

## INVESTIGATING ANAEMIA

The 3 classes of anaemia are:

- Blood loss
- Haemolysis
- Non-regenerative (failure of erythropoiesis)

Aim firstly to classify the anaemia into one of these 3 groups and then consider the differential diagnoses within each group:

Haemorrhage	Haemolysis	Bone marrow suppression
EXTERNAL	INFECTIOUS	Dietary iron deficiency
Guttural pouch mycosis	Piroplasmosis	Chronic haemorrhage
Ethmoid haematoma	Equine Infectious Anaemia	Chronic sepsis
Urinary (renal, bladder, urethral)	IMMUNE MEDIATED	Chronic hepatic disease
Coagulopathy (e.g. toxins or liver failure)	Primary (idiopathic)	Chronic renal disease
Severe ectoparasitism	Neoplasia (especially lymphoma)	Peripheral neoplasia
INTERNAL	Infectious ( <i>Piroplasmosis, EIA, Clostridial, Streptococcal</i> )	Myeloid neoplasia
Haemothorax	Penicillin administration	Myelofibrosis
Splenic rupture/neoplasia	Neonatal Isoerythrolysis	Immune mediated disease
Mesenteric vessel rupture	OXIDATIVE INJURY	Phenylbutazone toxicosis
Uterine artery rupture	Phenothiazine administration	Chloramphenicol toxicosis
Gastric/colonic ulceration	Onion or Red Maple toxicosis	Exogenous erythropoietin administration
Verminous arteritis ( <i>S. vulgaris</i> )	Thermal burns	Viral infections (Enteroviruses?)

### IMPORTANT CLINICAL SIGNS TO NOTE AND MONITOR

Behaviour - Depression/lethargy/poor performance – offers an indication of severity and acuity.

Mucus membrane colour - Peracute anaemias from haemorrhage or haemolysis usually show pallor. Pallor might also occur with chronic non-regenerative anaemias although this is variable. Subacute to chronic haemolytic anaemias are more likely to show jaundice.

Pyrexia - Common in cases of immune mediated (e.g. idiopathic IMHA, neonatal isoerythrolysis, paraneoplastic) or infectious causes (piroplasmosis, EIA).

Tachycardia/tachypnoea - as a compensatory response to poor oxygen carrying capacity.

Pigmenturia - Discoloured urine may be a result of intravascular haemolysis and clearance of free haemoglobin (haemoglobinuria), blood loss into urine (e.g. renal haemorrhage) or bilirubin excretion as urobilinogen.

## INVESTIGATING ANAEMIA

### ASSESSMENT OF HAEMATOLOGY

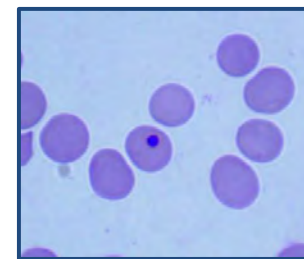
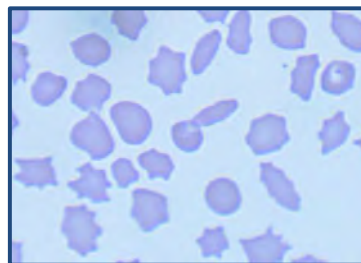
Erythrocyte size (MCV) and morphology may give an indication of the cause of anaemia. However, bear in mind that foals normally have small erythrocytes and donkeys very large erythrocytes. If reference ranges for adult animals are used, foals may appear to have a mild microcytosis.

- *low MCV* (microcytic anaemia) may accompany iron deficiency or chronic disease.
- *high MCV* (macrocytic anaemia) typically accompanies haemolytic causes of anaemia or haemorrhage and is an indicator of regeneration.
- *high MCH or MCHC* tends to suggest intravascular haemolysis (and free haemoglobin)

Erythrocyte size is a reasonable but not entirely reliable indicator of regeneration and, as reticulocytes are not released into the peripheral circulation in horses, assessment of bone marrow is a better means of classifying anaemia sub-types (see section on bone marrow collection).

