Anaemia

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Define the problem
Anaemia is a relatively common disorder with a multitude of causes. Animals with anaemia often present with pale mucous membranes - the first step is therefore to differentiate those animals with pale mucous membranes due to poor peripheral perfusion (i.e. hypovolaemic, cardiogenic or obstructive shock) and those who have a reduced red blood cell mass.

Is the anaemia primary or secondary?
Once it has been confirmed that true anaemia is the cause of the animal’s pale mucous membranes, it is important to assess whether the anaemia is the primary disease process or secondary i.e. is it causing the animal’s presenting clinical signs or is it secondary to an underlying disorder.

Often the clinical significance of mild to moderate anaemia is overestimated - mild to moderate anaemia is a common feature of many chronic disorders (more details later in this module) and anaemia of chronic disease is the most common cause of mild anaemia. Classification of the severity of anaemia is arbitrary but can be as follows in dogs for example: mild anaemia (packed cell volume (PCV) 30–36%), moderate anaemia (PCV 18–29%) and severe anaemia (PCV < 18%).

Clinical signs relate to rapidity of onset
The rapidity with which anaemia develops also will influence its clinical effect - for example a dog with a PCV of 20% may show only mild clinical signs if the anaemia is chronic, but will be more severely affected if the PCV has decreased to this level suddenly.

Is the anaemia regenerative or non-regenerative?
It is imperative to ascertain whether the anaemia is regenerative or non-regenerative.

The work-up and aetiologies for regenerative and non-regenerative anaemia are very different and inappropriate tests will be ordered and time wasted if the type of anaemia is not established as soon as possible.

How long does the bone marrow take to respond?
Be aware that it takes two to four days for the bone marrow to respond to acute anaemia therefore signs of complete regeneration may not be present if peracute red blood cell (RBC) loss (from haemorrhage or haemolysis) has occurred. However, usually within 24 hours there will be some evidence of a response even if it is not complete.

Assessment of anaemia
Reticulocyte counts
Signs of regeneration that can be seen on blood smear include polychromasia (variable colour of red blood cells), anisocytosis (variable sizes of RBCs), macrocytosis (increased numbers of large RBCs), and reticulocytosis (increased numbers of immature RBCs). Polychromatophils are the larger, ‘bluer’ immature red cells seen on blood films stained with romanowsky stains (e.g. Diff-Quik®) and they correspond to reticulocytes when the film is stained with a stain such as new methylene blue. The number of reticulocytes present is the most accurate means of quantifying the degree of regeneration present. One method of reporting the reticulocyte count is for it to be corrected for the PCV - once it is corrected (see formula below) a value greater than 1.0% indicates a regenerative anaemia in a dog; a value less than 1.0% indicates a non-regenerative or poorly regenerative anaemia.

\[
\text{Reticulocyte (\%) x patient PCV (\%)}
\]

Species PCV

Thus a dog with a PCV of 15% (0.15 L/L) and an uncorrected reticulocyte count of 3% has a corrected reticulocyte count of 1.125% indicating a mildly regenerative anaemia. Regeneration should always be interpreted in view of the degree of anaemia. As 15% is a severe anaemia, unless the drop in PCV has happened very recently (i.e. pre-regenerative state), you would expect regeneration to be more pronounced than this.

\[
\frac{3 \times 15 (or 0.15)}{40 (or 0.40)} = 1.125
\]

In cats a corrected reticulocyte count of >0.4% is considered regenerative.

Check with your laboratory as to whether they report corrected or uncorrected reticulocyte counts.

If your laboratory reports reticulocyte numbers as an absolute count, then there is no need to correct the value for the PCV (greater than 60,000 - 80,000 is regarded as indicative of some degree of regeneration in dogs). In cats an absolute reticulocyte count of >40,000 is considered regenerative (cats show less peripheral evidence of regeneration than dogs).

