Anaemia in dogs and cats (part 1)

Defined as a reduction in red blood cell mass, anaemia can be caused by red blood cell loss (haemorrhage, destruction) or reduced production; RCVS recognised and European veterinary specialist in small animal medicine, Polly Frowde MA VetMB DipECVIM MRCVS explores a logical approach to investigating anaemia in dogs and cats and summarises its main causes

This two part-review explores a logical approach to investigating anaemia in dogs and cats and summarises its main causes. We start with a general overview, then focus on non-regenerative anaemias in part 1; followed by regenerative anaemias in part 2. Treatment and prognosis will be discussed, but readers are directed to previous articles for detailed guidelines on transfution therapy.[1]

GENERAL APPROACH TO ANAEMIA:

HISTORY & PHYSICAL EXAMINATION

It is important to obtain a thorough general history, with particular attention to possible exposure to toxins, medications or infections (eg. any travel history or ticks), signs of bleeding (eg. melaena) and whether the patient has received a blood transfusion before (in case cross-matching will be required for any transfusion therapy). Owner observations are often non-specific, eg. lethargy, weakness, collapse. Pica may have been noted (eg. cats eating their litter).

During clinical examination, note how well compensated the patient seems, ie. whether they are tachycardic/weak, or whether these parameters seem surprisingly normal in the face of obvious pallor. This aids decision-making regarding the need for a transfusion, but can also give an indication of disease chronicity (chronic anaemia allows more time for adaptation, and is more likely to be non-regenerative). Poor body condition/cachexia also suggests a chronic disease process. Assess for lymphadenopathy, palpable masses or petechiae/ecchymoses.

Pallor is the hallmark of anaemia (see Figure 1) but can also be caused by poor perfusion (both processes may contribute to pallor in acute blood loss). Poor perfusion severe enough to cause pallor should be associated with prolonged CRT and weak pulses, whereas anaemic, normovolaemic patients have normal capillary refill time (CRT) and normal or bounding pulses (except after acute blood loss). A packed cell volume (PCV) can usually quickly differentiate between the two, however this test can be misleadingly normal in the face of acute haemorrhage (<24 hours) when interstitial fluid hasn't yet equilibriated with intravascular fluid. Repeat PCV assessment following initial crystalloid/colloid therapy for hypovolaemia should reveal the true extent of any anaemia. 'Haemic' murmurs (caused by reduced blood viscosity) are common with moderate-severe anaemia – typically soft, systolic (innocent) murmurs centred over the heart base. Any other abnormalities on cardiac auscultation warrant further

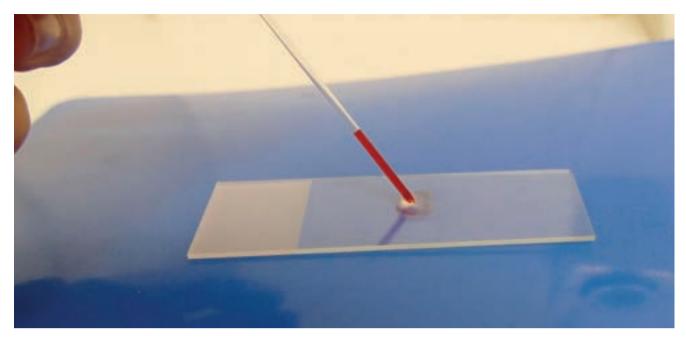Figure 1: Pallor: the clinical hallmark of anaemia, but not exclusive to it.

Figure 2: In-saline agglutination test: in-house.

investigation in case cardiovascular disease/poor perfusion is contributing to pallor (investigations could also be justified for an innocent murmur to ensure no extra risk factors for volume overload – alternatively, monitor closely during any transfusions and ensure that the murmur disappears once anaemia resolves).

DIAGNOSIS

Anaemia can be confirmed quickly by in-house tests, but it is important to be aware of their limitations. A PCV can provide a rapid, convenient assessment of red blood cell (RBC) mass. Perform on a well-mixed, appropriately-filled, clot-free blood sample and ensure that micro-haematocrit tubes are analysed in pairs and between 2/3 to 3/4 full, since under or over filling can result in falsely high or low readings, respectively. This should always be accompanied by a full haematology profile, including smear assessment, to help characterise the anaemia and screen for concurrent cytopenias, atypical circulating cells and microorganisms. Analysis by an external lab is strongly recommended for reliable white blood cell (WBC) differential counts and expert smear assessment, but can be combined with in-house cytology and automated counts when more immediate results are needed.