Blood smears should also be examined for:

- Schistocytes - haemolysis, DIC, neoplasia
- Heinz bodies or eccentrocytes - oxidant toxicity
- Howell Jolly bodies - regeneration
- Parasitaemia - *Babesia caballi*, *Theileria equi*
- Haemosiderophages - immune-mediated haemolysis?
- Spherocytes - immune-mediated haemolysis



### SERUM BIOCHEMISTRY

**Total serum proteins** - Blood loss may be associated with hypoproteinaemia (i.e. total protein <50g/L). Immune-mediated haemolysis is often associated with increases in globulins. Chronic inflammation, which is a frequent cause of non-regenerative anaemia, may be associated with mild hypoalbuminaemia and hyperglobulinaemia.

**Acute phase proteins** - Plasma fibrinogen and serum amyloid A (SAA) may be increased as a result of: inflammatory/neoplastic haemorrhagic lesions, immune-mediated haemolysis, infectious disease (e.g. piroplasmosis); inflammatory/neoplastic lesions causing bone marrow suppression.

**Bilirubin** - Haemolysis is typically associated with high total and indirect (unconjugated) bilirubin and normal direct (conjugated) bilirubin. Values are generally proportionate to the acuity of haemolysis and may be >200µmol/L in some cases. However, haemolysis is not the only cause of increased bilirubin. Anorexia in horses is associated with mild to moderate increases in total and indirect bilirubin (and normal direct bilirubin) that may occasionally be as high as 100µmol/L. Hepatic insufficiency is a further cause of increased total and indirect bilirubin as well as increased direct bilirubin. If direct bilirubin accounts for >25% of total bilirubin then hepatobiliary disease is likely.

## INVESTIGATING ANAEMIA

### INVESTIGATING HAEMORRHAGIC ANAEMIAS

There may be little initial change in haematological and serum biochemical markers following whole blood loss, as blood cells and plasma are lost in equal proportions and the spleen provides a reserve supply of cells. By 24 hours, a decrease in PCV and RBC may be observed and there is usually an accompanying hypoproteinaemia. Neutrophilia may be seen in some horses as a stress response. In horses with acute or sub-acute haemorrhage clinical signs may develop when PCV drops to 15-20%. In horses with more chronic haemorrhage clinical signs may not be seen until PCV drops to 12% or less.

Although very rare, iron deficiency anaemia may occur with chronic haemorrhage over weeks to months. Remember low serum iron is also a common marker of inflammation and does not always reflect deficiency. Measurement of low serum iron concentration and high total iron binding capacity (TIBC) provides evidence of iron deficiency, whereas both iron and TIBC are low in inflammatory diseases.

Consider the following to search for a source of possible blood loss:

**Rectal examination** – NB. Any faecal samples submitted for occult blood (see below) should precede rectal examination.

**Gastroscopy** – bleeding gastric ulcers. Not a common cause of anaemia but is possible – especially in foals/youngsters.

**Endoscopy** – for direct signs of bleeding and/or collection of tracheal wash/bronchoalveolar lavage to look for presence of haemosiderophages (normal in exercising horses, abnormal in sedentary horses).

**Ultrasonography** – haemothorax or haemoperitoneum may be evident as a cloudy, swirling effusion. Abnormal masses may also be evident.

**Abdominocentesis** – for the presence of increased red cell content, phagocytosed red cells or haemosiderophages.

**Urinalysis** – dipsticks will show “positive blood” if haemoglobinuria, haematuria or myoglobinuria is present. Centrifugation and examination of sediment should enable differentiation of haemoglobinuria from haematuria if the sample is fresh. Increased urobilinogen detected by standard urine dipsticks supports a diagnosis of haemolysis.

Faecal occult blood testing is neither sensitive nor specific in horses. Experimental studies have suggested that several hundred millilitres of blood given intragastrically is required to produce a positive faecal occult blood test. Furthermore, false positive results are possible after routine rectal examination.

