over several weeks.[2] Other indications of a good response to corticosteroids include the resolution of autoagglutination, an increase in reticulocyte count (if the anemia was initially nonresponsive), and decreased numbers of spherocytes on the blood smear. In the first few days of treatment, an increase in spherocytes may be observed because the mononuclear system is removing fewer antibody-coated RBCs. A change in the Coombs' test result from positive to negative is also an indication of response to therapy; however, in some dogs, the Coombs' test remains positive for several weeks after complete resolution of anemia.

In most cases, the prednisone dosage should start at 2 mg/kg orally twice daily. Once the hematocrit is above 30%, the dose may be decreased to 1 mg/kg twice daily in most cases. The dose is then tapered by 25% to 50% per month over a three- to six-month period depending on the hematocrit and severity of side effects. After three to four months, it is usually possible to move to an every-other-day dose, which minimizes adverse effects. The severity of adverse effects from corticosteroids varies widely, but small dogs generally tolerate higher doses better than large dogs, and the side effects are usually much less severe once the dose is less than 0.5 mg/kg once daily. If the disease is in remission after six months and a low prednisone dose (0.1 mg/kg) is being given every other day, the clinician can discontinue the drug. There is little benefit in further tapering such a low dose.

If hemolysis recurs after drug withdrawal, the clinician should reinstitute prednisone at the original dose and then slowly taper the medication to the dose used before relapse, with no further attempts to discontinue it. If the prednisone cannot be tapered enough to adequately resolve adverse effects, azathioprine should be added (2 mg/kg orally once daily) (*Table 1*). After four weeks of combination treatment, the clinician should attempt to taper the prednisone while continuing the azathioprine at the same dose. He or she should begin to taper azathioprine only after the prednisone has been successfully discontinued for four to eight weeks. Azathioprine should first be decreased to an every-other-day dose and then an every-third-day dose, with dose adjustments being made no more often than every eight weeks. At this point, total discontinuation should be considered if the animal is in complete remission and recovering from the first episode of disease. In animals that have previously relapsed, low-dose, every-other-day azathioprine should be continued throughout their lives.

Patients receiving azathioprine should be monitored for signs of bone marrow suppression and hepatotoxicosis. All patients on long-term immunosuppressive drug regimes should be routinely monitored for urinary tract infections and dermatophytosis.

Some IMHA cases do not respond to corticosteroids alone, and these dogs should start receiving a cytotoxic drug early in treatment. One dilemma in managing IMHA is whether all dogs should receive this additional drug right away or whether it is better to wait and identify the cases most likely to benefit. The advantage of starting a cytotoxic drug early is that, if the drug is necessary, no time

Table 1: Drugs Used in Managing Dogs with IMHA

Drug	Mechanism of Action	Dosage	Adverse Effects
Prednisone	Decreased Fc-mediated destruction of antibody-coated RBCs Decreased lymphocyte proliferation Stabilization of lysosomal membranes Inhibition of prostaglandin and leukotriene production	1-2 mg/kg orally b.i.d.	Iatrogenic hyperadrenocorticism Predisposition to infection
Azathioprine	Purine analog antimetabolite that disrupts DNA synthesis Has preferential effect on T lymphocytes	2 mg/kg orally once daily	Hepatopathy Bone marrow suppression Pancreatitis Gastrointestinal upset
Cyclophosphamide	Alkylating agent that blocks DNA and RNA synthesis and thereby reduces cell division	200 mg/m^2 once per week intravenously or orally or 50 mg/m^2 orally four days per week	Bone marrow suppression Sterile hemorrhagic cystitis Gastrointestinal upset
Cyclosporine	Selective T cell immunosuppression Reduces production of interleukin-2, thus blocking amplification of the immune response	5-10 mg/kg orally b.i.d. (Sandimmune — Novartis)* or 2.5-5 mg/kg orally b.i.d. (Neoral — Novartis)*	Gastrointestinal upset Gingival hyperplasia Papillomatosis Predisposition to infection (dose-related)
Danazol	Synthetic androgen Reduces RBC fragility Blocks Fc receptors on mononuclear phagocytic cells	5-10 mg/kg orally b.i.d.	
Human intravenous immunoglobulin	Blocks Fc receptors on mononuclear phagocytic cells Other immunomodulating effects poorly characterized in dogs	0.5-1.5 g/kg intravenously over six to 12 hours	Possibility of increased risk of thromboembolism Safety of multiple treatments not established