Cat reticulocytes
Cat reticulocyte counts are more problematical than dog reticulocyte counts and again it is important to know what your laboratory is reporting. Cat reticulocytes may be classed as punctate or aggregate. The punctate forms are the more mature and indicate past regeneration - they should not be included in the reticulocyte count. The aggregate form is the ‘true’ indicator of recent regeneration. The cut-off values given above for cats correspond to aggregate reticulocyte numbers.
Nucleated RBCs
The relative number of nucleated RBC (NRBC, also known as normoblasts) in comparison to the reticulocyte count may also be helpful in assessing anaemia. NRBCs will appear in several circumstances. Normoblastaemia will normally occur associated with intense regenerative anaemia (both haemorrhagic and haemolytic, but more frequent in the latter).

If, however, the anaemia is non-regenerative (reticulocyte count <60,000-80,000 absolute or 1.0% corrected in a dog) but is accompanied by more than a few normoblasts (i.e. greater than five NRBC per 100 WBC) this is regarded as an inappropriate response because there are disproportionately more of the immature cells (NRBCs) present than the more mature cells (reticulocytes).

This can occur in following conditions:
1. Peracute anaemia.
2. Normal Miniature Schnauzers and Dachshunds (inconsistent finding).
3. Splenic disorders (with or without anaemia) especially haemangiosarcoma.
4. Lead toxicity (usually little or no anaemia).
5. Bone marrow disorders (disorders of production – myelophthisis, dyserythropoiesis, leucoerythroblastic response. There is variable anaemia).
6. Some inflammatory liver conditions (mechanism not clear, uncommon).

As usual, all clinical information should be used to interpret unexpected findings such as high numbers of NRBCs.

Again cats are different - the appearance of a few or, sometimes, numerous late normoblasts in the peripheral blood of sick cats, (with conditions other than lead toxicity) and without accompanying evidence of intensified erythrogenesis, is a phenomenon unique to the cat.

Having determined whether the anaemia is regenerative or non-regenerative, the differential diagnosis for each type may be considered.

Spherocytosis
While large numbers of spherocytes in a blood smear are virtually pathognomonic for immune mediated haemolytic anaemia, small numbers can occur in patients with a mononuclear phagocytic neoplasm, microangiopathic haemolysis or zinc toxicity. Hereditary spherocytosis has been rarely reported and occasionally haemolysis due to hypophosphataemia in diabetic patients can be associated with mild spherocytosis. The absence of spherocytes does not preclude a diagnosis of immune-mediated haemolytic anaemia (IMHA), particularly in cats as the small nature of cat RBCs makes the presence of spherocytes a difficult judgement call. Finally, if spherocytes are present in IMHA, it doesn't tell you if the immune mediated process is primary (i.e. idiopathic) or secondary to an underlying disease that is triggering the abnormal immune response (e.g. lymphoma).

Microcytosis
The most frequent causes of microcytosis (MCV less than normal or visibly small cells on blood smear examination) are iron deficiency anaemia or portosystemic shunts. Some dog breeds can also have small cells normally (Akita's and Shiba Inu).

Macrocytosis
If the MCV is greater than normal consider regeneration, breed effect (Poodles tend to have larger RBCs) and feline leukemia virus (FeLV) infection in cats.

Schistocytosis
The presence of schistocytes or fragmentocytes (fragmented RBCs) occurs in disorders where RBCs are damaged by shearing, turbulent blood flow or intrinsic RBC abnormalities.

Mechanisms & diseases that may be associated with schistocytosis

<table>
<thead>
<tr>
<th>Shearing by fibrin strands:</th>
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<tbody>
<tr>
<td>• Microangiopathic haemolytic anaemia.</td>
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<td>• Disseminated intravascular coagulation.</td>
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<tr>
<td>• Haemangiosarcoma.</td>
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<td>• Glomerulonephritis.</td>
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<td>• Myelofibrosis.</td>
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<td>• Haemolytic uremic syndrome.</td>
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<td>• Hypersplenism.</td>
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<th>Turbulent blood flow:</th>
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<td>• Congestive heart failure.</td>
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<td>• Valvular stenosis.</td>
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<td>• Caval syndrome in heart worm disease.</td>
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<tr>
<td>• Haemangiosarcoma.</td>
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<th>Intrinsic RBC abnormalities:</th>
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<td>• Chronic doxorubicin toxicosis.</td>
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<td>• Severe iron deficiency anaemia.</td>
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<td>• Pyruvate kinase deficiency.</td>
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<td>• Congenital &amp; acquired dyserythropoiesis.</td>
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Heinz bodies
Heinz bodies form whenever there is oxidative injury to erythrocytes. They are often found in small numbers in healthy cats but are almost always indicative of pathology in dogs.