Following haemorrhage, PCV is expected to increase at around 0.7% per day and regeneration should be complete within a month. However, other factors that affect PCV (hydration status, excitement etc.) may confound assessment based on PCV alone.

## INVESTIGATING ANAEMIA

### INVESTIGATING HAEMOLYTIC ANAEMIAS

Most haemolytic anaemias in UK will be immune mediated or infectious.

Intravascular haemolysis is more acute and characterised by free haemoglobinaemia and haemoglobinuria

Extravascular haemolysis is considerably more common and the only indication that it is occurring may be slowly progressive anaemia and the presence of spherocytes. Haemoglobinuria is not present.

Immune-mediated haemolytic anaemia (IMHA) is usually insidious in onset and clinical signs may include pyrexia, lethargy and weight loss. Haematological analysis may reveal reduced RBC count, increased spherocytes, macrocytosis, anisocytosis and biochemical analysis may reveal increased bilirubin concentration. Identification of antibodies on erythrocytes with a Coombs test provides further evidence of IMHA.

Primary idiopathic immune mediated anaemia in the absence of recognised causes does occur although investigations should be performed into potential underlying causes. Many infectious diseases, drugs and neoplastic diseases have the potential to trigger immune mediated haemolysis

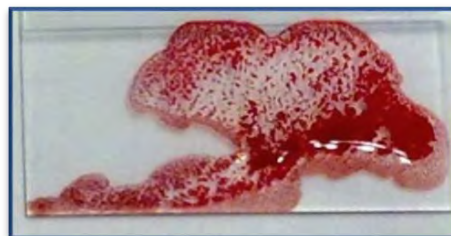
Equine infectious anaemia (NOTIFIABLE!) should be considered as a potential cause of haemolytic anaemia in imported horses or horses which have received imported blood products. Clinical signs may be vague and can include recurrent pyrexia, weight loss and oedema in addition to anaemia. Thrombocytopaenia is the most profound haematological abnormality. The diagnosis may be confirmed by the traditional Coggins test, by ELISA or by PCR.

Piroplasmosis is another potential cause of haemolytic anaemia in imported horses (e.g. France, Spain, Scandanavia, Americas) and is caused by infection with *Babesia caballi* or *Theileria equi*. The potential for tick-borne spread within the UK exists.

The Direct Coombs test is used to examine for antibody (IgG and/or IgM) or complement attached to erythrocyte membranes and may be positive in cases of immune-mediated anaemia. False negative results are very common.

#### Testing for autoagglutination

Autoagglutination and increased fragility of erythrocytes in saline may also provide evidence of IMHA and is worthwhile given the limitations of the Coombs test. The test is performed warm and cold. A drop of whole EDTA blood is added to a drop of normal saline on a slide and gently rocked and observed for grossly visible agglutination which often happens immediately or within 60 seconds (see image).



## INVESTIGATING ANAEMIA

### INVESTIGATING NON-REGENERATIVE ANAEMIAS

Sternal bone marrow aspirate and biopsy (see below) is a relatively straightforward procedure in horses and aids significantly in the investigation of anaemias (as well as cases showing other persistent abnormalities of leucocytes or platelets).

In normal horses there are similar numbers of cells from myeloid (WBC) and erythroid (RBC) series. The reference range of the ratio of myeloid to erythroid cells is typically 0.5 to 1.5. Higher numbers of erythroid series (low M:E ratio <0.5) infers a regenerative condition whereas lower erythroid series (high M:E ratio >1.5) infers non-regeneration.

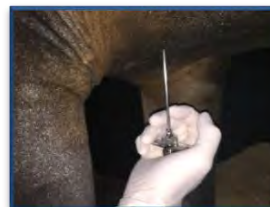
Failure of bone marrow may be caused by myelophthitic disease (destruction of normal bone marrow structure) or aplastic disease (functional failure of stem cells). Myelophthisis is most commonly caused by neoplastic disease (especially lymphoma) but may also be caused by proliferation of fibrous tissue within the bone marrow. Aplastic anaemia has been reported secondary to bacterial or viral infection, chronic renal or hepatic disease, neoplasia, irradiation, drug therapy or autoimmune disease.

