Clinical Pathology Basics for Equine Practitioners - Liver Disease

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Introduction

Clinical signs of liver disease in equines are often vague and non-specific, although there are some clinical symptoms which are highly suggestive of a primary hepatopathy, and these include hepatomegaly / microhepatica, icterus, ascites and hepatic encephalopathy. The more common non-specific clinical signs include depression, weight loss, anorexia, abdominal pain, polyuria/polydipsia (PU/PD). These non-specific signs are also frequently observed with many other diseases.

In the early stages of liver disease, sub-clinical presentation with non-specific signs is common, while specific hepatic symptoms are rarely observed. Therefore, serum biochemical profiling of hepatic function is extremely useful in identifying the presence or absence of liver disease and whether any hepatic dysfunction is acute or chronic. These biochemical changes may result from either primary liver disease or are secondary to other primary non-hepatic diseases. However, when these biochemical tests are interpreted in conjunction with clinical signs and other laboratory analyses, they allow for recognition of pattern changes, from which a number of diagnostic, prognostic and treatment decisions can be made.

The diagnosis of liver disease is based on abnormalities in serum enzymes that are partly or exclusively associated with hepatocytes and/or epithelial cells lining the biliary tree as well as some analytes that assess liver functionality and other more general indicators of inflammation.

Hepatic enzymes are divided into two categories

- Hepatocellular leakage enzymes (cytoplasmic, mitochondrial).
- Induced enzymes.

Hepatocellular leakage enzymes are soluble enzymes that occur normally in the cytoplasm (aspartate aminotransferase AST; alanine aminotransferase ALT, sorbitol dehydrogenase SDH) or mitochondria (glutamate dehydrogenase GLDH and aspartate aminotransferase AST) of hepatocytes. They are released with hepatocellular membrane damage with sublethal injury or hepatocellular necrosis. The serum activity of these enzymes depends on the number of hepatocytes injured, the severity of the injury and the half-life of the enzyme involved.

Induced hepatic enzymes (alkaline phosphatase ALP; γ-glutamyl transferase GGT) are typically membrane-bound and not released into the serum with increased membrane permeability. Increased serum enzymatic
activity is the result of enzyme induction, which is typically induced by cholestasis, drug or hormonal effects.

Total Bile Acids are the preferred liver analyte assessing hepatic function, while the general inflammatory indicators most commonly employed are plasma fibrinogen, serum amyloid A and protein electrophoresis.

EQUINE LIVER FUNCTION PROFILE

- Plasma fibrinogen (Na-citrate, EDTA anticoagulant)
- Serum amyloid A, serum protein electrophoresis, AST, SDH, GLDH, GGT, ALP, Total Bile Acids (Serum)
- Liver biopsy (10% buffered formalin).

Plasma fibrinogen

Acute-phase reactive protein, which increases in response to inflammation with elevations observed in the presence of tissue damage. The test can be performed on fresh, paired, non-haemolysed EDTA or serum samples, by subtracting of total serum protein levels from plasma protein levels. More accurate results are obtained from samples collected into sodium citrate anticoagulant to measure fibrinogen by direct coagulometric assay.

Serum amyloid A

This is a highly sensitive, rapidly reacting inflammatory protein, which is used for evaluating early responses to infection and their response to treatment. Most normal horses have zero measurable levels but with acute, particularly septic or toxic inflammation (hepatitis), levels increase rapidly within 24 hours. Raised levels also fall rapidly as inflammation subsides or when the infection/inflammation is controlled.
**Serum protein electrophoresis:**

This identifies elevations in specific globulin fractions

- Alpha 2 globulin - acute-phase inflammatory protein responses.
- Beta 1 globulin - Strongylus vulgaris and mixed strongyle larval activity.
- Beta 2 globulin – hepatopathy*.
- Gamma globulin - antibody responses to bacterial or viral infections.