Excessive onion and garlic ingestion can cause them to form as well as exposure to a variety of oxidants including paracetamol, excessive Vitamin K3, benzocaine-containing local anaesthetics, zinc and toilet bowl cleanser. They have also been observed in splenectomised animals, after corticosteroid therapy and in patients with severe splenic dysfunction.

Howell-Jolly bodies
The presence of Howell-Jolly bodies suggests regeneration or splenic dysfunction. Low numbers can be normal in cats.
Red blood cell morphology
(Pictures courtesy of Assoc. Prof. Paul Canfield, The University of Sydney)

Above: normal dog blood film.

Above: hypochromasia and microcytosis.

Above: polychromatic erythrocytes and a nucleated red blood cell.

Above: canine reticulocytes.

Above: acanthocytes and schistocytes.

Above: poikilocytosis.

Above: spherocytosis.

Above: Howell-Jolly body.
Regenerative anaemia
Haemorrhage or haemolysis?
Regenerative anaemia may be the result of haemorrhage or haemolysis.

Haemorrhage
If the haemorrhage is acute, there may initially be no change in the PCV as both plasma and RBCs have been lost. However, after a few hours the plasma will equilibrate and the reduced PCV will become apparent.

Acute haemorrhage is often associated with decreased plasma protein concentrations especially if the haemorrhage is external. If a patient has developed an acute anaemia and the plasma protein is high normal or above normal then haemolysis is more likely than external haemorrhage.

Chronic external haemorrhage (e.g. gastroduodenal ulceration, bleeding gastrointestinal tract (GIT) or skin tumour) will eventually cause iron deficiency although the anaemia often remains moderately responsive. Anaemia due to chronic gut haemorrhage is usually associated with mild to moderate hypoproteinaemia. Platelet count is often high normal or slightly elevated in chronic blood loss.

Internal haemorrhage
Internal haemorrhage into a body cavity may be difficult to detect if the volume is small.

With internal haemorrhage, the protein usually is less reduced than with external haemorrhage, because the protein is not ‘lost’ and plasma levels will increase more quickly after the bleed. Plasma levels will not usually however be above normal, however, unless the patient is also dehydrated.

Intra-thoracic haemorrhage will usually cause acute, specific signs of compromised respiratory function unless the volume of the bleed is small. However, intra-abdominal haemorrhage may be more difficult to identify clinically. It is worthy of note that intra-abdominal haemorrhage is rapidly resorbed back into the circulation (the peritoneum is a very large and efficient absorption surface) thus an acute splenic bleed of small volume, for example in a dog with haemangiosarcoma, may not be detectable by abdominocentesis within a few hours of the bleed.

Haemolysis
Haemolysis may occur extravascularly (RBCs are broken down in the reticuloendothelial system in the spleen predominantly, but also the liver and bone marrow) or intravascularly (RBCs are broken down within the bloodstream releasing haemoglobin) or both. Increased serum bilirubin is observed in the majority (but not all) cases of extravascular IMHA although overt clinical jaundice may be less common. The diagnosis of IMHA should therefore never be excluded on the basis of absence of overt jaundice.

Intravascular haemolysis can be easily demonstrated as the plasma of a centrifuged (and appropriately collected*) sample will be red if the intravascular haemolysis is moderate to severe. Haemoglobinuria is often also present – this can be differentiated from haematuria by centrifuging urine and observing the supernatant - it will be clear if haematuria is present, and red if haemoglobinuria is present.

*Haemolysis is commonly caused ex vivo by improper sampling methods such as strong suction during blood collection, small needle gauge or vigorous shaking of the blood tube.

