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
WBC count	6.1 x 10^9/L	9.8 x 10^9/L
Neutophils	***2.3 x 10^9/L (38%)***	3.7 x 10^9/L (38%)
Lymphocytes	3.3 x 10^9/L (54%)	***5.3 x 10^9/L (54%)***
Eosinophils	0.5 x 10^9/L (8%)	***0.8 x 10^9/L (8%)***

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.

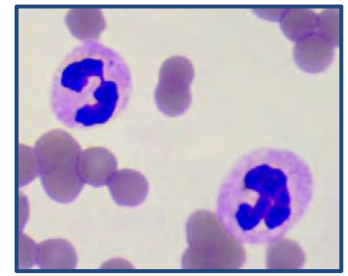
ASSESSING CHANGES IN THE LEUCOGRAM

NEUTROPAENIA

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

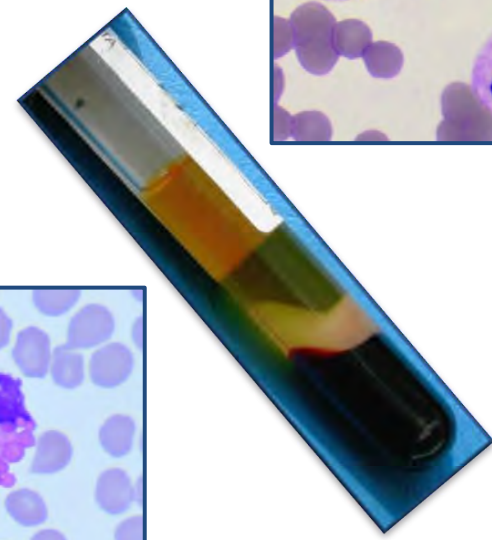
Neutropaenia is often seen in horses showing signs of relatively mild lethargy and suboptimal performance and is frequently attributed to viral challenge. In horses showing more marked signs of illness such as tachycardia and pyrexia then severe bacterial sepsis, endotoxaemia, loss of neutrophils into 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)

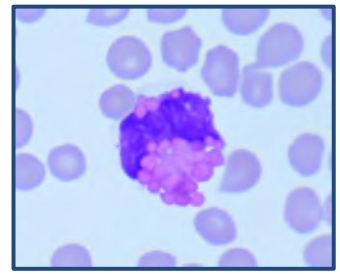


LYMPHOPAENIA

- 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

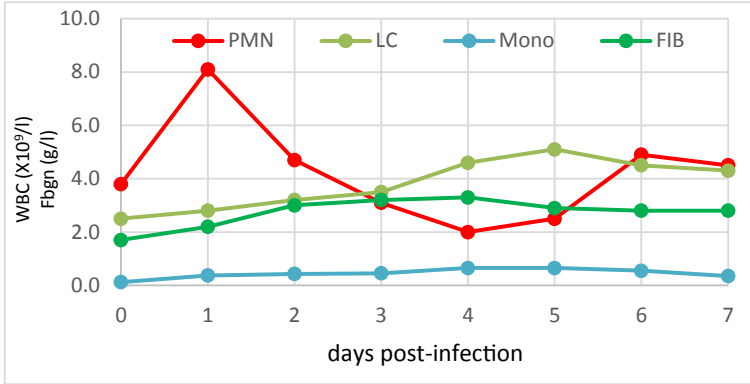
Infection	Bacteria
	Viruses
	Parasites
	Protozoa
Tissue Damage	Surgery
	Traumatic injury
	Exertional rhabdomyolysis/Atypical myopathy
Immune-mediated disease	Lymphangitis/vasculitis
	Immune-mediated haemolysis/thrombocytopaenia
	Pemphigus foliaceus
Endotoxaemia	Secondary to colitis or similar
Neoplasia	
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
Virus (typical):	Neutrophilia	neutropaenia/lymphocytosis/monocytosis
Bacterial (typical):	Neutrophilia	neutrophilia/monocytosis
Bacterial (severe):	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.