Whilst it is helpful to identify aplastic anaemia it is frequently impossible to determine the primary cause of the aplasia although a presumptive diagnosis may be made from the horse's history.

Aplasia associated with drug administration may be temporary or permanent. Phenylbutazone and chloramphenicol have been reported as causes of aplasia.

#### Performing a bone marrow aspirate and biopsy

- Collected from the sternum on the midline at the level of the points of the elbows in a horse standing square (see image).
- Following sedation and sterile preparation a 4 inch, 18-21 gauge spinal needle can be slowly "drilled" into the sternum and then the stylet removed and a 2 to 5 ml syringe attached containing a bead of EDTA solution in the hub.
- A *brief and gentle* vacuum is then applied to the syringe in an attempt to obtain a *single drop* of bone marrow in the hub.
- Air-dried smears are then prepared and submitted for evaluation.
- If a free flowing sample is obtained this is likely to be heavily blood contaminated and unsuitable and if this is the case then a site slightly caudal or cranial to the original site is chosen and the procedure repeated. If no sample is obtained despite several attempts then the needle may be sitting in an intersternbral space rather than in a sternebra itself and the needle should be positioned a few centimetres in front or behind the previous site.
- Ultrasound can be utilised to determine the position of the sternebrae but is not necessary
- Bone marrow biopsy is performed at the same site with an 8 gauge Jamshidi needle which collects a small core of biopsy for a better evaluation of cell numbers within bone marrow. Biopsy provides considerably more information than aspirate alone.



## ASSESSING CHANGES IN THE LEUCOGRAM

Interpretation of white blood cell data can be subjective rather than based on objective evidence. When samples are submitted they are initially interpreted with an automated machine and then results are confirmed via microscopy by our haematologist.

It is more meaningful to consider the absolute numbers of the different types of leucocytes rather than their relative percentages. It is only when the total individual leucocyte numbers are normal that the relative differential cell counts might be helpful. For example, the two cases below have the same differential percentages but case 1 shows a neutropaenia whereas case 2 shows a lymphocytosis and eosinophilia. It is also important to interpret the results in light of other inflammatory markers such as Serum Amyloid A and Fibrinogen.

	Case 1	Case 2
<b>WBC count</b>	6.1 x 10 <sup>9</sup> /L	9.8 x 10 <sup>9</sup> /L
<b>Neutrophils</b>	<b><u>2.3 x 10<sup>9</sup>/L (38%)</u></b>	3.7 x 10 <sup>9</sup> /L (38%)
<b>Lymphocytes</b>	3.3 x 10 <sup>9</sup> /L (54%)	<b><u>5.3 x 10<sup>9</sup>/L (54%)</u></b>
<b>Eosinophils</b>	0.5 x 10 <sup>9</sup> /L (8%)	<b><u>0.8 x 10<sup>9</sup>/L (8%)</u></b>

The relative concentrations of cells within the circulating and peripheral pools will be influenced by factors such as stress and excitement, therefore the conditions of sampling must be considered when interpreting results. Samples should ideally be collected at least six hours after exercise or stressful incidents and not immediately post feeding. If this is not possible, the results should be interpreted cautiously.

The typical glucocorticoid-mediated response that accompanies the stress of exercise and competition (as well as exogenous corticosteroid administration) results in neutrophilia, lymphopaenia and eosinopaenia and an overall increase in leucocyte numbers. However, this effect may be balanced during high-intensity exercise or short-term excitement (such as during venepuncture) by increased blood volume and lymphocyte release secondary to splenic contraction.

### NEUTROPHILIA

- Inflammatory Diseases
- Endogenous (stress, or exogenous glucocorticoids)
- Catecholamines (acute excitement/fear)

Neutrophilia is usually the result of an acute or chronic inflammatory response. This may be caused by infectious (viral, bacterial or parasitic) or non-infectious disease (e.g. myopathies, surgical trauma, immune-mediated disease, neoplasia). Given that stress is a cause of neutrophilia, cross-checking against acute phase proteins (Serum Amyloid A and Fibrinogen) and globulins may help determine the “inflammatory versus stress” question.