Immune-mediated haemolytic anaemia
In dogs, the most common cause of intravascular or extravascular haemolysis is immune-mediated disease.

Primary vs. secondary
IMHA may be primary (idiopathic) or secondary e.g. to drug reaction, neoplasia (e.g. lymphoma) or some infections. Primary (idiopathic) disease is most common in dogs. In the cat, immune-mediated haemolytic anaemia was generally considered to be rare in the past, however it is now recognised relatively frequently in specialist centres. As with dogs, secondary causes of IMHA should be considered and ruled out during investigation. Although secondary IMHA is probably more common in cats compared to dogs, primary IMHA is being reported with increasing frequency in cats.

Immunoglobulin type
Immune-mediated destruction of RBC may be via IgG or IgM. As IgM is a larger molecule, it is most frequently associated with intravascular haemolysis. Evidence of autoagglutination of RBC on a slide is more commonly present with intravascular haemolysis, but may be seen with extravascular haemolysis too (partly because both mechanisms can co-exist). The absence of auto-agglutination does not rule out IMHA. To perform a slide agglutination test place one drop of EDTA blood and one to two drops of saline on a glass slide and gently mix. The appearance of tiny ‘clumps’ is compatible with autoagglutination.

Spherocytosis
The presence of spherocytosis in the blood film is common in immune-mediated haemolytic anaemia. However, small numbers of spherocytes may also been seen in other situations where RBC membranes are damaged – see paragraph earlier in the notes. They may be absent in some cases of immune-mediated haemolytic anaemia. Other RBC morphological changes that are observed in IMHA are polychromasia and other changes consistent with regeneration. Ghost red blood cells may be seen in dogs and cats with intravascular haemolysis (very faint red cell with thin rim of cytoplasm, not to be confused with hypochromatic cells seen in iron deficiency).

Splenomegaly
Animals with immune-mediated haemolytic anaemia often have splenomegaly due to increased phagocytic activity in the spleen. Other potential reasons for splenomegaly include the spleen as a site of extra-medullary haematopoeisis (i.e. a site of regeneration) or even neoplasia such as lymphoma.

WBC
Animals with immune-mediated haemolytic anaemia will also usually have a neutrophilia which can be extremely high and almost look leukaeic (thus called a leukemoid-erythroid response). This response is partly due to the
massive tissue (i.e. RBC) destruction that is occurring and also secondary to marked bone marrow stimulation which can be relatively non-specific.

**Non-regenerative haemolytic anaemia**

A small proportion of dogs and some cats are reported to have immune-mediated non-regenerative anaemia because of antibody directed against red blood cell precursors in marrow. Diagnosis of this disorder requires demonstration, by bone marrow biopsy, of apparent maturation arrest. This is often called pure red cell aplasia (PRCA) (although this term is not that helpful, many pathologists use it). Non-regenerative IMHA is relatively common in anaemic cats and used to be linked to FeLV. Now the majority of cats with PRCA are FeLV negative.

**Microangiopathic anaemia**

Microangiopathic anaemia is a variant of haemolysis where the RBC's are physically broken down because they are forced through tortuous passages.

Causes include:
- Caval syndrome.
- Splenic torsion.
- Haemangiosarcoma.

RBC morphological changes that are usually observed on blood films include schistocytosis (fragmentation) and evidence for regeneration. Spherocytosis may also be observed.

**Congenital haemolytic anaemia**

Haemolytic anaemia may also occur in certain breeds with congenital defects of red blood cell metabolism e.g. pyruvate kinase deficiency (Basenjis, West Highland White terriers) and phosphofructokinase deficiency (English Springer Spaniels - suffer acute, exercise-induced episodes of haemolysis). Usually affected dogs have clinical signs from a relatively young age.

**Infectious haemolytic anaemia**

Mycoplasmosis or haemoplasmosis (infection usually with *Mycoplasma haemofelis*) is a potential cause of haemolytic anaemia in the cat in the UK. It should be noted that if a cat has been reported to have FeLV. Now the majority of cats with PRCA are FeLV negative.

