An Emergency Clinician's Approach to Anaemia

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Introduction

Anaemia is defined as a decreased PCV, haemoglobin and RBC in a normally hydrated animal. The causes of anaemia can be both a challenge to diagnose and treat. Anaemia can be acute or chronic; both of which may present to in an emergency situation. While our main goal is to treat the anaemia, we must first attempt to determine the cause of the anaemia. In doing this, we will improve our decision making process and ultimately how we treat the reduced RBC mass and the underlying disease. Thrombocytopenia and coagulopathies have been discussed previously and will not be covered in this article. The focus of this article is to discuss the diagnosis of anaemia and blood products available for treatment and where it is important other chemotherapeutic modalities that will help improve the patients' condition.

Overview of Erythropoiesis

Erythropoiesis occurs extravascularly in the bone marrow. Stem cells under the influence of erythropoietin give rise to rubriblasts, which mature over 3-4 days into metarubricytes and reticulocytes. Reticulocytes remain in the marrow for a further 2-3 days before being released into the peripheral circulation where final maturation occurs. It takes 5 days from stem cell stimulation to reticulocyte release from the bone marrow.

Mature RBC's survive in the canine circulation for approximately 120 days before being removed by the spleen, in cats the RBC survival time is approximately 70 days.

Causes of Anaemia

Haemorrhage

Surgery
Coagulopathy
- von Willebrands Disease/thrombocytopathia
- Thrombocytopenia
- Haemophilia A, B/ decreases in other factors
- DIC

Ectoparasitism
Gastrointestinal (hookworms, neoplasia, ulceration)
Neoplasia (haemangiosarcoma)

Haemolysis

Antibody mediated
- Warm-reactive IgG AIHA
- Cold reactive IgA AIHA
- Transfusion reaction

Congenital
- Phosphofructokinase deficiency (English springer spaniels, American cocker spaniels)
- Familial non-spherocytic, haemolytic anaemia (Poodles)
- Elliptocytosis
- NADH methaemoglobin fructase deficiency
- Haemolytic anaemia secondary to RBC membrane defect (Beagles)
- Pyruvate kinase deficiency (Beagles, Basenjis, West Highland white terriers, giant schnauzers, Abysinians)
- Vitamin B_{12} deficiency (Giant schnauzers)
- Predisposition to oxidation injury, high erythrocyte potassium, and low glutathione levels (Akitas, Shebas)

Toxin/Drug induced
- Propylthiouracil
- Lead
- DL-methionine
- Cephalosporins
- Fenbendazole
- Dapsone
- Gold salts
- Modified live virus vaccines
- Oxidants
- Onions
- Acetaminophen
- Methylene blue
- Phenacetin
- Propylene glycol
- Phenol compounds (mothballs)
- Benzocaine
- Hydroyurea
- Vitamin K_3

Parasites
- Feline haemotrophic mycoplasmas
- Babesiosis
- Erlichosis

Microangiopathic
- Splenic torsion
- Vena cava syndrome
- Haemangiosarcoma

Decreased Production

Bone marrow disorder
- Haematopoietic neoplasia
- Myelofibrosis
- Idiopathic aplastic anaemia
- Irradiation
- Myelodisplasia

Systemic disease
- Anaemia of chronic disease/inflammation
- FeLV
- Parvovirus
- Renal failure
- Liver disease
- Endocrine disease (hypothyroidism, hypoadrenocorticism)
- Neoplasia

Toxin/Drug
- Oestrogen
- Chemotherapy
- Phenylbutazone
- Trimethoprim-sulphadiazine
- Griseofulvin
- Quinidine
- Thiacetarsamide
- NSAIDs

Nutritional
- Mineral deficiency (Iron)
- Vitamin deficiency (B complex)
- Inadequate protein intake

Diagnosis of Suspected Anaemia in the ICU and Emergency Centre or Your Vet Clinic

1. History

History can be very useful, for example a recently vaccinated dog may have developed IMHA, and an outdoors cat may have *Mycoplasma haemofelis*. Older animals may have neoplasia; there may be a history of trauma or previous disease. You should ask if the animal has had access to toxins, and what the animal has been fed (i.e., onions). List all drugs that the animal is receiving. During the initial consultation I will ask the client the same questions several times, using a slightly different approach to jog their memories and ensure that I collect the information I require. A full history may give us vital information on causes and the progression of the disease. Often more chronic anaemia's will present in acute decompensation.

2. Clinical Examination

Do the basics thoroughly, remember that pink mucous membranes do not rule out anaemia. Often affected animals are tachycardic, tachypnoeic, and have pronounced peripheral pulses. Though they can be clinically normal if they have compensated for the degree of anaemia, in this situation they may only be less active than normal.

Look at the mucous membranes, conjunctiva and sclera. These may be pale, jaundiced, or both and petechiae may be observed. May be icteric with haemolysis.