A left shift indicates the release of juvenile neutrophils (Band neutrophils or in extreme cases metamyelocytes). This indicates an acute infectious/inflammatory condition which can include cellulitis/lymphangitis, colitis, endotoxaemia peritonitis and pleuritis.

## ASSESSING CHANGES IN THE LEUCOGRAM

### NEUTROPAENIA

- Excessive demand/sequestration (e.g. septicaemia, bacterial peritonitis, pleuritis, colitis)
- Endotoxaemia
- Viral infection (mid-late phase response)
- Myelophthisis/bone marrow suppression

Neutropaenia is often seen in horses showing signs of relatively mild lethargy and suboptimal performance and is frequently attributed to viral challenge. In horses showing more marked signs of illness such as tachycardia and pyrexia then severe bacterial sepsis, endotoxaemia, loss of neutrophils into an effusion (e.g. peritonitis, pleuritis) or into inflamed bowel (e.g. colitis) are further possible common causes of neutropaenia.

Neutropaenia is an occasional consequence of bone marrow suppression. As the leucocyte with the shortest lifespan, the neutrophil population may be the first to be noticeably reduced by bone marrow failure before reductions in platelets and red cells are observed.

### LYMPHOCYTOSIS

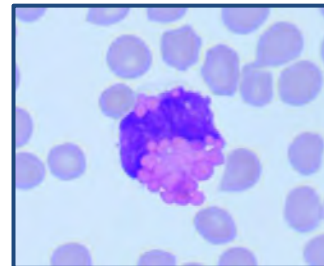
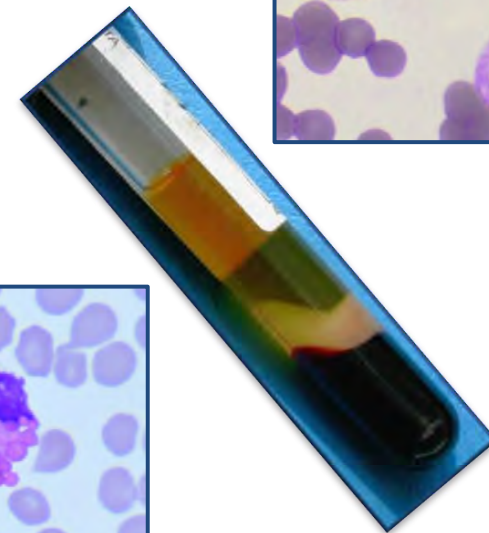
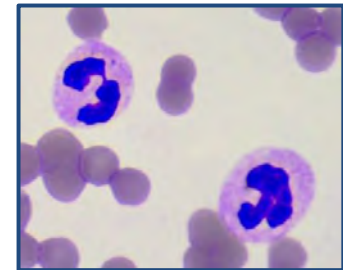
- Infectious diseases (primarily mid-late phase viral)
- Catecholamines (acute excitement/fear)
- Dramatic elevations can be due to a generalized lymphoma and in these cases the lymphocytes will be assessed for neoplastic changes (see extreme thickness of 'buffy coat' in image)

### LYMPHOPAENIA

- Infectious diseases (e.g. early EHV or severe bacterial infections)
- Endogenous (stress, or exogenous glucocorticoids)
- In foals a severe lymphopaenia can be indicative of genetic immunodeficiency syndromes.

### EOSINOPHILIA

- Hypersensitivity diseases (e.g. sweet itch, urticaria)
- Diseases of the intestine, skin and lung
- Inflammatory diseases (see neutrophilia)
- Parasitic larval migration (very rare!)



## ASSESSING CHANGES IN THE LEUCOGRAM

Eosinophils have many general roles in host defence and eosinophilia is often seen as a non-specific component of a systemic inflammatory reaction. Eosinophils are attracted by mast cell degranulation and have therefore been associated with antigen-antibody interactions in tissues rich in mast cells such as the skin, the respiratory tract and the intestine.