**Drugs and toxins**

Drugs such as sulphonamides, penicillin and methimazole have been associated with haemolytic anaemia, usually immune-mediated. Paracetamol will cause haemolysis in cats, and zinc has been reported to cause severe haemolysis in both cats and dogs. History (drugs) and plain radiography (zinc foreign body) can rule out these potential causes.

**Non-regenerative anaemia**

There are numerous causes of non-regenerative anaemia, the most common being:

**Anaemia of chronic disease**

Anaemia of chronic disease is usually clinically innocuous and does not require treatment. However, it is an indication that there is underlying disease present and warrants a vigorous search for its cause. Endocrine (e.g. hypothyroidism), immune-mediated and neoplastic diseases may be underlying. 'Anaemia of chronic disease' is usually mild with a PCV of 25-36% in dogs and 18-26% in cats. It should resolve if the underlying systemic disease is being treated successfully.

Other causes of non-regenerative anaemia include:

**Chronic kidney disease (CKD)**

This is more likely with advanced stages of CKD (IRIS stages three and four). Simple to rule out by checking urea, creatinine and urine specific gravity.

**Bone marrow disorders**

- Clues to bone marrow disease are severe, non-regenerative anaemia often with a chronic history but possibly recent deterioration once the compensatory mechanisms aren't adequate to maintain sufficient tissue oxygenation.
- Red cells are often normocytic and normochromic.
- The haemogram often contains major clues such as other cytopaenias (e.g. thrombocytopenia, neutropenia). Lymphopenia is relatively common and usually seen as part of a stress leukogram.
- If the animal has leukaemia or myelodysplasia abnormal circulating cells may be seen on blood smear.

**Bone marrow disorders include:**

- Infection: parvovirus, panleukopaenia, ehrlichiosis.
- Toxic damage: oestrogen, chemotherapy.
- Immune-mediated damage (pure red cell aplasia).
- Neoplasia: lymphoma, multiple myeloma, mast cell disease, leukaemias. Neoplastic cells compete for space, nutrients and release inhibitory factors. This is known as myelophthisis.
- Myelodysplasia: Abnormal maturation or production of cells in the bone marrow.
- Myelofibrosis: normal haemopoetic tissue is replaced by fibrous tissue. The cause of this is not always known.

**Iron deficiency**

Iron deficiency is a relatively uncommon cause of anaemia in small animals but it can be overlooked as it doesn't tend to fit into the neat classification of regenerative or non-regenerative anaemia. It can be moderately regenerative to non-regenerative in nature. Fortunately iron deficiency
anaemia is fairly characteristic usually resulting in:
- Microcytosis (low MCV).
- Hypochromasia (low MCHC).
- Poikilocytosis.
- Thrombocytosis if due to haemorrhage.

Iron deficiency in cats and early iron deficiency may be normocytic and normochromic. The most common cause of iron deficiency in adult small animals is chronic gastrointestinal blood loss. In young cats and dogs it may be caused by severe flea infestation.

**Interpretation of the leukogram in non-leukaemic disorders**

Interpretation of differential leukocyte counts should always be based on the absolute number of cells not the relative percentage distribution. Each leukocyte class behaves independently of the other classes in relation to production, function, distribution and response in disease. Thus percentage values are irrelevant – it is the absolute number that reflects what the particular type of WBC is doing.

Neoplasia and immune-mediated disease may cause leukocytosis, but usually only infection or severe tissue necrosis will result in changes such as significant numbers of toxic neutrophils and substantial increases in band neutrophils.

A normal white blood cell count may occur with any type of pathology but is less likely with significant bacterial infection. However, it can never be assumed that a normal WBC count completely rules out the possibility of a bacterial infection. In acute, severe infection (such as pneumonia, septic peritonitis) the increased demand for white blood cells may initially result in normal or even decreased circulating numbers. This will change if serial testing is performed over a 24-48 hour period and the animal will usually appear systemically unwell.

If an animal is persistently pyrexic, has no other localising signs and has a normal white blood cell count with normal appearing neutrophils (i.e. no toxic changes), it is more likely to have immune-mediated disease or neoplasia than infection. However, ‘walled-off’ infections such as hepatic, pancreatic or retroperitoneal abscesses are possible.

