

## DETECTION OF INFLAMMATION IN PERIPHERAL BLOOD SAMPLES

Inflammatory diseases are commonly encountered in equine practice and blood samples are a frequent starting place in the detection and elimination of harmful systemic inflammatory processes. These are often associated with infection although many non-infectious causes of inflammation exist also (below).

### Examples of causes of systemic inflammation

|                         |   |
|-------------------------|---|
| INFECTION               | - Bacteria ( <i>strangles, peritonitis, respiratory disease, Lyme disease</i> ) |
|                         | - Viruses   |
|                         | - Parasites ( <i>nematodes, cestodes</i> )                                      |
|                         | - Protozoa ( <i>Cryptosporidium</i> )   |
| TISSUE DAMAGE           | - Surgery   |
|                         | - Traumatic injury  |
|                         | - Exertional rhabdomyolysis   |
| IMMUNE-MEDIATED DISEASE | - Lymphangitis/vasculitis   |
|                         | - Immune-mediated haemolysis  |
|                         | - Pemphigus foliaceus   |
| ENDOTOXAEMIA            |   |
| NEOPLASIA               |   |
| OTHER                   | - Inflammatory bowel disease  |
|                         | - NSAID toxicosis   |
|                         | - Sand enteropathy  |

An indication of the presence, severity and chronicity of inflammation may be gained from examination of **white blood cells, platelets, albumin, globulins, fibrinogen, serum amyloid A** and possibly other acute phase proteins and serum iron.

### 1. HAEMATOLOGY

#### a) Interpretation of the Leucogram

Interpretation of white blood cell data can often be somewhat subjective and based on opinion rather than hard and fast objective evidence. Nevertheless there are certain clear rules that can be followed to help in the initial interpretation and subjective views can be superimposed in an attempt to refine the final interpretation. The following discussion considers some of the aspects important in reaching a final interpretation of a leucogram.

In the first instance it is much more meaningful to consider individually 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.

|     | <u>case 1</u>                  | <u>case 2</u>                  |
|-----|--------------------------------|--------------------------------|
| WBC | 6.1 x 10 <sup>9</sup> /L       | 9.8 x 10 <sup>9</sup> /L       |
| PMN | 2.3 x 10 <sup>9</sup> /L (38%) | 3.7 x 10 <sup>9</sup> /L (38%) |
| LC  | 3.3 x 10 <sup>9</sup> /L (54%) | 5.3 x 10 <sup>9</sup> /L (54%) |
| Eos | 0.5 x 10 <sup>9</sup> /L (8%)  | 0.8 x 10 <sup>9</sup> /L (8%)  |

The most common leucogram abnormalities are neutrophilia, neutropaenia, eosinophilia and monocytosis.

### **Neutrophilia (PMN > 6.7 x 10<sup>9</sup>/L)**

Neutrophilia is the commonest leucogram abnormality and is usually the result of an acute or chronic inflammatory response. This may be caused by infectious (viral, bacterial or parasitic) or non-infectious (eg. azoturia, surgical trauma, immune-mediated disease, neoplasia) disease. The typical effect of glucocorticoids on the leucogram is neutrophilia associated with a lymphopaenia and eosinopaenia. This may be related to stress, Cushing's disease or therapy with exogenous glucocorticoids. Therefore this type of leucocyte pattern in sick horses may be a non-specific result of the primary condition rather than a specific indicator of an inflammatory aetiology. Cross-checking against acute phase proteins and globulins may help determine the "inflammatory versus stress" dilemma. Short term excitement perhaps associated with venepuncture of a nervous horse or transport immediately before sampling can also result in neutrophilia but lymphocytosis may be more likely in these cases.

### **Neutropaenia (PMN < 2.4 x 10<sup>9</sup>/L)**

Neutropaenia is often seen in horses showing signs of relatively mild lethargy and suboptimal performance and is frequently attributable 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 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 (platelets and red cells being the other cells primarily affected).