*Serum samples should be used for protein electrophoresis, as raised fibrinogen levels in heparinised plasma samples will cause confusing rises in beta 2 globulins.

**Aspartate Aminotransferase (AST)**

Elevations of AST are documented with both acute and chronic hepatopathy. Although AST is mostly cytosolic, significant concentrations are also found in mitochondria. However, more severe cell injury or cell death is required for the release of mitochondrial AST. AST is found in many tissues including liver, striated muscle, intestine, kidney and erythrocytes. There is currently no method available to differentiate between the various AST tissue sources. Therefore, AST has low specificity for liver disease, although in the majority of liver disease cases it will be increased. The half-life has been reported at 7 to 8 days in horses.

Increased serum AST activity is observed with both reversible and irreversible injury to hepatocytes and can be seen following necrosis, ischemia, enzyme induction (phenobarbitone, corticosteroids), drug-induced hepatotoxicity, cholestasis or trauma.

Likewise, serum AST is increased following myocyte injury. In either case, the definitive disease process cannot be identified, only that cellular injury in muscle or liver has occurred. Because serum AST activity cannot differentiate between hepatocellular or myocyte injury, further testing using organ-specific enzymes such as SDH or creatine kinase (CK) is indicated. Markedly increased serum AST and SDH suggest acute or active hepatocellular injury, and markedly increased serum AST with modest to moderate SDH activity suggests chronic hepatic injury or recovery from acute liver injury.

An increased serum AST together with CK is a clear indication of muscle damage. The sensitivity of serum AST activity in horses has been reported as 72% for hepatic necrosis and 100% for hepatic lipidosis. For a correct diagnosis, determination of AST activity should be complemented with other hepatic indicators, such as SDH, total bile acids and GGT among others.

Careful sample collection and handling is important as hemolysis leads to increased AST activity, while improper handling leading to enzyme degradation results in artefactual reduction in AST levels. Serum samples can be stored for up to 24 hours in a refrigerator without appreciable loss of AST activity. The AST activity in frozen serum samples is also fairly stable, as long as the serum is not frozen and thawed repeatedly.

**Sorbitol Dehydrogenase (SDH)**

This is an enzyme found almost exclusively in the cytoplasm of hepatocytes and considered liver-specific, although in rare instances elevations can be seen in horses with skin conditions and enteropathy. It is considered a good indicator of active hepatocellular disease plus moderate to severe cholestasis. Unfortunately, SDH is highly labile and activity declines rapidly after blood collection. Analysis needs to be performed within 4 to 6 hours post collection and so this limits the usefulness of SDH as a diagnostic test.
Glutamate Dehydrogenase (GLDH)

GLDH as a cytosolic enzyme with elevations seen in the presence of acute hepatocellular damage. If hepatic damage persists for any period, the GLDH will return to normal. This is a mitochondrial enzyme found mainly in liver, with lesser amounts documented in heart muscle, kidney and intestine. The GLDH activity in these non-hepatic tissues is relatively small compared to that found in liver. It is a relatively stable enzyme and is a suitable liver function test replacement for sorbitol dehydrogenase (SDH), in samples transported to a diagnostic laboratory.

In horses, increases in serum GLDH activity are considered liver specific. Its relatively short serum half-life of 14 hours indicates that increased levels are associated with acute active hepatic damage (ischemia, hepatic toxicity). Non-specific increases of GLDH are documented in young foals, and so it should be interpreted with caution in this age group.

The sensitivity of GLDH activity for detection of hepatic necrosis, hepatic lipidosis, and hepatic cirrhosis has been reported at 78%, 86%, and 44%, respectively. The sensitivity is considered higher than that of SDH and comparable to AST.

Gamma Glutamyl Transferase (GGT)

GGT is predominantly associated with the brush border or microvilli on the canalicular surfaces of hepatocytes, biliary epithelial cells, renal tubular epithelial cells, pancreatic acinar cells and mammary gland epithelial cells. Increased GGT activity in serum is due to enzyme induction involving hepatocytes or biliary epithelium, increases of serum GGT activity are not generally associated with primary injury to the pancreas or kidney. Elevations in serum levels are seen in the presence of acute hepatitis, chronic liver cirrhosis and in very rare cases of pancreatitis. Following injury, the mean average half live of equine GGT is 3 days.