Establishing the anaemia as regenerative or non-regenerative greatly assists diagnostic workup. Reticulocyte counts give an objective assessment of regeneration (see Table 1), but macrocytosis, hypochromia and polychromasia are all very supportive and may provide sufficient evidence if obvious. Reticulocytes take up to five days to mature following acute RBC loss, therefore repeat counts may be necessary if it is unclear whether anaemia is truly non-regenerative versus 'pre-regenerative' at initial presentation. However, if acute/pre-regenerative anaemia already seems unlikely (eg. the patient presents with a severe but well compensated anaemia), further investigations for non-regenerative anaemia (eg. bone marrow biopsy) may be

justifiable without waiting for reticulocyte re-assessment. If immune-mediated anaemia is suspected, an in-saline agglutination test is simple to perform: place a drop of saline onto a slide, then add a smaller drop of ethylenediamine tetra-acetic acid (EDTA) blood using a micro-haematocrit tube (see Figure 2). Swirl gently in a circular motion assessing for gross agglutination, then perform microscopy (using a cover slip) to rule out rouleaux formation and assess for micro-agglutination (see Figure 3). If positive, this negates the need for a Coomb's test (since anti-RBC antibody has already been demonstrated).
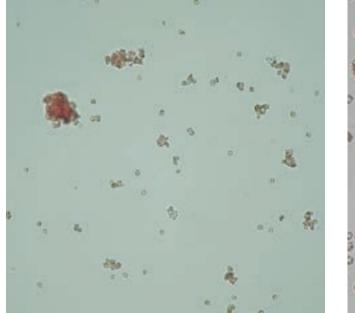
A full biochemistry profile is always indicated to screen for concurrent abnormalities (including consequences of the anaemia, or findings suggestive of another underlying disease process).

Low protein levels are consistent with blood loss and hyperbilirubinaemia is supportive of haemolysis, but neither 'markers' are 100% sensitive or specific for these processes. FIV/FeLV testing should always be performed in anaemic cats. Urinalysis should also be performed as part of a minimum database and to screen for evidence of haemolysis (haemoglobinuria). Haemoglobinuria can be differentiated from haematuria by urine centrifugation/sediment examination (revealing discoloured supernatant, rather than a pellet of RBCs with clear supernatant); and from myoglobinuria (rare) by demonstrating that the plasma is

Table 1: Canine and feline absolute reticulocyte counts.

Regeneration	Canine reticulocytes absolute count	Feline aggregate reticulocytes absolute count
None	60	<15
Weak	150	50
Moderate	300	100
Strong	>500	>200

Acute, severe anaemia should stimulate a moderate to strong regenerative response by approximately day five (failure to do so indicates non-regenerative anaemia).
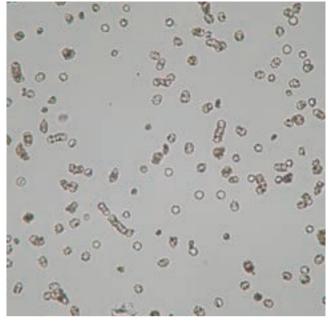
Figure 3: Autoagglutination (left) versus Rouleaux formation (right). Photo: Roger Powell.

also haemolysed (not expected with myoglobinaemia). Further tests (eg. diagnostic imaging) will then be indicated, as determined by initial findings.

NON-REGENERATIVE ANAEMIA

Regardless of cause, non-regenerative anaemia often develops gradually, allowing more time for the body to compensate. Clinical signs can therefore be relatively mild considering the severity of the anaemia (eg. a dog with PCV <15% at initial presentation and normal heart rate is more likely to have non-regenerative anaemia). Anaemia may not be the most clinically significant abnormality, eg. anaemia of chronic disease; pancytopenia.