Palpate abdomen for organomegaly, masses or fluid. A four quadrant abdominocentesis may detect haemorrhage. The chest should be auscultated, and if necessary perform bilateral

thoracocentesis. Clinically significant haemorrhage will not clot, as it is usually defibrinated at the time of examination.

3. Bedside Tests

Perform a PCV, TP and ACT on all anaemic patients. Ensure you look at the serum for signs of haemolysis, icterus, and agglutination before breaking the tube. Recording the total protein may help to differentiate haemolytic and non-regenerative anaemia from blood loss and assess for dehydration. Remember an animal may only become anaemic after treatment for hypovoalemia or dehydration. Assessing the clotting time allows the diagnosis of coagulopathies such as rodenticides or DIC. These tests can be done on presentation and give quantitative information about the underlying disease process. You need 2 ml of blood. Examining a drop of blood diluted in saline for agglutination can be useful.

4. Haemogram

This should be done on all patients. It can be done in-house (Vetscan), smear examination, or at your reference laboratory.

Look in the EDTA tube and on the smear for evidence of agglutination.

All in-house haematology should be confirmed with a smear examination. Two stains are required (1) Diff Quick (Wright-Geimsa) and (2) new methylene blue. Diff quick allows assessment of RBC morphology, platelets and WCC differentials. New methylene blue is used for reticulocyte counts and detection of *Mycoplasma haemofelis*. Reticulocyte counts are the gold standard in evaluating an animal's response to anaemia. In cats aggregate reticulocytes should only be accounted, not punctate reticulocytes. Aggregate reticulocytes look like dog reticulocytes and reflect bone marrow activity. A blood smear examination can be performed in any clinic with very little equipment.

The reference laboratory can confirm your smear examination findings.

RBC indices MCV, MCH and MCHC are used to evaluate overall red cell size and haemoglobin concentration. The terms macrocytic, normocytic and microcytic reflect MCV and RBC size, while hypochromic and normochromic refer to MCH, MCHC, and haemoglobin concentration. An elevated MCH or MCHC is usually the result of *in-vitro* or *in-vivo* haemolysis.

Interpreting instrument-derived RBC indices (from Vet Clinics of NA 33, 1207-1022, 2003)

MCV	MCHC
Macrocytosis or increased MCV Reticulocytosis Acute blood loss >3 days Acute haemolytic anaemia >3 days Early iron deficiency of young animals RBC agglutination FeLV Artefact Numerous large platelets or WBC's measured as RBC's (severely anaemic cats)	**Hyperchromasia or increased MCHC** Haemolysis Spectrophotometric interference Lipaemia Heinz bodies WCC >50 x 10^6/ml Icterus Spherocytosis Transfusion with haemoglobin-based oxygen carriers
Microcytosis or reduced MCV Absolute iron deficiency Diet deficiency of young Chronic blood loss Ineffective iron use Anaemia of chronic disease Portosystemic shunts RBC crenation Overanticoagulated sample Acute reaction of RBC's to hypotonic fluid (Hyperglycaemia, azotemia) RBC fragments or platelets measured as RBC's	**Hypochromasia or reduced MCHC** Reticulocytosis Acute blood loss >3 days Acute haemolytic anaemia >3 days Early iron deficiency of young animals Absolute iron deficiency Diet deficiency of young Chronic blood loss Ineffective iron use Anaemia of chronic disease Copper deficiency Vitamin B6 deficiency

Regenerative versus non-regenerative anaemia. This is important, as it will determine therapeutic, diagnostic and prognostic options. An absolute reticulocyte count of 60 x 10^6/ml for dogs and 40 x 10^6/ml for cats are the minimum values for indicating a regenerative response.

Blood loss and haemolytic anaemia's may be non-regenerative in the first 3-5 days. Repeat the haemogram and reticulocyte count 1-2 days post presentation.

In summary, Blood loss presents as a microcytic, hypochromic anaemia with hypoproteinemia; Haemolysis presents as a macrocytic anaemia with reticulocytosis, sometimes icterus and spherocytosis; FeLV associated anaemia is macrocytic, normochromic.

5. Other Bloods

Biochemistry and urinalysis: This may be used to determine a cause of anaemia such as chronic renal failure or determine other organ involvement as a result of the anaemia. For example ALT, ALP suggests either liver disease or hypoxia from anaemia. Bilirubin may indicate liver disease or haemolysis. Urinalysis will assess renal function and identify a potential source of blood loss.

Serology: All anaemic cats should be FIV and FeLV tested as these may directly contribute to the cause of the anaemia or allow blood parasites to establish disease or prevent regeneration.

Cross match and blood typing: EDTA blood should be collected for blood typing in cats or cross match in both dogs and cats. Cross match should be performed in any auto-immune disease or where repeat transfusions are required as the "best" unit of blood can then be given. Cats **must**

be typed (ideally) or cross-matched prior to any transfusion. In Australia approximately 30% of the cat population is Type B.

Coomb's test: This may be a useful test if immune mediated disease is suspected.