Peripheral eosinophilia is seen fairly commonly in association with hypersensitivity reactions such as sweet itch. Eosinophilia is very rarely found in association with intestinal parasitism in horses. Eosinophils undoubtedly play a role in host defence against parasitic infections but are found local to the parasite. Lungworm infection (also very rare) is usually associated with an eosinophilia in tracheal washes or bronchoalveolar lavage samples. Encysted cyathostomins are associated with eosinophilic infiltrates in caecal, colonic and sometimes rectal biopsies. The widely held association between parasitism and circulating eosinophilia stems from times when intra-arterial strongyles were prevalent.

### ***MONOCYTOSIS***

Monocytosis is a non-specific inflammatory indicator seen to rise in both acute and chronic inflammatory conditions and tissue damage. Granulomatous diseases and chronic bacterial infections can lead to monocytosis.

### ***BASOPHILIA***

Basophilia is very uncommon in the horse and when it does occur is attributed to non-specific hypersensitivity responses.

### ***THROMBOCYTOSIS***

Platelets are a useful inflammatory marker as they tend to increase in the presence of inflammation due to cytokine stimulation of the bone marrow. They do not act as an acute phase response but increase in most cases of chronic, persistent inflammation. High platelet counts will frequently be seen in association with abscessation and also with non-infective inflammatory conditions such as neoplasia.

### ***THROMBOCYTOPAENIA***

Thrombocytopaenia is often seen as an artefact following collection in EDTA. If this is the case then there will frequently be clumping of the platelets and this will be commented on. If the thrombocytopaenia is suspected to be real then it can be rechecked on a sodium citrate tube to ensure accuracy. It can be due to lack of production (bone marrow disease), consumption (DIC) or destruction (autoimmune).



## ASSESSING CHANGES IN THE LEUCOGRAM

### COMMON CAUSES OF INFLAMMATION

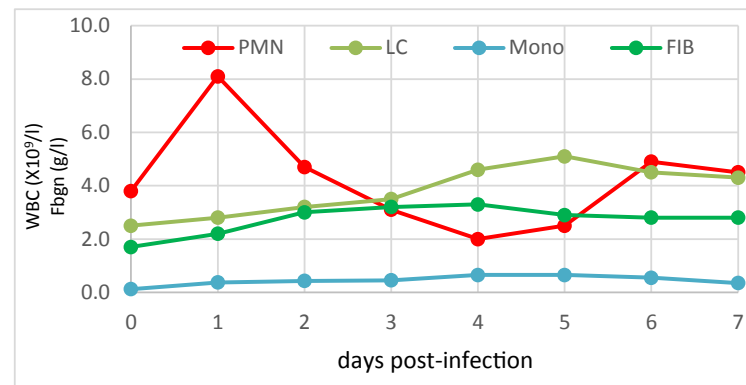
Infection	Bacteria
	Viruses
	Parasites
	Protozoa
Tissue Damage	Surgery
	Traumatic injury
	Exertional rhabdomyolysis/Atypical myopathy
Immune-mediated disease	Lymphangitis/vasculitis
	Immune-mediated haemolysis/thrombocytopenia
	Pemphigus foliaceus
Endotoxaemia	Secondary to colitis or similar
Neoplasia	
Other	Inflammatory bowel disease
	NSAID toxicosis
	Sand enteropathy

### IS IT A BACTERIAL OR VIRAL INFECTION?

The most consistent haematologic finding associated with the early stages of viral infections (i.e. when clinical signs are most marked and blood samples are most likely to be taken) is a neutrophilia. This has been demonstrated in association with many types of viral infection in adult horses and is indistinguishable from bacterial infections on the basis of haematology. However, later in the course of disease mild neutropaenia and possibly lymphocytosis and monocytosis would typify viral disease, whereas bacterial infections more typically remain neutrophilic and may develop a monocytosis. If, however bacterial infection is severe then neutropaenia may occur. Chronic bacterial conditions are frequently associated with a thrombocytosis by contrast to viral conditions. In addition, acute phase protein responses tend to be milder with viral diseases (e.g. SAA <50 mg/L) than with bacterial (e.g. >100 mg/L).