Although mild to moderate increases in serum GGT are of limited diagnostic or prognostic value, it is nevertheless very unusual to find significant hepatopathy in horses in the absence of increased serum GGT. Additionally, marked increases in serum GGT concentration are associated with a poor prognosis.

Alkaline Phosphatase (ALP)

Elevations in this brush border enzyme are most commonly observed with chronic biliary obstruction (cholestasis). High levels are also seen with abnormalities of bone metabolism and intestinal malfunction. Although many tissues or cell types have some ALP activity, cells from liver, bone, kidney, intestinal mucosa, and placenta have the greatest ALP activity. These various isoenzymes can be differentiated by certain reference laboratories.

Cholestasis causes significant increases in serum of ALP in horses and frequently precedes the development of hyperbilirubinemia. ALP increases within 48 hours of liver damage and the highest increases are observed with cholangitis, biliary cirrhosis and extra bile duct obstruction. Increases in ALP are also reported following administration of some drugs (phenobarbital, corticosteroids and others). Care needs to be taken when interpreting ALP levels in pregnant mares, due to placental origin sources of this enzyme. Caution should also be applied when evaluating ALP levels in growing animals, where normal values are 2 or 3 times higher than the reference values of adults, because of increased bone turnover associated with physical growth. Increases in bone ALP can also be observed in
any age animals with lytic or proliferative bone lesions. Horses with colic may have increased levels of intestinal ALP.

**Total Bile Acids (TBA)**

TBA are considered the preferred assay for evaluation of hepatic function. Equines lack a gall bladder and so secrete bile continuously through the biliary tract. Normally, most bile acid recycles back through the liver, with 1-2% going through the intestines. Measuring TBA is a much better guide to hepatobiliary status than bilirubin assays. High bile acid levels occur with severe hepatic dysfunction and liver failure and serve as important diagnostic and prognostic liver function tests in horses. It should be noted that increased TBA values can be found due to prolonged fasting (>3 days). The higher the TBA levels the less favourable the prognosis.

**Liver Biopsy**

Liver biopsy is considered the gold standard for the ante mortem diagnosis of liver disease as it provides valuable information about aetiology, treatment and prognosis. Liver biopsy is required to classify the nature of the hepatic pathology and is used to refine the prognosis in conjunction with the biochemical parameters. Various liver biopsy histopathology scoring systems have been developed, with the degree of fibrosis and bile duct hyperplasia being reported as important prognostic indicators.

**Other less frequently employed biochemical parameters for liver disease**

**Triglycerides:** these are good indicators of hepatic lipidosis, as increased serum concentrations are associated with lipid accumulation in the liver, interfering with the normal function of the hepatocytes.

**Bilirubin:** increased serum bilirubin levels are not specific for hepatic disease in the horse and they are documented with decreased hepatic functional mass, cholestasis, hemolytic disease or anorexia. Increase in plasma bilirubin associated with anorexia is due to a decrease in hepatic uptake and conjugation of bilirubin by the liver.

**Urea:** Although the majority of equine hepatopathy cases have normal serum urea concentrations, decreased serum urea is associated with more severe hepatopathies and has prognostic relevance.