Blood Products

In Australia, we have access to canine blood banks. The two products available are packed red blood cells (PRBC's) and Fresh frozen plasma (FFP). In practice clinicians have access to both canine and feline whole blood.

PRBC's are produced by separating the red blood cells from plasma by centrifugation. PRBC's have a higher haematocrit than whole blood and are useful for blood loss, haemolytic anaemias, bone marrow dysfunction and were volume overload is a concern. FFP contains protein and clotting factors. Using blood products allows better use of the whole blood collected from a donor, they limit potential transfusion reactions and can be stored (PRBC's for up to 35 days, FFP 1 year).

Whole blood is very useful in very small dogs or puppies, and in cats. It can be used in any situation where anaemia is affecting tissue oxygen delivery or clotting factors are required. Fresh whole blood is blood collected and used within 6 hours of collection and it contains RBC's, clotting factors, proteins and platelets. Stored whole blood is blood transfused greater than 6 hours post collection. Clotting factors V and VIII concentrations decrease with storage and platelet viability is lost. Whole blood should be discarded after 24 hours, as it is difficult to guarantee sterility especially in cats. It can be used to replace protein loss but the haematocrit may increase.

Blood products can be imported into New Zealand from The University of Melbourne Blood Bank by contacting Prof. B.Parry or Dr C. Deague on +61-3-9731-2000.

Haemoglobin-based oxygen-carriers. Oxyglobin has been used safely overseas as a PRBC substitute but it is unavailable locally. It has been suggested to be of greatest benefit where there are no compatible RBC's available. For further information refer to Vet Clinics of North America, SA Practice 33 (2003) 1277-1293.

Indications for Transfusions in Cats and Dogs

Why transfuse?

Our primary goal is to improve the delivery of oxygen to the tissues in order that they function normally. Three factors determine adequate tissue oxygen delivery, the haemoglobin concentration and oxygen saturation and cardiac output. Red blood cells provide haemoglobin and improve oxygen saturation. Arterial haemoglobin is responsible for 98% of the body's oxygen transport.

When to transfuse?

This decision should not be made purely on the patients PCV or haemoglobin concentration because these may be spurious in an anaemic patient, for example a hypovolaemic patient may initially have normal blood volume parameters. Ongoing haemorrhage initially may be missed due to acute splenic contraction. No threshold PCV or haemoglobin concentration has been determined.

In people when the haemoglobin concentration falls below 3 g/dl or the PCV falls below 12% they are at risk of developing multiple organ failure. In animals a transfusion is necessary if the PCV is between 10-12%.

Thorough observation and recording of clinical signs allows us to determine whether the patient is compensating or decompensating for the degree of anaemia. Clinical signs of decompensation include weakness, tachycardia, tachypnoea, reduced mentation and bounding femoral pulses. An elevated blood lactate >4 mmol/l may suggest tissue hypoxia.

Transfusions will only support the patient until the underlying disease is adequately treated or controlled.

In the ICU setting the optimal PCV for oxygen delivery is 27%, above this there is no benefit. Transfusing large volumes of RBC products to raise the PCV to normal ranges will not benefit the patient; rather it will inhibit erythropoiesis and may cause multiple organ failure or disseminated intravascular coagulation secondary to breakdown of the RBC's.

In summary look at the patient and determine the optimal time to transfuse based on clinical and laboratory data and the underlying disease process. Specific critical care cases will be discussed later.

Transfusion Reactions

Transfusion reactions, though rare, can be minimised by selecting of the optimal blood component, thorough pretransfusion screening (cross match or blood typing, immune mediated disease), and consideration of the transfusion triggers, and correctly administering the blood products.

1. Administration

I premed patients with adrenaline @ 1 ml/10 kg SQ and chlorpheniramine @ 1 mg/kg SQ prior to transfusion. All blood products should be within their use by date, and have been stored correctly. If the product does not look right, don't use it (black instead of red). Use a giving set with a clot filter, and give the product within 4 hours of storing at room temperature. Throw away partially used bags after 24 hours. Ensure asepsis is maintained.

2. Immune-mediated transfusion reactions

The most serious is acute haemolysis secondary to donor-recipient incompatibility or previous sensitisation secondary to previous transfusion or pregnancy. This will be seen in cats when type A blood is given to a type B recipient. Avoid by cross matching or blood typing.

Delayed haemolysis can be observed in dogs as a result of extravascular haemolysis, usually caused by prior sensitisation to RBC's.

Occasionally the patient may get a transient fever due to reactions with donor granulocytes.

Should an acute immunologic transfusion occur, stop the transfusion and give dexamethasone @ 4 mg/kg IV or prednisolone sodium succinate @ 10 mg/kg IV. Fluid therapy will help to remove immune complexes and prevent organ failure.

3. Nonimmune-mediated transfusion reactions

Haemolysis can occur due to poor product storage or administration. 0.9% NaCl is the IV fluid of choice during the transfusion. Bacterial contamination can result in haemolysis of the blood or septic shock.

When large volumes of RBC's are given rapidly, or whole blood is given to normovolaemic patients (cardiac disease, renal failure, or pulmonary disease) volume overload can occur. Clinical signs are those of congestive heart failure (cough, cyanosis, tachypnoea, or dyspnoea). If volume overload is a potential risk transfuse at a rate less than 4 ml/kg/hr.

Citrate toxicity may occur when large volumes of whole blood are given. This may lead to hypocalcaemia. Treat as for hypocalcaemia if this should occur.

Blood Product Dose Recommendations

Blood Product	Rate	Volume	Frequency
Fresh whole blood	6 ml/min	12-25 ml/kg	q 24 hours
Stored whole blood	6 ml/min	12-25 ml/kg	q 24 hours
Packed red blood cells	6 ml/min	5-10 ml/kg	q 24 hours
Fresh frozen plasma	6 ml/min	5-10 ml/kg	q 8-12 hours

Rules of thumb

2 ml/kg of whole blood will raise the PCV by 1%

1 ml/kg of PRBC will raise the PCV by 1%

Current Recommendations on Selected Anaemia's in Small Animals

1. Feline Haemotrophic Mycoplasmas

This disease was formerly known as *Haemobartonella felis*, which causes feline infectious anaemia. However, recent studies of 16S RNA it has resulted in the organism being reclassified as a mycoplasma. Two variants have been identified *Mycoplasma haemofelis* (major pathogen) and *Mycoplasma haemomintum*, which vary in virulence. The organism is a 0.5 μm gram negative rod, coccus or disc shaped obligate erythroid parasite, which is epierythrocytic.

Epidemiology

The epidemiology is poorly understood. Identified risk factors include age, gender, housing indoors-outdoors, and the presence of fleas (fleas have been shown to transmit the parasite). The disease has a peak incidence at 4-8 years old.

Male cats may be at greater risk due to fighting. Infection is seasonal mainly in spring and summer; hence association with fleas, but it may be due to increased roaming outdoors. Transmission can be via lactation, in-utero, iatrogenically, haematogenous arthropod? (Little correlation) or orally. It is transmitted in infected RBC's.

Pathogenesis

Pathogenesis is divided into pre-parasitic, acute, recovery and carrier phases. The mechanism of parasite attachment to the erythrocyte membrane is unknown, but ultimately there are humoral and cell-mediated immune responses. The organism itself does not cause erythrocyte destruction; however, the clinical signs are related to immune-mediated erythrocyte destruction and extravascular haemolysis, which can be severe in older cats. IgG antibodies can be detected 2 weeks post infection, with the period of maximal clinical signs occurring with the period of initial Ab production. Ab binding to the organism on the RBC activates the compliment cascade resulting in removal by the spleen. The reticuloendothelial system will also entrap RBC-*M. Haemofelis* pairs not targeted by IgG and remove the parasite before returning the RBC to the circulation. These RBC's may be more susceptible to osmotic lysis. Anaemia and parasitemia last 18-30 days, the acute anaemia observed may be in part due to the sequestration of the RBC's in the spleen. Infected cats are usually Coombes test positive after approx. 15 days, most likely due to B-lymphocyte stimulation.

After recovery from acute *M. haemofelis* infection, the organism is sequestered in the spleen. Long term immunity is not understood, but it is thought the organism is completely eliminated over time.

Splenectomy, bacterial infections, and drug-induced immunosuppression are unlikely to exacerbate the disease. Splenectomy will result in a transient reappearance of the organism but no disease. Concurrent infection with FeLV may enhance FeLV virulence. Infections with *M. haemofelis* alter the cats' immune status with immune mediated anti-self responses and increased susceptibility to infectious diseases (FeLV). Cyclical changes may occur in the PCV and number of infected erythrocytes.

Clinical Findings

Approximately 50% of naturally infected cats are clinically ill. The disease is progressive, with the development of mucous membrane pallor, dyspnoea, open-mouth breathing, weakness, anorexia, depression, jaundice and fever. Hepatosplenomegaly and mesenteric lymphadenopathy may be observed. Infected animals commonly become carriers (reservoir is yet to be identified).

Affected cats have a regenerative anaemia with reticulocytosis, macrocytosis, and metarubricytosis. Occasionally the anaemia can be normocytic, normochromic. Clinical signs are apparent when the PCV falls below 20% and approx. 50% will have a parasitaemia. Variable leucocyte responses are observed. Cats with concurrent *M. haemofelis* and FeLV have a macrocytic, hypochromic anaemia, there m also be evidence of myeloproliferative disease, aplastic anaemia or dyshaematopoetic disorders associated with FeLV infection.

Diagnosis

Blood smears stained using Wright-Geimsa (Diff quick) or New-methylene blue (best) and examined under the microscope, looking dark blue inclusions within the RBC's. Care when using Wright-Geimsa stains as the parasite may be confused with Heinz bodies. Smears may be negative due to cyclical behaviour of the disease.

A PCR has been developed that detects the 16S rRNA gene but this technique is experimental.

Treatment

If affected cats are untreated there may be up to 33% mortality. No treatment completely eliminates the organism.

A whole blood transfusion may be given if severely anaemic and the cat is not compensating for degree of anaemia. Transfuse if the PCV is ≤10% otherwise there is a high risk of multiple organ failure. While many cats will benefit from a transfusion due to their innate ability at activity restriction if ill, they will cope with a more severe anaemia without transfusion, so do not panic if a donor cat is unable to be located.

Doxycycline @ 5-10 mg/kg q 24 hours for 2-3 weeks. Enrofloxacin is also equally effective @ 5-10 mg/kg q 24 hours for 14 days. Enrofloxacin has been reported to cause blindness in cats when dosed higher than 5 mg/kg/day, twice daily dosing @ 2.5 mg/kg may reduce this risk.

Corticosteroids are required for the immune-mediated aspects of the disease, prednisolone @ 1 mg/kg q 12 hr initially, then taper over 3 weeks, Alternatively, you can use dexamethasone @ 0.3 mg/kg q 24 hr for 2-3 days, then 0.15 mg/kg q 24 hr for 2-3 days then 0.15 mg/kg q 48 hrs for 2-3 treatments.

Prognosis

For uncomplicated *M. haemofelis* induced anaemia the prognosis is good with up to 75% survival with treatment. Without treatment 65% of acutely ill cats will survive. Recovered cats are carriers for an unknown duration. There is a less favourable prognosis for recovery if the cat has an underlying immune disease (FeLV, FIV).

2. Canine Immune-mediated Haemolytic Anaemia

This is another common disease emergency and critical care clinicians diagnose and treat on a regular basis. IMHA is still one of the most fatal entities in veterinary practice with little changes in mortality in the past 20 years. It can be a challenging disease to treat. In a nutshell the disease is due to the increased destruction of erythrocytes by auto-antibodies. Erythrocyte destruction may be primary (Antibody (Ab) binds to unaltered endogenous erythrocyte membrane antigens) or secondary (co-exists with another disease, Ab binds to exogenous antigens adherent to erythrocyte membranes). Secondary causes of IMHA include drugs, neoplasia, toxins and infectious diseases. Haemolysis may be intravascular or extravascular. Canine primary IMHA accounts for 60-75% of all cases of IMHA.

Pathophysiology

All breeds are affected, with possible genetic predisposition in Old English Sheepdogs, Cocker spaniels, Poodles, Bichon frise, Rough-coated Collies, Doberman pinschers, Miniature pinschers, Lhasa apsos and Shih Tzus. The disease is seen more frequently in spayed, middle aged female dogs, though this may be negated when adjusted for age. Dog erythrocyte antigen 7 was may be associated with a protective effect for disease development. Elevated oestrogen levels are thought to favour the development of auto-immune disease. Some studies report a seasonal incidence, though this is unlikely.

The disease is characterised by a shortened red cell survival time with an immune response that destroys RBC's by coating them with compliment or immunoglobulin.

Two types of IMHA can be identified on basis of lab studies; the majority are mediated by coating the red cell with incomplete and warm-type auto-antibodies. **Warm-type antibodies** react optimally between 35-40 DegC (i.e., body temperature). **Incomplete antibodies** do not agglutinate RBC's in saline, as there is insufficient antibody binding to the erythrocyte membrane to cause agglutination or haemolysis, but affected erythrocytes are removed by the reticuloendothelial system (spleen, liver, bone marrow). Most warm incomplete antibodies detected by the direct antiglobulin test are IgG, rarely IgA or IgM.

Cold antibodies react optimally below 30 DegC. Virtually always IgM. These may cause agglutination or haemolysis at lower body temperatures in the periphery of the body (ear tips, paws, tail). Haemolysis can be intravascular (uncommon) or extravascular. This form of IMHA occurs very rarely.

Intravascular haemolysis is due to complement binding to IgM or very high titres of IgG Ab. IgM agglutinates RBC's and it activates the compliment cascade, however IgM can detach after compliment activation and bind to other RBC's. IgM complexes are primarily removed by macrophages in the liver (20% by spleen).

Extravascular haemolysis is due to IgG incomplete warm antibodies binding to Fc receptors on macrophages and monocytes resulting in phagocytosis and lysis of the RBC's within the spleen and liver. Complement components on the RBC's, in addition to IgG, increases the rate of destruction by phagocytosis. The severity of the haemolysis due to RBC auto-antibodies is dependent on Ab titre, avidity for auto-antigen class, their ability to fix complement, antigen density on the RBC membrane and the state of activation of the macrophage system.

