Proceedings of the
European Veterinary Conference
Voorjaarsdagen

Amsterdam, the Netherlands
Apr. 18 - 20, 2013

Next Meeting:

Apr. 17 – 19, 2014 - Amsterdam, the Netherlands

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INTERPRETING CLINICAL CHEMISTRY: BIOCHEMISTRY PROFILES

Clinical chemistry profiles are often performed in the clinical setting for several different reasons, including the investigation of illness, poor performance, as a part of routine health checks and subsequently as follow-up of a previously identified abnormality.

Frequently the tests that will be performed are pre-determined by the diagnostic laboratory, however consideration should be given to any additional analysis required prior to the submission of samples. In addition to the basic biochemistry profile which usually includes plasma protein concentrations, serum enzymes activity and bilirubin concentration, additional tests such as acute phase proteins, electrolytes, triglycerides, glucose, lactate, ammonia or bile acids may also be required.

Plasma proteins
Total protein is comprised of (predominantly) albumin, globulin and to a lesser extent fibrinogen.

Increased total protein may occur in:
- Dehydration (both albumin and globulins will increase)
- Chronic infections (globulins increase)
  False increase: if total solids are measured on a refractometer, other substances can interfere e.g. with hyperlipaemia as measure fats as ‘solids’

Decreased protein concentrations:
- Reduced globulin concentrations are rare
- Albumin decrease: usually due to loss into GIT e.g. protein losing enteropathy
- Can also be lost via the kidneys or into the pleural/peritoneal space if disease in these cavities is present

Serum enzyme activities
Aspartate transferase (AST)
- Enzyme found in liver and muscle cells
- Elevated levels reflect muscle or liver damage therefore need to interpret alongside other muscle (CK) and liver enzymes (SDH or GLDH)
- Peaks 24 hours following muscle injury and slower to decrease than CK

Creatine Kinase (CK)
- Enzyme found mainly in skeletal muscle
- Also myocardial and brain isoforms
- Increased concentrations reflect muscle damage, which may be primary e.g. rhabdomyolysis or secondary e.g. to prolonged recumbency
- Short half-life (4-6 hours) therefore reduces rapidly after muscle damage

Sorbitol dehydrogenase (SDH)
- Enzyme found in liver cells
  Specific for hepatocellular damage
  Short half-life therefore reflects acute liver damage

Gamma glutamyl transferase (GGT)
- Enzyme found in the bile ducts of the liver and increases are associated with diseases affecting the biliary tract
- Although there are other sources, e.g. renal tubular cells and pancreas, it is considered a sensitive indicator of liver disease

Alkaline phosphatase (ALP)
- Increased levels caused by cholestasis or drug administration
- Not specific for liver disease as also present in bone, intestines, kidney, placenta and WBC’s.
- Young animals have 2-3 times higher levels
**Internal Medicine**

### Miscellaneous chemistries:

**Urea and creatinine**

Creatinine is released from muscles at a fairly constant state and filtered by the kidneys without reabsorption. Urea is synthesized by the liver and also filtered by the kidneys, hence these two variables are often interpreted together to assess renal function, in conjunction with other indices of hydration status e.g. PCV/TP/Lactate/urine specific gravity (USG).

Increased urea and creatinine (azotaemia) may be:
- **Pre-renal**: Due to reduced circulating volume (high PCV and USG)
- **Renal**: Due to reduced filtration by the kidneys (USG low)
- **Post-renal**: Resulting from obstruction to urinary outflow e.g. ruptured bladder

**Bilirubin**

- Produced in the breakdown of red blood cells in the blood (unconjugated form)
- Unconjugated bilirubin is then removed from the blood by the liver and modified for disposal in bile (conjugated)
- Increased unconjugated bilirubin: increased breakdown of red blood cells (haemolysis), inappetence or liver disease
- Increased conjugated bilirubin: obstruction to the outflow of bile

**Bile acids**

- Removed from the blood by the liver
- Marker of liver function

**Triglycerides**

- Reflect mobilisation of fat in the body during prolonged inappetence
- Hyperlipaemia can be a life threatening consequence of diseases that result in anorexia
- Serum may be visibly opaque
- Common in ponies, donkeys, pregnant and lactating mares

### When to suspect:

#### Dehydration

- Increased PCV/Hct
- Increased TP/TS
- Increased lactate
- Increased creatinine/BUN

#### Liver disease

- Increased liver enzymes: AST, SDH, GGT, ALP
- Increased bilirubin
- Increased ammonia
- Increased globulins
- Abnormal clotting times

#### Renal disease

- Increased creatinine and BUN
- Anaemia
- Decreased albumin
- Electrolyte abnormalities
- Urinalysis

#### GIT disease

- Low white cell count
  - Neutropaenia
  - Left shift
  - Toxic changes
- Increased globulins
- Decreased albumin
- Electrolyte abnormalities

#### Muscle disease

- Increased CK
- Increased AST
INTERPRETING CLINICAL CHEMISTRY: HAEMATOLOGY PROFILES

Haematology profiles provide information about red and white blood cell parameters and platelets counts. They are frequently performed to evaluate the inflammatory and infectious status of a patient.