**Basic Liver Reference Ranges - Adult Horse***

<table>
<thead>
<tr>
<th>Assay</th>
<th>Units</th>
<th>Mean</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrinogen</td>
<td>g/L</td>
<td>2.1</td>
<td>0.3-3.9</td>
</tr>
<tr>
<td>Serum Amyloid A</td>
<td>mg/L</td>
<td>1.3</td>
<td>0-20</td>
</tr>
<tr>
<td>Beta-2 Globulin</td>
<td>g/L</td>
<td>5.3</td>
<td>1.7-8.9</td>
</tr>
<tr>
<td>AST</td>
<td>iu/L</td>
<td>263</td>
<td>102-350</td>
</tr>
<tr>
<td>SDH</td>
<td>iu/L</td>
<td>&lt;5.1</td>
<td>&gt;5.1</td>
</tr>
<tr>
<td>GLDH</td>
<td>iu/L</td>
<td>3</td>
<td>1-10</td>
</tr>
<tr>
<td>GGT</td>
<td>iu/L</td>
<td>16</td>
<td>1-40</td>
</tr>
<tr>
<td>ALP</td>
<td>iu/L</td>
<td>204</td>
<td>147-261</td>
</tr>
</tbody>
</table>

*These are only basic guidelines for adult horses. Reference ranges are influenced by age (foals, yearling, adults), breed (thoroughbred, non-thoroughbred), activity (competition, riding, stud) and reproduction status.

References.

01. Which of the following clinical signs would be considered highly specific of a primary hepatopathy?
   a. Depression.
   b. Weight loss.
   c. Microhepatica.
   d. Abdominal pain.
   e. Polyuria/polydipsia.

02. Of the following enzymes would be considered an induced hepatic enzyme?
   a. Aspartate aminotransferase AST.
   b. Alkaline phosphatase ALP.
   c. Glutamate dehydrogenase GLDH.
   d. Sorbitol dehydrogenase SDH.
   e. Aspartate aminotransferase AST.

03. Which of the following assays is the most sensitive assay for hepatic functionality and provides prognostic information?
   a. Serum Amyloid A.
   b. Plasma fibrinogen.
   c. Protein electrophoresis.
   d. Serum bilirubin.
   e. Total Bile Acids.

04. For coagulometric plasma fibrinogen determination which of the following blood tubes must be used for blood sample collection?
   a. Serum tube without clot activator.
   b. Serum tube with clot activator.
   c. EDTA blood tube.
   d. Sodium citrate blood tube.
   e. Heparin blood tube.

05. Which of the following statements about Aspartate Aminotransferase (AST) is incorrect?
   a. AST may be elevated in both acute and chronic hepatopathies.
   b. AST is found in the cytoplasm as well as mitochondria of hepatocytes.
   c. More severe damage is required for the release of cytosolic AST.
   d. AST is also found in intestine and kidney.
   e. The half life of AST in the horse is 7 to 8 days.

06. Elevated Glutamate Dehydrogenase (GLDH) levels should be interpreted with caution in which of the following situations?
   a. Suspected hepatotoxicity in adult horses.
   b. Suspected degenerative hepaticopathy in young foals.
c. Suspected degenerative hepatopathy in adult horses.
d. Suspected hepatic lipidosis in adult horses.
e. Suspected hepatic ischemia in adult horses.

07. The most significant / highest increase in Alkaline Phosphatase (ALP) would be expected in which of the following situations?

a. Cholangitis with biliary cirrhosis.
b. Administration of corticosteroids.
c. In pregnant mares.
d. In young growing horses.
e. Horses with colic.

08. In addition to hepatic dysfunction, raised Total Bile Acids (TBA) is also reported in which of the following conditions?

a. Acute pancreatitis.
b. Colic.
c. Administration of phenobarbitone.
d. Advanced pregnancy.
e. Fasting for > 3 days.

09. In liver biopsies which of the following lesions is considered the most important prognostic indicator?

a. Hydropic degeneration.
b. Fatty change.
c. Pigment accumulation.
d. Degree of fibrosis.
e. Proliferation of sinusoidal kupfer cells.

10. Which of the following biochemical abnormalities has the most prognostic relevance?

a. Elevated triglycerides.
b. Raised serum bilirubin levels.
c. Decreased serum urea.
d. Raised Aspartate Aminotransferase (AST).
e. Raised gamma globulin levels.