* Adjust dosage based on trough concentrations (collect blood sample immediately before next dose) 24 to 48 hours after starting treatment (goal 500 ng/ml). Adding ketoconazole at 5 mg/kg orally b.i.d. reduces dosage and cyclosporine treatment cost.

is lost waiting for a response to glucocorticoids. In addition, cytotoxic drugs are often necessary for long-term disease control. A disadvantage of starting these drugs early is the risk of adverse effects.

Azathioprine is the most commonly used cytotoxic drug for dogs with IMHA. Unfortunately, there are no controlled prospective studies evaluating azathioprine use in the management of IMHA patients. In a retrospective study of 70 IMHA cases, five dogs treated with azathioprine and prednisone survived longer than 16 dogs treated with prednisone alone (median 974 days vs. 57 days, respectively).[3] However, in another study of 88 dogs with IMHA, azathioprine treatment was not associated with a significant decrease in mortality.[4] Retrospective studies should be interpreted with caution, however, because many factors associated with poor prognosis also influence treatment choice. In addition, dogs that survive longer are more likely to be treated with azathioprine than are dogs that die early in the course of the disease. In another series of 16 dogs treated concurrently with prednisone and azathioprine (half the dogs also received danazol), one-year survival was only 38%, which is no better than that reported in other IMHA studies.[5]

Because azathioprine is often needed for long-term treatment, and because its adverse effects are usually less severe than those associated with long-term corticosteroid use, many clinicians use azathioprine and corticosteroids in all dogs immediately. Others add azathioprine only in cases with a poor prognosis (indicated by intravascular hemolysis, hyperbilirubinemia, autoagglutination, nonregenerative anemia, increased serum alkaline phosphatase activity). However, there are no prospective studies confirming the merit of this approach. In our hospital at Purdue University, we add azathioprine early in cases that fail to respond to five to seven days of glucocorticoids, in dogs that need more than two blood transfusions or infusions of a hemoglobin-based oxygen carrier (HBOC), and those with evidence of intravascular hemolysis.

Cyclophosphamide has also been recommended as an acute treatment for IMHA, but mounting evidence suggests that adding this drug is associated with a poorer prognosis. One small prospective randomized study evaluated the use of prednisone alone vs. prednisone and cyclophosphamide in initial IMHA treatment.[6] In this study, dogs treated with prednisone alone had a better outcome than those treated with the combined drugs. In the two retrospective studies discussed earlier, dogs treated with cyclophosphamide also had shorter survival times.[3,4] In our hospital, we rarely use cyclophosphamide to treat IMHA, reserving it for dogs that cannot tolerate oral azathioprine because of

persistent vomiting or gastrointestinal disease. Based on my experience, cytotoxic drugs in combination should be avoided because of the potential for severe immunosuppression and susceptibility to infection.

Other drugs used less commonly to manage IMHA in our hospital include cyclosporine, danazol, and human intravenous immunoglobulin. Cyclosporine is a more expensive and potent immunosuppressive drug used in dogs that have failed to respond to prednisone and azathioprine in combination. The drug is being used more widely to treat refractory IMHA, but there are few studies reporting its efficacy. In one study, cyclosporine was an effective treatment for two of three dogs with refractory IMHA; however, one dog died of sepsis.[7] In a retrospective study of 88 dogs with IMHA, dogs that received adjunctive cyclosporine did not survive significantly longer than dogs that did not.[4] Danazol has also been used as an adjunctive therapy for IMHA, but using this drug with prednisone and azathioprine did not improve outcome in a randomized, double-blind study.[5] Human intravenous immunoglobulin is a preparation of normal polyspecific IgG obtained from the plasma of healthy blood donors and has been used to treat canine IMHA.[8] The major benefit of this treatment seems to be short-term improvement in the hematocrit, which allows time for other treatments to become effective. Splenectomy has been reported to be effective in a small number of refractory IMHA cases, but this procedure may be detrimental if a patient's spleen is a significant source of hematopoiesis. Splenectomy should be reserved for those dogs in which all other treatment modalities have failed.

Treating tissue hypoxia

Most dogs with acute severe IMHA need oxygen-carrying support because it can take several days of treatment for the hematocrit to improve. Oxygen supplementation alone is of limited benefit in dogs with severe anemia. The need for additional oxygen-carrying support depends not only on the severity of the anemia, but also on the rapidity of onset, chronicity of the anemia, and severity of concurrent diseases such as pulmonary thromboembolism and gastrointestinal blood loss. Severe tissue hypoxia can exacerbate IMHA complications, such as DIC and thromboembolic disease. In general, transfusion or infusion should be considered when the dog is unable to compensate for anemia at rest, which is indicated by tachycardia, tachypnea, anorexia, lethargy, or weakness. Most dogs with acute IMHA and a hematocrit of less than 15% will benefit from increased oxygen-carrying capacity.

Options for oxygen-carrying support include packed RBCs or a HBOC (Oxyglobin® Solution—Biopure). Whole blood transfusion is less ideal because of unnecessary antigens contained in plasma, but this is an acceptable alternative if packed RBCs are not available. When administering packed RBCs, only blood from universal donors should be administered, and cross-matching should be performed before transfusion in any dog that has received prior transfusions. If multiple transfusions are necessary, administer blood from the same donor if possible. The advantages and disadvantages of packed RBCs and purified hemoglobin are discussed elsewhere in this monograph. In our hospital, we use both products in dogs with IMHA.

Preventing thromboembolism

Reported mortality of dogs with primary IMHA ranges from 26% to 70%, with thromboembolism being the cause of death in at least 30% to 60% of cases.[3,4,9,10] The cause of thromboembolism in canine IMHA is unknown, but increased concentration of procoagulant factors, decreased concentration of fibrinolytic or anticoagulant factors, vasculitis, DIC, enhanced platelet reactivity, and the presence of antiphospholipid antibodies may play a role.[9] In recent studies of dogs with IMHA, hemostatic abnormalities consistent with ongoing DIC were common at the time of clinical presentation. Decreased antithrombin activity was present in 50% of the dogs with IMHA, and the majority of dogs with IMHA had increased fibrinogen concentrations.[9] Clinical factors associated with increased risk of pulmonary thromboembolism include hyperbilirubinemia, intravenous catheter placement, and a negative Coombs' test result.[10] Because of the high incidence of thromboembolic disease in IMHA, many clinicians routinely use heparin, but the efficacy is unknown. In our hospital, dogs with IMHA are treated with heparin sodium at a starting dose of 150 to 200 units/kg subcutaneously every six to eight hours (see case study, page 10) and the dose is then adjusted to prolong the activated partial thromboplastin time by 25% to 50% of baseline. We have also used plasma transfusions to prevent DIC. However, studies in our hospital have shown increased mortality in dogs routinely treated with plasma compared to historical controls in which plasma was administered only to dogs with overt DIC. Therefore, we administer plasma only to dogs in overt DIC. Low-dose aspirin may also help prevent thromboembolism in dogs with IMHA. In our hospital, we often add aspirin (0.5 mg/kg every 12 to 24 hours) to the treatment regimen during heparin withdrawal. If a thromboembolic event does occur in a dog with IMHA, the long-term prognosis is generally poor.

Providing supportive care

Aggressive supportive care is critical to a good outcome in dogs with IMHA. Detection and treatment of underlying disease, detection of complications associated with immunosuppressive drug therapy, and good nursing care may positively influence the outcome.

It is important to detect and treat underlying disease in dogs with IMHA because these conditions may influence both treatment and prognosis. Immunosuppressive therapy is usually still necessary in dogs with secondary IMHA, but the duration of immunosuppression may be shorter if an underlying cause is identified. If an infectious cause is identified, the addition of cytotoxic drugs should be avoided.

Complications of immunosuppressive drug therapy include bone marrow suppression, infection, gastrointestinal ulceration, and iatrogenic hyperadrenocorticism. Gastrointestinal hemorrhage can contribute to anemia in dogs with IMHA; hemorrhage may occur because of high doses of corticosteroids or because of concurrent thrombocytopenia, vasculitis, ischemia, or other disease. It is important to recognize occult gastrointestinal hemorrhage because the resulting anemia can be confused with a failure to respond to IMHA treatment. Gastrointestinal hemorrhage treatment includes protectants such as sucralfate, H_2-blockers (*e.g.* famotidine), and prostaglandin analogs such as misoprostol. Unfortunately, none of these drugs have been demonstrated to decrease the incidence of corticosteroid-induced gastrointestinal ulceration. In our hospital, we use these drugs commonly to treat dogs with IMHA while they receive corticosteroids, but in my opinion there is little evidence to support their efficacy in this regard.

Case study: Floppy

This case illustrates the complex diagnostic tests and treatment required to manage severe IMHA. The issues raised include: When is it appropriate to use a cytotoxic drug in dogs with IMHA? What factors determine which type of oxygen-carrying support to implement? What is the significance of hemoglobinuria? Is plasma appropriate to use in dogs with IMHA and DIC? Should a crossmatch be performed before blood transfusion in all dogs with IMHA?

Signalment: 4-year-old spayed female cocker spaniel

History: One-day history of lethargy, jaundice, anorexia, and dark-red urine

Physical examination findings: 34.1 lb (15.5 kg), body condition score 3.5/5, temperature 102.4° F (39.1° C), heart rate 120 beats/min, respiratory rate 40 breaths/min, 5% dehydrated. Pale and icteric mucous membranes, and mild otitis externa and seborrhea sicca.

Minimum database

CBC*	Patient's Values	Normal Values
Total protein (g/dl)	8.0	6-8
PCV (%)	26 (manual)	37-55
Hb (g/dl)	8.4	12-18
RBC (x10⁶/µl)	Inaccurate due to agglutination	5.5-8.5
MCV (fl)	75.5	60-75
MCHC (g/dl)	32.3	32-36
WBC (x10³/µl)	25.9	6-17
Seg (x10³/µl)	21.24	3-12
Band (x10³/µl)	2.33	0-0.3
Metamyelocytes (x10³/µl)	0.26	0
Lymph (x10³/µl)	1.55	1-5
Mono (x10³/µl)	0.52	0.15-1.35
Eos (x10³/µl)	0	0.1-1.25
Baso (x10³/µl)	0	0-0.1
Platelets (x10³/µl)	Clumped but adequate	200-400
Reticulocyte (%)	5.8 (no absolute count due to agglutination)	
Reticulocyte production index	1.68	> 2**

*Morphology: Enlarged platelets, 1+ anisocytosis, 1+ poikilocytosis, 2+ polychromasia, 1+ spherocytes, 1+ toxic neutrophils
**Consistent with regenerative response

Serum Chemistry Profile	Patient's Values	Normal Values
Glucose (mg/dl)	109	67-132
BUN (mg/dl)	20	7-32
Creatinine (mg/dl)	0.6	0.5-1.5
Phosphorus (mg/dl)	2.6	2.2-7.9
Calcium (mg/dl)	9.3	9.7-12.3
Sodium (mmol/L)	147	138-148
Potassium (mmol/L)	3.0	3.5-5.0
Chloride (mmol/L)	121	105-117
Total protein (g/dl)	6.5	4.8-6.9
Albumin (g/dl)	3.0	2.3-3.9
Globulin (g/dl)	3.5	1.7-3.8
ALT (IU/L)	72	3-69
ALP (IU/L)	173	20-157
GGT (IU/L)	11	5-16
Total bilirubin (mg/dl)	2.3	0.1-0.8
Unconjugated bilirubin (mg/dl)	2.1	0-0.3
Conjugated bilirubin (mg/dl)	0	0
Delta bilirubin (mg/dl)	0.2	0-0.7
Cholesterol (mg/dl)	285	125-301
Amylase (IU/L)	864	378-1033
Lipase (IU/L)	549	104-1753

Urinalysis

Color	pH	Glucose	Bilirubin	WBC/hpf
Dark red	6.5	Negative	1+	0-2
Specific gravity	**Protein**	**Ketones**	**Blood**	**RBC/hpf**
1.045	Negative	Negative	4+	0-2

Major problem list
Anemia (slightly regenerative)
Icterus
Inflammatory leukogram
Hemoglobinemia
Hypokalemia

Preliminary diagnosis: IMHA

Summary

Despite the many modalities used to treat canine IMHA, the mortality from this disease is still unacceptably high. Factors associated with higher mortality in some studies include intravascular hemolysis, hyperbilirubinemia, autoagglutination, nonregenerative anemia, severe leukocytosis, and increased serum alkaline phosphatase activity.[3,11-13] However, these factors are not particularly helpful for determining the prognosis in an individual patient. As mentioned earlier, controlled prospective studies are necessary to compare the efficacy of the many drugs available for treating IMHA. Aggressive supportive care and monitoring are necessary to prevent adverse sequelae such as DIC, sepsis, and thromboembolism.

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Additional diagnostic testing

Coagulation Profile	Patient's Values	Normal Values
Fibrinogen (mg/dl)	700	200-400
PT (sec)	8.8	5.5-7.9
PTT (sec)	23.1	11.4-16.4
FDPs (µg/ml)	10-40	<10

Crossmatch
One out of six donors compatible (patient control negative)

Coombs' test
Positive

Urine culture
Negative

Infectious disease titers
Ehrlichia canis <1:40
Babesia canis <1:40

Imaging studies
Abdominal radiography: Mild hepatomegaly and splenomegaly
Thoracic radiography: Normal
Abdominal ultrasonography: No significant findings

Final diagnosis: Primary IMHA with intravascular hemolysis and DIC

Treatment

Treatment	Dosage	Start day	End day	Justification
Lactated Ringer's solution (with KCl)	64 ml/hour 32 ml/hour	1 2	1 8	Replace initial volume deficit Maintain hydration
HBOC (Oxyglobin)	125 ml (8 ml/kg) intravenously b.i.d. 45 ml/hour first 30 minutes, 158 ml/hour remainder	2	6	PCV dropped to 15% on Day 2 Dog became weak Compatible blood donor not immediately available
Heparin sodium	133 units/kg (2,000 units) subcutaneously q.i.d. 200 units/kg (3,000 units) subcutaneously q.i.d.	1 2	1 Still receiving	Prevent progression of DIC Prevent pulmonary thromboembolism Dose increased based on PTT (27 seconds Days 5 to 8)
Prednisone	2 mg/kg (30 mg) orally b.i.d.	1	Still receiving	Prevent Fc-mediated RBC destruction
Azathioprine	1.7 mg/kg (25 mg) orally s.i.d.	1	Still receiving	Slow agglutination and hemolysis
Packed RBCs	250 ml intravenously	6	6	Compatible donor identified on Day 2 HBOC continued until severe hemolysis had subsided Packed RBCs administered for longer-duration oxygen-carrying capacity
Famotidine	0.66 mg/kg (10 mg) orally b.i.d.	1	Still receiving	Prevent gastrointestinal ulceration due to prednisone
Plasma	15 ml/kg intravenously—one dose	1	1	Prevent progression of DIC

Outcome

The dog was discharged on Day 10 with a hematocrit of 30%. Medications continued at home were prednisone, azathioprine, famotidine, and heparin at the final dosages listed in the treatment table. The dog was doing well clinically with a stable hematocrit (32%) at the recheck visit one week after discharge. The CBC showed a marked neutrophilic leukocytosis with a left shift and spherocytes were still present, so the prednisone dosage was not decreased. All other medications were unchanged as well. Two weeks after discharge, the hematocrit was 29%, the WBC count was decreasing, and spherocytes were absent. One week later the hematocrit was stable, prednisone dosage was decreased to 20 mg every 12 hours, azathioprine was left unchanged, and heparin was tapered over three days. At the one-month recheck, the hematocrit was 33%, the WBC count was normal with no left shift, the prednisone dosage was decreased to 15 mg every 12 hours, and azathioprine was unchanged.

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