Table 1: Classification of canine IMHA based on antibody type present

Class	Title	Characteristics
Class I	Autoagglutination	IgM mediated, uncommon, very poor prognosis, serum icteric
Class II	Autohaemolysis	IgM mediated, uncommon, very poor prognosis, serum red
Class III	Incomplete antibody	IgM/IgG mediated extravascular haemolysis. Antibody does not cause haemolysis directly. Most common, fair prognosis
Class IV	Cold reacting autoagglutination	IgM mediated, uncommon/rare
Class V	Cold reacting incomplete antibody	Rare, cold reacting, Coombes test to diagnose

Recent investigations have shown that oxidative stress may also play a role in both the pathogenesis and progression of the disease.

Clinical Signs and Diagnosis

The clinician must rule-out other causes of haemolysis or secondary IMHA. See causes of anaemia in this article. A thorough history is required including all drugs the dog has recently been exposed to or currently being used, access to toxins such as onions.

Dogs often present with weakness, lethargy, pallor, tachycardia, tachypnoea, sometimes icterus and splenomegaly and hepatomegaly. They can have a lymphadenopathy and a haemic murmur. Some animals will have a change in urine colour due to haemolysis.

Some animals on presentation will not be anaemic but will become anaemic with IV fluids or increased haemolysis.

Dogs may have a regenerative anaemia with reticulocytosis, spherocytosis, anisocytosis, polychromasia, and a varying degree of reactive leucocytosis. Some dogs on presentation will have a non-regenerative anaemia depending on how acute the haemolysis has occurred. This should become regenerative in 3-5 days after presentation, however if the antibodies or compliment are directed against erythrocyte precursors in the bone marrow regeneration may not occur. Up to 67% of patients have a thrombocytopenia; this has been associated with an increased mortality and increased risk of thromboembolic disease. Severe thrombocytopenia ($< 50 \times 10^9$/l) may occur concurrently. Often observe in-saline autoagglutination. Coomb's test will be positive in 60% of cases.

Should the patient fail to become regenerative, a bone marrow aspirate may be required to diagnose RBC aplasia or hypoplasia, neoplastic infiltration, or immune destruction of erythroid precursors.

Coagulation profiles provide evidence for concurrent DIC. IMHA dogs are in a hypercoagulable state and at high risk of thromboembolic disease and developing DIC.

Biochemistry reflects hypoxic- and haemoglobin-induced multiorgan damage with pre-renal azotemia, elevated liver enzymes and bilirubin.

Abdominal and thoracic radiographs should be performed to rule out neoplasia.

Prognostic Indicators

There is no difference in mortality associated with age, sex, PCV at presentation or autoagglutination. However, patients with icterus, autoagglutination, intravascular haemolysis, severe thrombocytopenia, hypoalbuminaemia and marked bilirubinaemia had a poorer prognosis for survival.

Therapy for IMHA

Supportive care

Judicious IV fluids should be used to maintain renal perfusion and assist with clearance of haemoglobin and bilirubin. IV catheters predispose the patient to thromboembolic disease but the benefits of fluid therapy outweigh the risks. Perform regular coagulation profiles to detect DIC early. Oxygen therapy may be of assistance but generally the RBC-haemoglobin is saturated due to the anaemia.

Transfusions

Attempt to cross match blood prior to transfusion, this can be difficult in patients with autoagglutination, but using the transfusion with the least reaction may limit *in-vivo* transfusion reactions. A retrospective study of IMHA cases at Werribee (T.Bassett) between 1990 and 2001 showed that 60% of all cases were cross-matched, with 90% receiving a matched unit of PRBC's, of these 25% exhibited some form of transfusion reaction. Haemolysis was most common.

When transfusing it is important to consider the survival times of the transfused RBC's as the underlying immune-mediated disease will inevitably shorten RBC survival time, sometimes a transfusion will last 24 hours.

When to transfuse? If a transfusion is required give it, as hypoxia will lead to multiorgan damage. PRBC's are preferred to avoid volume overload. There is no optimal PCV to indicate when transfusion should be given. Transfuse if the PCV ≤10%. To limit transfusion reactions, base your decision on the PCV and clinical signs suggesting decompensation. Failure to transfuse is more detrimental than transfusing. I try not to transfuse until the PCV is 11-12% as long the animal is compensating for the degree of anaemia and there are is no evidence of organ damage. Aim to give sufficient PRBC's to raise PCV to 20-24%, this will improve signs related to hypoxia and support the patient until regeneration begins.

If there is evidence of DIC give FFP.

Corticosteroids

These drugs have a rapid favourable effect on haemolysis due to their anti-inflammatory and immunosuppressive actions suppressing lymphocyte proliferation and interleukin-2 production, altered recognition for Fc receptors on macrophages, inhibiting the compliment cascade, and decreasing the production of immunoglobulins. Start prednisolone @ 1-2 mg/kg/day IV, IM, PO for 2 weeks, then slowly taper as improvement occurs. Alternatively dexamethasone can be used @0.5-1.0 mg/kg SID. You may be able to discontinue treatment after 2-4 months.

A word of warning, do not leave patients on high doses of prednisolone for greater than 2 weeks as haematochezia, malena and haematemesis can occur due to the ulcerative effects of steroids.

Azothiaprine

Required in autoagglutinating or non-regenerative forms of IMHA. We tend to start all our IMHA cases on both prednisolone and azothiaprine in order to reduce the doses of steroids after 2 weeks. Azothiaprine suppresses immune system by inhibiting cell division, T-cell cell-mediated immunity, and cytokine production. It is believed that azothiaprine prolongs the length of survival. Start @ 2 mg/kg/day PO initially, and then reduce to 2 mg/kg EOD after the first 7-10 days. Adverse effects are mild and include myelosuppression, gastro-enteritis, pancreatitis and elevation of liver enzymes. If the neutrophil count drops below 3000/µl stop drug until count returns to normal. Taper slowly over several months to prevent relapse.

Cyclophosphamide

This drug inhibits humoral and cell-mediated immune responses. It causes cross-linking of DNA in dividing and resting cells (T and B-cells) suppressing T-cell-dependant B-cell responses.

Adverse effects include anorexia, GIT signs, myelosuppression, poor hair growth and haemorrhagic cystitis. Two protocols have been suggested (1) a single IV or oral dose of 200 mg/m^2 during the initial treatment period or (2) 50 mg/m^2 PO or IV SID for 4 consecutive days each week until the PCV improves.

Reports in the literature suggest cyclophosphamide may be of little benefit in the treatment of IMHA (Burgess K, et al, J Vet Intern Med. 2000 Jul-Aug; 14(4):456-62 Mason et al, J Vet Intern Med. 2003 Mar-Apr; 17(2):206-12.).

Cyclosporine

Cyclosporine A suppresses the cell-mediated immune response by interfering with activation of lymphocytes and macrophages. The release of Il-2 from T-helper cells is also suppressed. Care must be exhibited when using any other drugs that use the cytochrome P-450 pathway as this will increase cyclosporine levels. Adverse effects include GIT signs and anorexia. Current recommended doses are 10 mg/kg SID-BID, with drug monitoring advised every 2-4 weeks (trough levels of 100-300 ng/ml). Stop cyclosporine when the patient has been in remission for at least 2 weeks.

Danazol

This is used in human IMHA. It is a synthetic androgen that acts as an immune modulator by normalising the suppressor/helper T-cell ratio and down regulating Fc receptors on macrophages. It is incorporated into the RBC membrane stabilising it and rendering it more resistant to haemolysis Danazol has a sparing effect on corticosteroids, reducing the long-term side effects of steroid use. It can be given alone or in combination with prednisolone. The dose used is @10 mg/kg/day. Once in remission, reduce to 5 mg/kg/day. Steroids use can be stopped once anaemia improves. Taper dose after 2-3 months of normal haemograms. A recent study showed no difference in canine survival time.

Anticoagulants

The pathophysiology of IMHA may result in pulmonary thromboembolism and DIC. IMHA can fulfil Virchow's triad (vascular stasis, hypercoagulability and endothelial damage) resulting in thrombi development. Heparin may help prevent thrombi formation in these patients but have no effect against existing clots. Heparin prevents the deposition of fibrin and platelets on the thrombin surface. Dose ranges of 75-300 IU/kg IV or SQ QID have been suggested; however the efficacy of this therapy is questioned. Therapeutic levels are believed to be achieved when the APTT is prolonged by 1.5-2.0 times normal, or the ACT is prolonged by 15-20 seconds. Heparin is unlikely to do any harm and in my experience rarely results in a prolongation of the ACT.

Gastric protectants

IMHA patients are at risk of developing gastric ulceration due to poor perfusion of the GIT and the effects of corticosteroids. Gastric protectants such as ranitidine, cimetidine, misoprostyl, omeprazole and sucralfate may be used at standard dosages.

Antibiotics

Antibiotics have no place in the treatment of IMHA unless the patient is showing signs of bacterial sepsis. Antibiotics have been known to cause or contribute to the development of

IMHA. A recent study by Miller *et al* (2004) was unable to demonstrate a bacteraemia on blood cultures from 12 dogs with IMHA. I routinely do not use antibiotics in IMHA patients and have observed no adverse effects.

Novel treatments

Splenectomy has been recommended for refractory IMHA. Removing the spleen removes a source of B-cells and activated macrophages resulting in decreased Ab production and RBC destruction. Care must be taken due to the animals being on long term immune-suppressant therapy prior to surgery.

Plasmapheresis has been used to separate RBC's from plasma, and the plasma filtered to remove excess Ab and immune complexes. This may be useful in the future with few reports in the literature.

Erythropoietin therapy is being studied as a possible alternative to blood transfusions; watch this space!

Summary

The diagnosis and treatment of anaemia in small animals is not daunting if a systematic approach is adopted. Many clues can be obtained from the history and the utilisation of the ACT and a blood smear. Once the underlying disease process has been identified the patient may be treated with definitive therapies (surgery, immune-suppressants or toxin withdrawal) and the symptomatic use of blood products.

Blood typing or cross-matching is recommended prior to transfusion in dogs and is a must in cats. The decision to transfuse must be based on the PCV and the clinical signs exhibited by the patient. Remember blood transfusions support the patient until definitive therapy can be applied. Finally IMHA is a common disease and they can be successfully managed with a combination of supportive therapy and immune suppressants, and they are fun to treat.

References and Suggested Reading

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Appendix A: In-house Laboratory Protocols

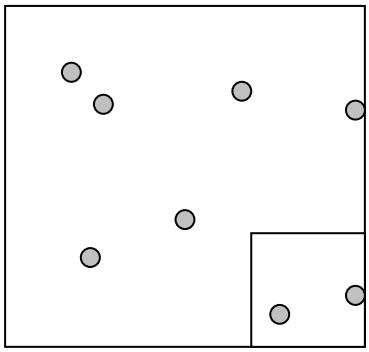
1. New Methylene Blue Staining Procedure

In a test tube add an equal volume of whole blood and new methylene blue (I add 2 drops of each) and incubate at room temperature for 10 minutes. Prepare a smear and examine once air dried.

To get a reticulocyte count all reticulocytes in a large square and the RBC's in a square 1/10 size of the original square (include RBC's and reticulocytes) See Figure 1. Record reticulocytes and RBC's separately. Additional fields are counted until at least 110 RBC's have been counted in the smaller square. Now calculate the percentage of reticulocytes as follows:

% Retics = (total retics in large square x 100) / (Total RBC's in smaller square x 9)

Figure 1: Schematic view of reticulocyte counts



2. ACT

Collect 2 ml of blood with minimal excitement and probing of the vessel, avoiding venous stasis, preferably from peripheral vein. Place the collected blood in a pre-warmed ACT tube (37°C in a water bath or in the umpit) and record the time taken for a clot to form. Normal ranges are from 90-120 seconds.

This test will be prolonged with a profound thrombocytopenia as platelet factors are needed for formation of the clot.

3. Blood Cross Match

(a) Obtain EDTA-anticoagulated blood from the recipient and the crossmatch tubing segments of blood from the donor blood packs.

(b) Centrifuge donor and recipient blood for 5 minutes.

(c) Using pipettes remove plasma and save in separate labelled tubes.

(d) Wash the red blood cells by adding 0.9% NaCl to the red cells to fill the tube. Resuspend the red cells in the saline by gently tapping the bottom of the tube with a finger.

(e) Centrifuge red cells and saline for 5 minutes. Pipette off saline, and discard.

(f) Repeat steps 4 and 5 twice.

(g) After the third washing of the red cells resuspend the red cells to a 3-5% solution. Add approx.5 ml saline.

(h) For each potential donor, mix 2 drops of recipient plasma and 1 drop of donor red cell suspension for the major cross match. Mix gently.

(i) For each potential donor, mix 2 drops of donor plasma with 1 drop of recipient red cell suspension for the minor cross match. Mix gently.

(j) For the recipient control, mix 2 drops of recipient plasma and 1 drop of recipient red cell suspension. Mix gently.

(k) Incubate the tubes at room temperature for 15 minutes.

(l) Centrifuge the tubes for 15 sec.

(m) Observe the plasma for haemolysis.

(n) Resuspend the centrifuged cells by shaking gently.

(o) Observe the red blood cells for agglutination. Both macroscopically and microscopically.

4. Feline blood donors and blood collection

The ideal feline blood donor should be an indoor cat that is FeLV and FIV negative and *Mycoplasma haemofelis* free and be blood typed. The cat should be 4.5-5 kg in body weight and have a pleasant disposition, with easily accessible jugulars (avoid brachycephalic cats).

Donor cats may need sedation; ketamine diazepam is effective or midazolam and isoflurane. It is recommended that an equal volume of saline or LRS be given to the cat to replace the blood volume removed as some cats can have hypotensive crises. Blood is collected aseptically (surgical scrub and sterile gloves) into 25 or 50 ml syringes with a 19 g butterfly needle attached, either 625 U heparin is mixed with 50 ml blood or 1 ml Acid Citrate Dextrose anticoagulant added for every 7-9 ml blood collected. As this is an open system blood should not be stored longer than 24 hours. Shoof offers feline blood collection systems where blood may be stored for up to 30 days.

5. Canine blood donors and blood collection

The ideal canine blood donor is a Greyhound or any large dog ≥27 kg, which is quiet. They should be heartworm negative and healthy at the time of donation. 1 unit 450 ml blood is collected. The blood should be collected aseptically (surgical scrub and sterile gloves) especially if storage is anticipated (up to 20 days). Ideally the blood bag should be gently rocked during collection to mix the anticoagulant and the bag weighed to ensure sufficient blood is collected and that the blood is flowing freely into the bag.