ANAEMIA OF CHRONIC DISEASE:

The most common cause of mild non-regenerative anaemia in cats and dogs, anaemia of chronic disease occurs with many disease processes – including hepatic and renal disease, neoplasia, chronic inflammation and several endocrinopathies (eg. hypothyroidism, hypoadrenocorticism, diabetes mellitus). It is usually normocytic, normochromic and mild-moderate (Hct typically >20-25% in dogs, >17% in cats) with two exceptions: congenital portosystemic shunts can cause microcytic anaemia (+/- hypochromic), and chronic kidney disease (CKD) can cause severe normocytic/chromic anaemia.

Various mechanisms (often multifactorial) contribute to the anaemia, depending on the underlying disease process. With anaemia of inflammatory disease (AID), inflammatory mediators promote iron sequestration leading to functional iron deficiency (with normal body stores). Specific treatment of the anaemia is rarely necessary as resolution largely depends on managing the underlying disease. Supportive measures, eg. antacids where gastrointestinal haemorrhage is a possible contributor (eg. CKD) could be considered. When anaemia of CKD seems severe enough to be contributing

to clinical signs, erythropoietin (EPO) therapy +/- iron supplementation can be considered. However, cross-reacting anti-EPO antibodies may develop, causing erythroid hypoplasia and deterioration in anaemia – this risk seems to be lower with Darbepoetin than other recombinant human erythropoetins.[2]

IRON DEFICIENCY ANAEMIA:

Iron deficiency anaemia develops as a result of chronic blood loss depleting iron stores. It is initially regenerative, but eventually becomes non-regenerative with microcytosis and hypochromia. Additional laboratory findings may include thrombocytosis and hypoproteinaemia. Young animals have poor iron stores and are especially prone to iron depletion. Iron analysis helps to confirm deficiency and guide therapy. The most common source of occult blood loss is the

Table 2: Anaemia of inflammatory disease versus iron deficiency anaemia.

	Anaemia of inflam-matory disease	Iron-deficiency anaemia
MCV/MCHC	**Normocytic/normo-chromic**	**Microcytic/hypo-chromic**
Serum iron	**Decreased**	**Decreased**
TIBC	**Normal to de-creased**	**Normal to In-creased**
Transferrin satura-tion	**Mildly decreased**	**Markedly de-creased**
Serum ferritin*	**Normal to increased**	**Decreased***
Bone-marrow iron stores	**Normal – increased**	**Decreased**

*Ferritin is also an acute phase protein so may be elevated in inflammatory conditions.
Important: since it is a mean value, mean corpuscular volume (MCV) may appear normal in the face of regenerative microcytic anaemia (due to concurrent microcytes and macrocytes), ie. in early iron deficiency. Smear assessment and increased red cell distribution width (RDW) can be helpful in this scenario.
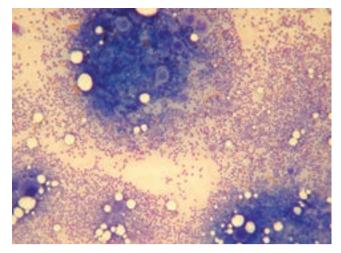
Figure 4: Bone marrow aspirates: cellular spicules. Photo: Roger Powell.

gastrointestinal tract and a faecal occult blood test may help confirm. Table 2 highlights the differences between an iron deficient state versus AID:

BONE MARROW DYSFUNCTION:

This can result from a variety of benign and malignant diseases, often manifesting as bi- or pancytopenias, in which case any concurrent neutropenia/thrombocytopenia may be more of a clinical problem than the anaemia. However, marrow dysfunction can also present solely as non-regenerative anaemia.

If a patient presents with non-regenerative anaemia too severe for anaemia of chronic disease and with no evidence of iron deficiency, bone marrow disease should be suspected and marrow sampling considered (concurrent cytopenias should automatically increase suspicion for bone marrow dysfunction). Bone marrow sampling techniques are beyond the scope of this article (readers are encouraged towards references such as the *BSAVA Guide to Procedures*). It is good practice to perform in-house cytology during marrow collection to ensure adequate marrow harvest (see Figure 4) +/- enable repeat sampling if unsatisfactory.

TOXINS/DRUGS ASSOCIATED WITH MYELOSUPPRESSION

These include chemotherapy, phenobarbitone, chloramphenicol, colchicine, methimazole, griseofulvin and oestrogen (exogenous sources, eg. human HRT tablets or endogenous e.g. sertoli cell tumour). Chronic lead poisoning can cause impaired RBC development and shorten mature RBC survival (RBCs may show basophilic stippling).

INFECTIOUS CAUSES OF MYELOSUPPRESSION
- Parvovirus – concurrent neutropenia common (+/- occasional thrombocytopenia);
- Ehrlichia – concurrent thrombocytopenia common +/- neutropenia or leukocytosis; and
- FIV, FeLV – concurrent cytopenias possible.

APLASTIC ANAEMIA
More accurately described as aplastic pancytopenia,

since usually associated with pancytopenia (sometimes bicytopenia), 'aplastic anaemia' can be idiopathic or caused by many of the toxins/infections listed above. Neutropenia commonly occurs first followed by thrombocytopenia +/- non-regenerative anaemia (reflecting the relative lifespans of neutrophils, platelets and RBCs).

Bone-marrow analysis reveals hypoplasia with 95% fat. reatment is supportive ,eg. transfusion therapy, antibiotics (+ manage any underlying disease/remove toxin exposure). Idiopathic disease seems poorly responsive to immunosuppression and the prognosis for this is guarded although spontaneous recovery has been reported.[3]

MYELOPHTHISIS
This term encompasses any process involving effacement of haematopoietic tissue in the bone marrow by abnormal tissue, most commonly fibrosis or neoplastic cells.
The pathogenesis is probably more complex than just 'crowding out' of normal cells (eg. there may also be competition for nutrients or inhibition by cytokines). Myelofibrosis can be primary (idiopathic) or occur secondary to neoplasia, chronic bone marrow inflammation (myelonecrosis), chronic drug toxicity (eg. phenobarbitone) or chronic erythropoietic stimulation (eg. chronic haemolysis: pyruvate kinase deficiency (PK) deficiency).

Non-regenerative anaemia is often the only cytopenia present.

Idiopathic myelofibrosis is sometimes responsive to immunosuppressive therapy, so this is worth trying if secondary causes have been ruled out as far as possible (using drug doses/protocols similar to those for immune-mediated haemolytic anaemia [IMHA]).[4] Bone-marrow aspirates are usually frustratingly acellular, so core biopsies are needed to confirm collagen effacement.

Neoplasms commonly associated with myelophthisis include: multiple myeloma, leukaemia, lymphoma, mast cell tumour (MCT) and histiocytic disease. Neoplastic cells will not necessarily be present in peripheral blood (even with some leukaemias).

OSTEOSCLEROSIS
This uncommon condition is characterised by excessive trabecular bone causing a reduction in the haematopoietic space (a thickened cortex can be seen on radiographs). It is seen with end-stage PK deficiency in dogs and FeLV infected cats. There is often concurrent myelofibrosis.

OSTEOPETROSIS
A rare inherited condition in puppies involving increased bone density, with similar consequences to osteosclerosis (non-regenerative anaemia or pancytopenia).

MYELODYSPLASTIC SYNDROME
This group of diseases is characterised by proliferation of abnormal (but non-neoplastic) stem cells in the marrow. Myelodysplastic syndrome (MDS) can be primary: caused by acquired mutations in stem cells (therefore onset is often in middle-old age); or secondary: underlying causes in animals

are not well established (apart from FeLV in cats), but in humans include drugs/toxins and radiation therapy. One or more cytopenias may be present and clinical signs are usually insidious.

Bone-marrow analysis reveals clonal dysplastic changes in one or more cell lines, with increased numbers of precursor cells (eg. rubriblasts, myeloblasts). Clonal proliferation remains <20% of overall marrow cellularity, helping differentiate MDS from leukaemia (although MDS can sometimes transform into acute myeloid leukaemia). Treatment options include: supportive care (eg. antibiotics, blood transfusion, EPO) or specific drug therapy (various protocols have been tried and specialist advice is recommended). The prognosis is variable (weeks to years) and dependent on the 'subtype' of MDS.[5]

DYSMYELOPOIESIS

Primary (congenital) dysmyelopoiesis occurs in dogs but only causes benign morphological changes without impacting on cell function, eg. macroplatelets in Cavalier King Charles Spaniels; macrocytosis in Poodles.

Secondary dysmyelopoiesis however, can cause clinically significant cytopenias. It is characterised by non-clonal dysplasia (usually lower numbers of dysplastic cells than seen with MDS and without the increase in precursors, but distinguishing between the two can be challenging). Triggers include: drugs (eg. phenobarbitone), infection (leishmania), neoplasia, immune-mediated disease, iron deficiency and toxins (heavy metals). When secondary to drugs, it occurs during treatment (unlike 2' MDS, which usually occurs years later). Successful treatment relies on controlling underlying disease where possible (complete recovery can occur).

VITAMIN B12 DEFICIENCY

Anaemia is more common with congenital B12 deficiency (eg. Border Collies, Giant Schnauzers) than acquired deficiency (e.g. secondary to gastrointestinal disease, exocrine pancreatic insufficiency).

Since B12 is required for normal DNA synthesis, deficiency impairs erythropoiesis resulting in normocytic non-regenerative anaemia (+/- other cytopenias, eg. mild neutropenia), often with myelodysplastic features.

Clinical signs can also include gastrointestinal signs and encephalopathy (hyperammonemia).

Once confirmed (by demonstrating low serum levels), treatment is straightforward: parenteral B12 supplementation (this will need to be lifelong with congenital deficiency) +/- management of underlying disease with acquired deficiency.

NON-REGENERATIVE IMHA (NR-IMHA)

Although IMHA is more commonly a process involving destruction of peripheral RBCs, immune-mediated disease sometimes primarily targets RBC precursors in the bone marrow, causing non-regenerative anaemia. As with other non-regenerative anaemias, clinical signs can be mild for the degree of anaemia since this develops more gradually than in typical IMHA and doesn't involve the same acute inflammatory response.

NR-IMHA can be primary or secondary (eg. to infection, drugs etc.) and there may even be evidence of concurrent peripheral RBC destruction (spherocytes, auto-agglutination). Bone marrow sampling typically reveals maturation arrest – ie. a sudden 'halt' in RBC precursors which can occur at any stage of erythroid development, causing either overall erythroid hyper- or hypoplasia (depending upon the stage of erythropoiesis affected and any compensatory hyperplasia of preceding precursors). This is expressed as an altered M:E ratio - the ratio of myeloid to erythroid cells (normally between 0.5-2:1 in healthy dogs). M:E ratios can be increased (erythroid hypoplasia) or decreased (erythroid hyperplasia) in NR-IMHA. The ratio always needs to be interpreted in the context of overall marrow cellularity and peripheral haematology (eg. a decreased M:E ratio would also exist in regenerative IMHA, but in association with peripheral reticulocytosis).

Occasionally, disease targets the earliest stem cells, resulting in 'pure red cell aplasia' (defined as <5% erythroid precursors present in the marrow). NR-IMHA may be associated with secondary myelofibrosis, but there should not be any significant myelodysplasia and iron stores should be adequate (often increased). Treatment is very similar to that for IMHA (so will be discussed in part 2), however response rate is typically much slower (mean time till remission is four months and can be much longer).

Therefore, although the prognosis is generally considered better for NR-IMHA (28% mortality reported)[6] than IMHA, there can be a higher risk of side effects/opportunistic infections since patients are on high immunosuppressive doses for longer. Multiple blood transfusions may be necessary while awaiting a response, causing greater expense, risk of transfusion complications and challenges finding a compatible donor.

REFERENCES ON REQUEST

READER QUESTIONS AND ANSWERS

TRUE OR FALSE?
1. **OVER-FILLING A MICROHAEMATOCRIT TUBE CAN FALSELY LOWER THE PCV READING.**
2. **IRON DEFICIENCY ANAEMIA IS ALWAYS NON-REGENERATIVE.**
3. **ANAEMIA OF CHRONIC/INFLAMMATORY DISEASE IS ASSOCIATED WITH LOW SERUM FERRITIN LEVELS.**
4. **APLASTIC ANAEMIA INVOLVES AT LEAST TWO CYTOPENIAS.**
5. **MYELOFIBROSIS IS EASILY DIAGNOSED ON BONE MARROW ASPIRATES.**

ANSWERS: 1: TRUE, 2: FALSE, 3: FALSE, 4: TRUE, 5: FALSE