## ASSESSING CHANGES IN THE LEUCOGRAM

	Early	Mid-late
<b>Virus (typical):</b>	Neutrophilia	neutropaenia/lymphocytosis/monocytosis
<b>Bacterial (typical):</b>	Neutrophilia	neutrophilia/monocytosis
<b>Bacterial (severe):</b>	neutrophilia/ neutropaenia	neutrophilia/neutropaenia/ (thrombocytosis- chronic)



Response to EHV infection (Mason et al 1990)

## ACUTE PHASE PROTEINS

The acute inflammatory response results in a widespread and complex cascade of cytokine and lymphokine production (interleukins, interferons, eicosanoids etc). “Acute phase proteins” (APP) is the collective term for proteins which are synthesised and released from the liver in response to inflammatory cytokines. These proteins include fibrinogen, serum amyloid A, ceruloplasmin, C-reactive protein, haptoglobin, activin A and several others. A panel of these acute phase proteins are used in human clinical pathology, however in many veterinary laboratories fibrinogen is the only APP which is measured. The LEH Laboratory offers serum amyloid A which is frequently a more sensitive indicator of inflammation than fibrinogen alone and may be more useful in monitoring responses to infection in the first few days of disease.

### **FIBRINOGEN**

Fibrinogen can only be measured in plasma (EDTA or citrated). It is normally less than 4.0 g/L and may rise as high as 10-15 g/L in severe inflammatory cases. Therefore the “pathological range” is approximately 4 x the physiological range. Plasma fibrinogen concentrations may take 24-48 hours to start to increase above normal ranges following initiation of an acute inflammatory response and can take 7 days to peak.

### **SERUM AMYLOID A (SAA)**

SAA responds more rapidly than fibrinogen (within 24 hours) and may therefore be more helpful in assessing acute inflammatory disease. Furthermore, most normal horses have SAA concentrations close to 0 and with severe inflammatory disorders this can rise to approximately 1000 mg/L. Compared with fibrinogen this allows a ‘grading’ of severity of the *inflammatory process and more sensitive monitoring of progress*.

### **GLOBULINS**

Globulins are often seen to increase in inflammatory disease as most of the acute phase proteins are included in the globulin fraction. There is no evidence of any benefit of subclassifying globulins further using serum protein electrophoresis as this technique rarely provides further useful diagnostic or prognostic information unless a severe hyperglobulinaemia is seen which could be secondary to a neoplasm. In addition to being a non-specific indicator of chronic inflammation, hyperglobulinaemia is seen commonly with hepatopathy.

### **ALBUMIN**

Serum albumin is often referred to as a “negative acute phase protein” as albumin levels can fall slightly with inflammation (especially in chronic cases) as amino acids are utilized for synthesis of acute phase proteins. Hypoalbuminaemia as a result of inflammation tends to be mild.

Hepatopathy is also a potential cause of mild hypoalbuminaemia. Marked hypoalbuminaemia (<20 g/L) is almost invariably indicative of loss of albumin rather than merely reduced synthesis and the most likely causes are protein-losing enteropathy or loss into an effusion. Though uncommon, protein losing nephropathy as a result of glomerular disease is another potential cause of hypoalbuminaemia.

## INVESTIGATING BLEEDING DISORDERS

- May involve clotting factors and/or platelets
  - clotting factor problems
    - Hepatic failure and DIC are commonest
  - platelet problems (numbers or function)
    - Immune mediated thrombocytopenia and DIC are commonest

Clinical signs of bleeding disorders may include petechial or ecchymotic haemorrhages, persistent bleeding after venipuncture, recurrent/persistent epistaxis or multiple haematomas. Terminology often describes problems with clotting factors as clotting disorders and problems with platelets as bleeding disorders.



Hepatic disease – Although the liver produces a large number of factors important in coagulation, clinical bleeding disorders are uncommon as a consequence of hepatic disease – quite severe hepatic failure is needed before any problems arise.

Disseminated Intravascular Coagulation (DIC) – a procoagulant status leads to consumption of clotting factors with thrombotic disease (e.g. at catheter sites) followed by haemorrhages (eg epistaxis post nasogastric tubing). Associated with sepsis, endotoxaemia and neoplastic diseases may all lead to vasculitis and coagulopathy (relatively common).

### Specific factor deficiencies

- Vitamin K deficiency may occur as a result of ingesting rat poisons
- Factor VIII deficiency (classic haemophilia) has been identified in Thoroughbreds, Standardbreds, Quarter Horses and Arabs
- Deficiency of Von Willebrand's factor has been identified in Thoroughbreds and a Quarter Horse
- Prekallikrein deficiency has been identified in Belgian horses, American Miniature horses and a Quarter Horse

### Thrombocytopenia

- Immune-mediated (primary or secondary to infection/neoplasia/drugs) – may be associated with immune mediated anaemia
- DIC
- Bone marrow disease (see anaemia)
- chronic excessive haemorrhage
- Equine Infectious Anaemia (NOTIFIABLE!)
- Anaplasmosis
- Piroplasmosis

### Functional platelet disorders

- Glanzmann's thrombasthenia is a rare congenital disorder of platelet function

## INVESTIGATING BLEEDING DISORDERS

### **DIAGNOSTIC TESTS**

Tests available in practice are somewhat crude and comprise prothrombin time (PT), activated partial thromboplastin time (aPTT) and bleeding time. Further functional tests are used as research tools but are not available commercially. All tests should be compared contemporaneously with a control horse as reference ranges are unreliable due to the number of external factors that may affect the test. Values greater than 20% above the control are considered abnormal.

Tests of clotting factors – PT and aPTT are used to assess functionality of the clotting factor cascade. Most clotting disorders (e.g. liver failure or DIC) will have abnormalities in both pathways although changes in PT often precede changes in aPTT.

PT - (extrinsic pathway) is usually around 10-12 secs and depends on factors I, II, V, VII, and X and will be affected by coumarin-type anticoagulants, vitamin K deficiency, liver damage and general consumptive coagulopathies (DIC). Factor VII has the shortest half-life of all clotting factors so generalised clotting disorders may be detected by prolonged PT prior at an early stage followed by prolonged aPTT.

aPTT - (intrinsic and common pathways) is usually around 30-45 secs and depends on factors I, II, V, VIII, IX, X, XI, & XII and will be affected by heparin treatment, general consumptive coagulopathies (DIC), von Willebrand disease, haemophilia and probably severe hepatic failure.

Platelet count - is usually  $>100 \times 10^9/L$  but is very susceptible to artefactually low measured values. If thrombocytopenia is identified in EDTA samples then measurement should be repeated on a citrate sample as the likelihood of artefactual platelet clumping is reduced when citrate is used as an anticoagulant. Bleeding disorders are generally associated with platelet counts  $< 20 \times 10^9/L$ . Primary differentials for thrombocytopenia include immune-mediated disease, DIC and liver failure.

Bleeding time - is a crude test of platelet function whereby a small stab incision is made in the skin and blood removed every 30 secs with tissue (without touching the skin). Bleeding should cease within 5 minutes in normal horses. Bleeding time is affected by several processes including thrombocytopenia, thrombasthenia, DIC, vasculitis, aspirin, liver and kidney failure. Worth comparing with a control horse.

D-Dimer is a product of fibrin breakdown and is increased in horses with colic, laminitis, jugular thrombosis and other inflammatory disorders. Increased concentrations may therefore give an indication of coagulopathy. D-dimer concentrations in peritoneal fluid may also be measured as an indicator of intra-peritoneal fibrinolytic activity in horses with intestinal disease. In plasma, concentrations above 1000 ng/ml are considered to indicate a coagulopathy and in colic cases a concentration  $> 4000$  ng/ml is associated with reduced likelihood of survival.