When submitting blood for a haematology profile, samples should be collected into appropriate anticoagulant, i.e. EDTA, and if additional tests such as acute phase protein measurements are required additional samples may be necessary, e.g. sodium citrate for fibrinogen or plain serum for serum amyloid A. If a delay in submission to the laboratory is anticipated, making a fresh blood smear is recommended to allow for accurate evaluation of white blood cell morphology.

White blood cells

The two main leucocytes in the circulation are the neutrophils and the lymphocytes, in addition monocytes, basophils and eosinophils are also present.

Neutrophils:
Neutrophils phagocytose (engulf and destroy) foreign material, especially bacteria, and have a short life span in the circulation (approximately 6 hours).

- Neutrophilia (increased neutrophils):
  - Bacterial infections especially chronic bacterial infections such as Strangles
  - Glucocorticoid response e.g. stress, steroid administration or PPI D
- Neutropaenia (decreased neutrophils):
  - Increased demand e.g. Acute severe infection or inflammation, especially involving the GIT. A ‘left shift’ (increased concentration of immature or band neutrophils) or toxic changes may also be seen.

Bone marrow suppression

Lymphocytes:
There are two main types of lymphocytes the B and T cells. B cells form plasma cells which produce antibodies, whilst the T cells are involved in cell-mediated immunity.

- Increased lymphocyte counts are rare and occur with chronic infection, lymphosarcoma or lymphocytic leukaemia.
- Low lymphocyte counts can follow acute infection, a corticosteroid response or reduced production, which can occur in several rare hereditary immunodeficiency syndromes e.g. Severe Combined Immunodeficiency of Arabian foals.

Eosinophilia may form part of the non-specific inflammatory response. A systemic increase in eosinophils in response to parasites is rare, as the eosinophilic response is usually more local. Hypersensitivity reactions, hypereosinophilic syndromes or myeloproliferative disease should all be considered as unusual causes of increased levels.

Monocytes are not very helpful when evaluating the health status of the patient. They are transformed to macrophages within tissues, and an increased number can occur in response to increased demand. Basophils are also rarely identified in the systemic circulation.

Red blood cells

PCV/HCT
Packed cell volume is a measure of the volume of red blood cells in plasma. Normal ranges vary between breeds, with “hot bloods” having a higher PCV than pony and “cold-blood” breeds

- Increased PCV (Polycythaemia)
  - Relative: Reduced circulating volume e.g. dehydration or stress
  - Absolute: Increased RBCC without reduced circulating volume. E.g. Chronic hypoxia, hepatic disease, neoplasia,
• Decreased PCV (Anaemia)
  - Blood loss (Usually 12hrs before PCV reduces; hypoproteinaemia accompanies)
  - Haemolysis
  - Reduced production e.g. Chronic disease, iron deficiency or bone marrow disease (rare!)

As immature red blood cells are not released into the circulation a bone marrow aspirate is the accurate way of determining if anaemia is regenerative or not. However, other red cell parameters such as MCV, MCH and MCHC in conjunction with serum iron and iron binding capacity may be used to evaluate this, of these MCHC is the most reliable.

**Mean cell Volume (MCV) - Increased suggests regeneration**
Mean Corpuscular Haemoglobin (MCH) = Mean haemoglobin concentration per red blood cell. Increased in regeneration/haemolysis; reduced in iron deficiency

Mean Corpuscular Haemoglobin Concentration (MCHC) = Mean haemoglobin concentration in a given volume of red blood cells. Reduced in iron deficiency and increased with haemolysis

**Platelets**
Platelets are formed from megakaryocytes in the bone marrow and have an important role in haemostasis. Clumping of platelets can lead to erroneous results therefore evaluation of a blood smear may be useful to confirm platelet numbers.

**Thrombocytopenia (decreased platelets)**
• Decreased production e.g. Bone marrow disease
• Increased destruction e.g. immune mediated
• Increased consumption – DIC (most common) or haemorrhage

Thrombocytosis (increased platelets) is much less common than thrombocytopenia, occurring secondary to chronic, persistent infections and in neoplastic conditions.

**Acute phase proteins**
The acute phase proteins are produced by the liver in response to cytokines such as IL-6. They are useful indices of systemic inflammation used in the diagnosis and monitoring of disease. The two main acute phase proteins measured in the horse are serum amyloid A (SAA) and fibrinogen, which respond over different timescales, SAA responding much more rapidly whilst fibrinogen usually has a lag time of several days.
A PRACTICAL VIEW ON THE WORK-UP OF EQUINE HEADSHAKING SYNDROME

Headshaking syndrome in horses is not a new condition; it may well have been around for as long as we have known the domesticated horse. So why is it a problem?

Severe headshaking can make a horse dangerous to ride or handle. In a study of 100 horses, a possible cause was found in only 4, and when treated 2 stopped headshaking. Therefore, in 98% of horses with headshaking, there appears to be no gross pathology. This has, understandably, been interpreted that most headshakers are disobedient, require disciplining, and are not covered by veterinary or life insurance. In fact, it is likely that most headshakers are suffering from, often severe, facial pain but this is often not recognised or understood. Disciplining these horses in error is obviously a significant welfare issue.