**Neutrophilia**

Neutrophilia should always be assessed in light of changes in other WBC, to determine whether the neutrophilia is stress-related (associated with lymphopenia, eosinopenia and monocytosis) or inflammation. Stress-related neutrophilia is usually mild but the count can be as high as twice normal (18 x 10⁹/L).

**Neutrophilic left shifts**

In a regenerative left shift there is a neutrophilic leukocytosis accompanied by immature neutrophils, but segmented neutrophils predominate.

**What constitutes a left shift?**

If the number of band neutrophils is above 1.0 x10⁹/l, this is a confirmed left shift. If the number is below 1.0 x10⁹/l but above the reference range (usually stated as 0.5 x 10⁹/l), then one has to compare this with the actual number of segmented cells – if the ratio of bands: segmented cells is 1:16 or less for dog and 1:12 or less for cat (divide the total number of segmented cells by the number of band cells), then a left shift is present.

In degenerative left shifts, the number of immature neutrophils exceeds the number of segmented neutrophils. This indicates an inadequate marrow response and is associated with severe inflammatory and infectious disease.

**Toxic neutrophils**

Toxic neutrophils contain degenerative changes such as Doehle bodies, cytoplasmic basophilia, foamy vacuolated cytoplasm and toxic granules. Toxic neutrophils are assumed to be functionally compromised and usually indicate severe inflammation or infection. Toxic neutrophils are most obvious in severe bacterial infections, septicaemia/sepsis, endotoxaemia, acute inflammation and extensive burns.

The severity of toxic changes is a subjective assessment by the clinical pathologist and a report of only 1+ toxicity is not necessarily evidence for infection or severe inflammation.

**Hypersegmented neutrophils**

Hypersegmented neutrophils have five or more distinct lobes and most commonly reflect prolonged blood transit time. They are usually seen in the late phases of inflammation, during corticosteroid therapy and in patients with hyperadrenocorticism. Hypersegmentation also occurs as an in vitro degenerative change and can occur in EDTA specimens that are more than several hours old or have been exposed to high ambient temperatures prior to film preparation.

**Neutropenia**

Neutropenic animals are often pyrexic although a focus of infection may not be evident. Neutropenia may be the cause of the pyrexia or a consequence of the underlying pathology that is causing pyrexia.

Causes of neutropenia include:
- Bone marrow dysfunction.
  - Drugs.
  - Neoplasia.
  - Maturation defects.
- Viral infection parvovirus feline immunodeficiency virus (FIV), FeLV.
- Serious bacterial infection (neutrophils will show toxic changes and there will be a degenerative left shift).
  - Septicaemia.
  - Pyometra.
  - Prostatic abscess.
  - Pyothorax.
  - Peritonitis.
- Immune-mediated neutropenia.

**Lymphocytes**

**Reactive lymphocytes**

Reactive lymphocytes are observed in low numbers in healthy animals and should only be reported by the pathologist if
they comprise greater than 5% of the lymphocyte population. They are non-specific indicators of antigenic stimulation and are observed in inflammatory and infectious disease and after vaccination.

**Lymphopenia**
Lymphopenia may suggest viral infection or stress. This is a relatively common finding on canine haematology results.

**Lymphocytosis**
Lymphocytosis can occur in excitement (especially in cats), when there is an immune component to the inflammatory response and in hypoadrenocorticism. Lymphocytosis is not that common in sick dogs.

**Monocytosis**
Monocytosis may occur with chronic, granulomatous infection (e.g. bacterial, fungal), with stress or where there is a strong immune component to the inflammatory response especially where the macrophages are involved in phagocytosis of antigen-antibody complexes.

**Eosinophils**
Eosinophilia most commonly occurs with allergy, parasitic disease, inflammatory bowel disease (of any type as bowel inflammation increases the ability of antigens to gain access to the systemic circulation), eosinophilic syndromes (e.g. eosinophilic granulomas or plaques in cats, eosinophilic bowel disease), hypoadrenocorticism and neoplasia.