### **Eosinophilia (Eos > 0.6 x 10<sup>9</sup>/L)**

Eosinophilia is often a fairly non-specific finding as the eosinophil has 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 skin, respiratory tract and intestine and peripheral eosinophilia is certainly seen fairly consistently 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. Hence intra-arterial *S. vulgaris* larvae (although very rare) may well be associated with a peripheral eosinophilia, lungworm infection is usually associated with an eosinophilia in tracheal washes or bronchoalveolar lavage samples, encysted cyathostomes are associated with eosinophilic infiltrates detectable in caecal, colonic and sometimes rectal biopsies.

### **Monocytosis (Monos > 0.6 x 10<sup>9</sup>/L)**

In a similar fashion to neutrophilia and eosinophilia, monocytosis is a non-specific inflammatory indicator seen to rise in both acute and chronic inflammatory conditions and tissue damage.

### **b) Thrombocytosis (Platelets > 250 x 10<sup>9</sup>/L)**

Platelets are a frequently overlooked but quite useful inflammatory marker. Platelet numbers tend to increase in the presence of inflammation probably as a result of generally increased bone marrow activity. Hence they do not act as an acute phase reactant but do increase in most cases of chronic, persistent inflammation. High platelets counts will frequently be seen in association with abscessation (eg Strep equi, Rhodococcus) and also with non-infective conditions such as neoplasia).

## **Prime considerations in interpretation of the leucogram**

These lists are not intended to be exhaustive and comprehensive but rather to serve as an aide memoire for the more common causes of abnormalities of the leucogram seen in the adult horse.

### **Neutrophilia (PMN > 6.7 x 10<sup>9</sup>/L)**

- Inflammatory diseases
- Endogenous/exogenous glucocorticoid (stress, equine Cushing's disease)
- Catecholamines (acute excitement/fear)

### **Neutropaenia (PMN < 2.4 x 10<sup>9</sup>/L)**

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

### **Lymphocytosis (LC > 5.0 x 10<sup>9</sup>/L)**

- Infectious diseases (primarily mid-late phase viral)
- Catecholamines (acute excitement/fear)

### **Lymphopaenia (LC < 1.1 x 10<sup>9</sup>/L)**

- Infectious diseases (eg. early EHV)
- Endogenous/exogenous corticosteroid (stress, equine Cushing's disease)

### **Eosinophilia (Eos > 0.6 x 10<sup>9</sup>/L)**

- Hypersensitivity diseases (eg. sweet itch, urticaria)
- Diseases of intestine, skin and lung
- Inflammatory diseases (see neutrophilia)
- Parasitic larval migration (rare)

### **Monocytosis (Monos > 0.6 x 10<sup>9</sup>/L)**

- Inflammatory diseases (see neutrophilia)

### **Is it possible to differentiate bacterial and viral disease on the basis of haematology?**

The most consistent haematologic finding associated with the early stages of viral infections (ie. the time when we are usually asked to examine the horse and take a diagnostic blood sample) is a neutrophilia (PMN > 6.5 x10<sup>9</sup>/L). 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 then mild neutropaenia and possibly lymphocytosis and monocytosis would typify viral disease, whereas bacterial infections more typically remain neutrophilic with, possibly, a monocytosis, unless severe whereupon neutropaenia may occur. Chronic bacterial conditions are frequently associated with a thrombocytosis whereas viral conditions do not generally cause this. Additionally acute phase protein responses also tend to be milder with viral diseases (e.g. SAA <50 mg/L) than with bacterial (e.g. >100 mg/L).

|                      | <u>early</u>              | <u>mid-late</u>                                      |
|----------------------|---------------------------|--|
| VIRUS (typical):     | neutrophilia              | - neutropaenia/Lymphocytosis/monocytosis             |
| BACTERIAL (typical): | neutrophilia              | - neutrophilia/monocytosis                           |
| BACTERIAL (severe):  | neutrophilia/neutropaenia | - neutrophilia/neutropaenia/(thrombocytosis-chronic) |

## **2. ACUTE PHASE PROTEINS**

The acute inflammatory reaction results in a widespread and complex cascade of cytokine and lymphokine production (interleukins, interferons, eicosanoids etc..) leading to many potentially detectable changes in the blood. "Acute phase proteins" are a wide array of proteins which are synthesised and released from the liver in response to inflammatory cytokines (especially interleukin-6) released from leucocytes. These proteins include **fibrinogen, serum amyloid A, caeruloplasmin, C-reactive protein, haptoglobin** and several others. A large selection of these acute phase proteins is commonly used in human clinical pathology although only fibrinogen is assayed widely in veterinary laboratories. The Liphook Equine Hospital Laboratory is one of only a few veterinary laboratories to offer serum amyloid A as an additional acute phase protein and further proteins are under investigation such as C-reactive protein, haptoglobin and procalcitonin.

### **Fibrinogen**

Measured in plasma (EDTA or citrated) and normally less than 3.7 g/L and may rise as high as 10-15 g/L in severe inflammatory cases. Therefore the 'pathophysiological range is approximately 4 X. Values greater than 10 g/L must always be regarded seriously and carry a guarded (but not necessarily poor) prognosis. Fibrinogen responds to acute inflammation relatively sluggishly and may not be outside the reference range for 24-48 hours following initiation of an acute inflammatory response.

### **Serum amyloid A**

Has been used at the Liphook Equine Hospital Laboratory for many years now. We have found the test both reliable and sensitive to acute inflammation in horses and it has become our preferred diagnostic and monitoring acute phase protein in cases such as viral/bacterial respiratory disease, peritonitis, colitis etc.... Most normal horses have SAA concentrations close to zero and with severe inflammatory disorders this can rise to approximately 1000 mg/L creating a massive pathophysiologic range. Compared with fibrinogen this allows a far greater '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 little if any benefit in subclassifying globulins further using protein electrophoresis as this technique almost never provides any further useful diagnostic or prognostic information unless globulin levels are extreme (eg >90 g/L). Hyperglobulinaemia as well as indicating chronic inflammation (of all causes) is commonly seen in cases of hepatopathy also.

## **Albumin**

Serum albumin is often referred to as a “negative acute phase protein” as albumin levels are often seen to fall slightly with inflammation (especially in chronic cases) as synthesis of positive acute phase proteins listed above is accelerated at the expense of albumin synthesis. Albumin concentration is a key diagnostic factor in weight loss assessment. Marked hypoalbuminaemia (<20 g/L) is almost invariably indicative of protein-losing enteropathy (eg. larval cyathostomiasis, inflammatory bowel disease, NSAID toxicity or alimentary lymphoma) whereas mild hypoalbuminaemia may also indicate hepatopathy or a non-specific effect of chronic inflammatory disease. Protein losing nephropathy cases are rarely encountered but should not be forgotten.

### **Is it possible to diagnose parasitism on the basis of haematology or serum protein electrophoresis?**

Nematode infections in the adult horse were once typified by intra-luminal adult worms and intra-arterial larval migration associated with *Strongylus vulgaris*. These were often associated with an eosinophilia detectable in blood samples in response to intra-arterial larvae and also, in some instances, a detectable increase in  $\beta_1$ -globulins (especially IgG<sub>(T)</sub>). Parasitological surveys in more recent years have consistently confirmed the decline of *S. vulgaris* (almost to the point of extinction) in conjunction with a domination of parasitic burdens by members of the cyathostome genus (small strongyles) which consistently comprise almost 100% of nematode eggs detected in equine faecal samples in this country. Cyathostome infection results in encystment of larvae locally in the caecal and colonic wall but is not associated with larval parasitic migration. An eosinophilia is not associated with cyathostome infections and a raised  $\beta_1$ -globulin fraction is a very occasional and non-specific finding.

Several research studies have failed to confirm any clinically useful relationship between serum protein electrophoresis and parasitism in horses. Normal concentrations of IgG<sub>(T)</sub> and  $\beta_1$ -globulins are usually found in parasitised adult horses and ponies although changes may be more likely in young horses. In a recent investigation of horses with chronic diarrhoea, less than half of horses with parasitic diarrhoea had raised  $\beta_1$ -globulins and this finding was also common in horses with non-parasitic disease. ‘Cyathostomiasis’, the acute diarrhoea and weight loss syndrome associated with en masse larval emergence, is consistently associated with a neutrophilia, hypoalbuminaemia and hyperfibrinogenaemia (all unfortunately non-specific findings), but other than this blood samples taken from parasitised horses show no consistent abnormalities in haematology or protein analyses.