The facial pain syndrome suffered by most headshakers with no gross pathology bears many clinical resemblances to human facial pain syndromes, notably trigeminal neuralgia. This is usually in sudden in onset during middle age and, at least initially, there is a specific trigger for the attacks of pain which is often likened to an electric shock across the face. Most horses seem to suffer an acute onset of signs in early middle age. Typically they show sudden, sharp, vertical twitches as well as predominantly vertical headshaking and this is accompanied by nose rubbing or snorting. A trigger may be involved in some horses who appear only seasonally affected.

The first stage of investigation is to take a thorough history; for example headshaking due to bad riding is unlikely to be seen with every rider or show a seasonal pattern. The horse should be observed to headshake; for example horizontal or rotator headshaking is less likely to stem from facial pain.

The next stage would be prove the presence of facial pain. As long as the horse is headshaking consistently, diagnostic local analgesia at the caudal portion of the infraorbital nerve (in earlier publications this procedure has been called the posterior ethmoidal nerve PET block) should be performed. If headshaking significantly improves following administration of local anaesthetic then a diagnosis of headshaking due to facial pain is made. As with all diagnostic local analgesia, there is a risk of false negative results; in two studies, a likely false negative response was found in 6/17 (35%) and 4/27 (15%) horses. Unpublished results in a cadaver head study suggest false negative responses may be acquired due to displacement especially by non-experienced operators. It can therefore be possible to make a diagnosis of headshaking due to facial pain from history, observation and investigation to exclude other causes.

Once a diagnosis of facial pain is reached, investigations should be performed to determine a cause for that facial pain. These should include clinical, oral and ophthalmic examinations, endoscopy of the upper respiratory tract including guttural pouches and radiographic imaging of the head. Computed tomography is ideal, giving information far superior to that of conventional radiography.

In most cases, no pathology causing the facial pain is determined, even on these extensive investigations. In these cases a diagnosis of headshaking due to facial pain from a suspected trigeminal neuropathy may be made.

TREATMENT DECISIONS IN EQUINE HEADSHAKING SYNDROME: HOW TO ASSESS A FAIR PROGNOSIS AND HOW TO MANAGE

Although a diagnosis of headshaking due to trigeminal neuropathy may be made by exclusion, any diagnosis reached by exclusion remains somewhat unsatisfactory. Similar to orthopaedic examinations, a diagnosis of headshaking due to facial pain may be reached through diagnostic local analgesia. The trigeminal nerve is responsible for sensory innervations to the head. It is easily accessible rostrally, following emergence from the infra-orbital canal. Instillation of local anaesthetic at this position is simple to perform, but rarely useful. Also, neurectomy performed at this rostral location has a poor success rate. Likely the site of the neuropathy is further caudal within the nerve. Accessing the nerve at its most caudal point would be ideal but this is unreachable. It is however possible to access the infraorbital nerve just before it enters the infraorbital canal. Here is the caudal nasal branch of this nerve which innervates the caudal part of the nasal passages and ethmoidal regions. This branch is suspected to be important in the pathogenesis of headshaking (1). The technique of diagnostic local analgesia at this position has previously been termed the posterior ethmoidal (PET) block (2,4).

The technique for this procedure is described by Newton et al in 2000 and description and video are available in the iPhone application developed by equine advances Ltd and recently published by the British Equine Veterinary Association (BEVA). If headshaking significantly improves following administration of local anaesthetic a diagnosis of headshaking due to facial pain is made. As with all diagnostic local anaesthesia, there is a risk of false negative results; in two studies, a likely false negative response was found in 6/17 (35%) and 4/27 (15%) horses (1,2). Unpublished results in a cadaver head study suggested false negative responses may be acquired due to misplacement especially by non-experienced operators. Possible complications include haematoma, infection or even temporary blindness if the local anaesthetic is sufficiently misplaced as to affect the optic nerve. Treatment of headshaking due to a suspected trigeminal neuropathy is challenging. The first intervention should be using a nose-net. This is non-invasive and this may be beneficial; in 25% of cases there is up to 70% relief. Various pharmaceuticals have been used, including cyproheptadine and carbamazepine. Results have been inconsistent although some individuals may benefit. These drugs are costly for long-term management, prohibited in competition and may result in drowsiness.

Short-term resolution of signs was achieved with bilateral sclerosis of the caudal part of the infraorbital nerve and caudal nasal nerve in 5/5 cases. This has led to the technique where platinum coils are placed caudally in the infraorbital canal, with a view to compressing the nerve and therefore modifying nerve function. This has led to an initial success rate of 63% but recurrence is possible, leading to an ultimate success rate of 49% in 58 horses with a median follow-up time of 18 months. However, after surgery 67% of horses have suffered mostly short-term side effects of nose-rubbing, sometimes leading to self-trauma and sometimes accompanied by worsening of headshaking. Administration of gabapentin and acepromazine may help in management. However, non-resolution or severity of these side-effects has led to euthanasia in 4/58 horses. Therefore, we recommend surgery only where euthanasia is the only other option.

References: