

## **INTERPRETATION OF BLOOD TESTS FOR LIVER DISEASE**

Tests performed on blood samples with the intention of investigating liver disease may be subdivided into serum enzymes and indicators of residual hepatic functional capacity.

### **1. Serum Enzymes**

Increased serum concentrations of several intracellular enzymes have been reported to be useful in establishing the diagnosis and prognosis of hepatopathies in the horse. These include alanine aminotransferase (ALT), alkaline phosphatase (AP), arginase, aspartate aminotransferase (AST), gamma glutamyltransferase ( $\gamma$ GT), glutamate dehydrogenase (GLDH), iditol dehydrogenase (IDH) and lactate dehydrogenase (LDH). The four most commonly assayed liver-derived serum enzymes comprise **AP, AST, GLDH and  $\gamma$ GT**. Relative increases in serum concentrations of liver derived enzymes may infer the underlying nature of the liver disease. Experimental studies have suggested a primarily biliary source for  $\gamma$ GT, hepatocellular sources for arginase, ALT, AST, GLDH, IDH and LDH and both biliary and hepatocellular sources for AP.

#### ***Alkaline phosphatase (AP)***

Increased serum AP concentration has the strongest association with failure to survive of any enzymes although increased serum AP concentration is neither consistently increased in liver disease nor liver specific. In addition to hepatobiliary sources, serum AP is known to be derived from tissues such as bone, intestine, inflammatory cells and placenta and these possible sources should be considered in interpretation of increased serum AP concentrations.

#### ***Gamma Glutamyl Transferase ( $\gamma$ GT)***

Although mild to moderate increases of serum  $\gamma$ GT (eg. up to 100 iu/L) are of limited diagnostic or prognostic value, it is nevertheless very unusual to find significant hepatopathy in horses in the absence of increased serum  $\gamma$ GT. Additionally, marked increases in serum  $\gamma$ GT concentration (eg. >400 iu/L) are associated with a poor prognosis. Modest increases in serum concentration of  $\gamma$ GT should be interpreted with great caution as examination of liver biopsy specimens in such cases often fails to reveal significant underlying liver disease. The pancreas, or even kidneys, could potentially be the source of increased serum  $\gamma$ GT in the absence of hepatopathy although renally-derived  $\gamma$ GT is widely accepted to appear in urine and not serum. Another possible explanation is that increases in serum concentrations of liver-derived  $\gamma$ GT may result from insults too minor to result in detectable histopathology. For example horses with large colon obstructions are often reported with increased  $\gamma$ GT perhaps as a result of direct pressure applied to the liver by the distended and heavy colon. Furthermore, cases of liver disease are not infrequently seen where increasing concentrations of serum  $\gamma$ GT may be noted despite clinical evidence of improvement of the hepatopathy, perhaps as a consequence of reparative processes or biliary hyperplasia leading to increased serum  $\gamma$ GT.

#### ***Aspartate Aminotransferase (AST)***

AST is derived from widespread tissue sources and has low specificity for liver disease although the majority of liver disease cases will have increased serum AST.

#### ***Glutamate Dehydrogenase (GLDH)***

Although serum GLDH is supposed to be entirely derived from the liver, only a moderate specificity for liver disease has been found probably due to fairly mild and innocuous hepatic insults resulting in increased serum GLDH concentrations. Its relatively short serum half life might suggest an association between GLDH levels and the currently active degree of hepatic insult. The prognostic usefulness of GLDH is debatable and very high values are not uncommonly encountered in horses that recover uneventfully.

## **2. Serum biochemical evaluation of hepatic function**

Serum concentrations of several biochemical substances have been reported to reflect the capability of the liver to perform its normal functions. These are primarily endogenous and exogenous substances which accumulate in the blood as a result of failure of extractive and processing functions normally performed by the liver; and other substances whose serum concentrations are normally maintained by hepatic biosynthesis. They include various amino acids, ammonia (NH<sub>3</sub>), bile acids, bilirubin (total (Tbil) and direct (dBil)), fibrinogen, globulins, glucose and urea. Additional functional biochemical tests performed on blood samples and found to be useful in the investigation of liver disease include blood clotting times (activated partial thromboplastin time (APTT) and prothrombin time (PT)). These functional biochemical parameters might theoretically be more closely associated with the remaining mass of functional liver cells and therefore better reflect remaining hepatic function in comparison to estimation of serum liver-derived enzymes. Abnormalities of these functional parameters might therefore be more useful in the differentiation of hepatic failure from cases of adequately compensated hepatic disease and consequently have both diagnostic and prognostic value.

### ***Serum globulins***

Hyperglobulinaemia is a common finding in association with hepatic insufficiency probably resulting from systemic immunostimulation by intestinal-derived antigenic material following loss of the protective barrier of Kupffer cells. When increased serum globulins are found in association with other clinicopathological indicators of liver disease, this is a strong indication that the liver insult has been significantly harmful and the magnitude of the increase in serum globulin concentration has prognostic importance. Serum globulin concentrations greater than 45 g/L are concerning and values as high as 60-70 g/L are occasionally seen and warrant a guarded prognosis.

### ***Serum bile acids***

The main limitation of the usefulness of serum bile acid estimation is that liver disease must be quite severe before increased concentrations are detected and most liver disease cases will be found to have normal serum bile acid concentration at the time of initial presentation. Additionally anorexia and inappetence can increase serum bile acids as high as 20-30 µmol/L in the absence of liver disease. Hepatopathy cases with serum bile acid concentrations greater than 20 µmol/L are less likely to survive than those with lower values and *chronic* cases with bile acid concentrations above 100 µmol/L are almost invariably fatal. However this author has seen cases of *acute* hepatopathy with bile acid concentrations as high as 360 µmol/L that have survived.

### ***Serum albumin***

Although albumin is synthesised by the liver, its relatively long serum half-life explains the rarity of marked hypoalbuminaemia in equine liver disease which greatly limits its clinical usefulness. Serum albumin concentrations below 20 g/L are very rarely encountered even in severe hepatopathies. Nevertheless, even when present to a moderate degree, hypoalbuminaemia is a useful prognostic indicator in equine hepatopathy cases.

### ***Bilirubin***

Failure of the liver to take up, conjugate and excrete bilirubin may lead to increased serum concentrations of unconjugated and/or conjugated bilirubin. Anorexia and haemolysis are additional causes of unconjugated hyperbilirubinaemia. Horses may have serum unconjugated bilirubin concentrations greater than 200 µmol/L due to anorexia alone although more typically values of less than 150 µmol/L are expected. The magnitude of increased unconjugated bilirubin concentrations associated with acute haemolytic disease is very variable but can be greater than 500 µmol/L. The majority of equine liver disease cases have normal or only moderate increases in serum bilirubin concentration (typically 50-150 µmol/L) and the unconjugated fraction usually greatly exceeds the conjugated fraction. Cases of liver disease in the horse in which serum conjugated bilirubin represents greater than 25% of total bilirubin are very likely to be suffering from obstruction of the biliary tract.

### ***Urea & Creatinine***

Low serum urea concentrations have been recognised previously in association with liver failure and have been suggested to indicate reduced hepatic synthesis of urea from ammonia. However one study found no association between urea concentration and hyperammonaemia in equine hepatopathy. 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. Interestingly, a similar relationship was found in one study between low serum creatinine and prognosis. The association of low urea *and* low creatinine with a poor prognosis may suggest that uncontrolled diuresis rather than reduced hepatic synthesis is the underlying mechanism behind low blood urea in some cases of liver failure. Polydipsia and polyuria have rarely been reported in horses with liver failure although anecdotally it is well known that such clinical signs are frequently not recognised by horse owners.

### ***Blood clotting times***

Hepatic insufficiency is associated with a decrease in the synthesis and function of the majority of procoagulant, anticoagulant and fibrinolytic proteins in addition to reduced platelet numbers and function. Despite the complexities of effects on individual proteins, the net effect of hepatic failure on haemostasis is invariably impairment of coagulation as determined by prolonged APTT and PT, although clinical evidence of coagulopathy is less commonly seen than is clinicopathological evidence of coagulopathy in horses with hepatic insufficiency. Clotting times are best compared with a simultaneously sampled control horse rather than simply interpreted from absolute values. Clotting times >30% more than control are indicative of significant coagulopathy.

### ***Plasma amino-acids***

Derangements in serum concentrations of amino acids is commonly associated with hepatic insufficiency comprising increased concentrations of methionine and the aromatic amino acids (AAAs) tyrosine and phenylalanine and decreased concentrations of the branched chain amino acids (BCAAs) valine, leucine and isoleucine. Increased AAAs result from reduced hepatic clearance whereas decreased BCAAs results from increased muscular metabolism. Although clinical usefulness of these parameters has been established in the horse, limited availability of testing has greatly restricted their employment.

### ***Glucose***

Despite the central gluconeogenic role of the liver, hypoglycaemia is an uncommon consequence of hepatic failure with normal or high glucose concentrations being most usually found in adult horses. Foals with liver disease are far more likely to be suffering from hypoglycaemia.

### ***Ammonia***

Although plasma ammonia concentration is increased in nearly all cases of hepatic encephalopathy, the concentration does not necessarily correlate with severity of the disease. This apparent paradox may be explained by increased permeability of the blood brain barrier to ammonia in cases of hepatic encephalopathy.

Dynamic testing of liver function has been examined in horses using exogenous agents including bromosulphothalein, indocyanine green and radiopharmaceuticals although none appear to be widely used due to limited availability in contrast to other simple tests of hepatic function.

Table 1. The **diagnostic** usefulness of blood results demonstrating relationship between single test results and presence or absence of significant liver disease with derived values for probability (P) that test results are the same in horses with and without confirmed liver disease, sensitivity (S<sub>N</sub>), specificity (S<sub>P</sub>), positive predictive value (PPV) and negative predictive value (NPV); data from Durham *et al* 2003).

<b>Test</b>	<b>Test Result</b>		<b>Liver disease</b>		<b>P</b>	<b>S<sub>N</sub></b>	<b>S<sub>P</sub></b>	<b>PPV</b>	<b>NPV</b>
			<b>yes</b>	<b>no</b>					
<b>γGT</b> (n=81)	<b>&gt;55 iu/L</b>	<b>+</b>	<b>55</b>	<b>18</b>	<b>0.033</b>	<b>92</b>	<b>14</b>	<b>75</b>	<b>38</b>
	<b>≤55 iu/L</b>	<b>-</b>	<b>5</b>	<b>3</b>					
<b>albumin</b> (n=74)	<b>&lt;28 g/L</b>	<b>+</b>	<b>5</b>	<b>0</b>	<b>0.065</b>	<b>9</b>	<b>100</b>	<b>100</b>	<b>29</b>
	<b>≥28 g/L</b>	<b>-</b>	<b>49</b>	<b>20</b>					
<b>globulins</b> (n=74)	<b>&gt;36 g/L</b>	<b>+</b>	<b>30</b>	<b>4</b>	<b>&lt;0.001</b>	<b>56</b>	<b>80</b>	<b>88</b>	<b>40</b>
	<b>≤36 g/L</b>	<b>-</b>	<b>24</b>	<b>16</b>					
<b>AST</b> (n=68)	<b>&gt;388 iu/L</b>	<b>+</b>	<b>39</b>	<b>13</b>	<b>0.389</b>	<b>80</b>	<b>32</b>	<b>75</b>	<b>38</b>
	<b>≤388 iu/L</b>	<b>-</b>	<b>10</b>	<b>6</b>					
<b>AP</b> (n=60)	<b>&gt;522 iu/L</b>	<b>+</b>	<b>27</b>	<b>4</b>	<b>0.048</b>	<b>57</b>	<b>71</b>	<b>87</b>	<b>34</b>
	<b>≤521 iu/L</b>	<b>-</b>	<b>19</b>	<b>10</b>					
<b>GLDH</b> (n=58)	<b>&gt;8 iu/L</b>	<b>+</b>	<b>30</b>	<b>5</b>	<b>0.150</b>	<b>63</b>	<b>64</b>	<b>86</b>	<b>39</b>
	<b>≤8 iu/L</b>	<b>-</b>	<b>14</b>	<b>9</b>					
<b>Bile acids</b> (n=47)	<b>&gt;15 mmol/L</b>	<b>+</b>	<b>9</b>	<b>0</b>	<b>0.075</b>	<b>24</b>	<b>100</b>	<b>100</b>	<b>26</b>
	<b>≤15 mmol/L</b>	<b>-</b>	<b>28</b>	<b>10</b>					
<b>Urea(low)</b> (n=33)	<b>&lt;3.8 mmol/L</b>	<b>+</b>	<b>6</b>	<b>2</b>	<b>0.737</b>	<b>24</b>	<b>75</b>	<b>75</b>	<b>24</b>
	<b>≥3.8 mmol/L</b>	<b>-</b>	<b>19</b>	<b>6</b>					
<b>TBil</b> (n=31)	<b>&gt;48 μmol/L</b>	<b>+</b>	<b>4</b>	<b>0</b>	<b>0.093</b>	<b>17</b>	<b>100</b>	<b>100</b>	<b>26</b>
	<b>≤48 μmol/L</b>	<b>-</b>	<b>20</b>	<b>7</b>					

Table 2. The *prognostic* usefulness of blood results. Values compared against survival to 6 months (data from Durham et al 2003)

Variable	Category	Survivors		Non-survivors		Hazard ratio	95% confidence interval	P-value
		n	%	n	%			
<b>AP (iu/L)</b> <b>(n=80)</b>	<301	15	93.8	1	6.2	referent		
	301-600	23	82.1	5	17.9	3.09	0.36 - 26.46	0.303
	601-900	13	76.5	4	23.5	4.21	0.47 - 37.67	0.199
	<b>&gt;900</b>	<b>9</b>	<b>47.4</b>	<b>10</b>	<b>52.6</b>	<b>10.66</b>	<b>1.36 - 83.47</b>	<b>0.024</b>
AST (iu/L) (n= 94)	<350	16	84.2	3	15.8	referent		
	350-524	26	76.5	8	23.5	1.56	0.41 - 5.88	0.512
	525-700	10	58.8	7	41.2	3.03	0.78 - 11.72	0.109
	>700	21	87.5	3	12.5	0.81	0.16 - 4.02	0.797
<b>γGT( iu/L)</b> <b>(n=99)</b>	<100	16	88.9	2	11.1	referent		
	100-199	28	87.5	4	12.5	1.15	0.21 - 6.26	0.875
	200-399	16	72.7	6	27.3	2.59	0.52 - 12.82	0.245
	<b>&gt;399</b>	<b>16</b>	<b>59.3</b>	<b>11</b>	<b>40.7</b>	<b>4.54</b>	<b>1.00 - 20.49</b>	<b>0.049</b>
GLDH (iu/L) (n=61)	<12	26	86.7	4	13.3	referent		
	13-40	11	68.8	5	31.2	2.65	0.71 - 9.88	0.146
	>40	12	75.0	4	25.0	1.99	0.50 - 7.94	0.332
<b>TBA (mmol/L)</b> <b>(n=61)</b>	<11.0	28	93.3	2	6.7	referent		
	11.1-20.0	8	66.7	4	33.3	5.13	0.94 - 28.01	0.059
	<b>&gt;20.0</b>	<b>10</b>	<b>52.6</b>	<b>9</b>	<b>47.4</b>	<b>9.69</b>	<b>2.09 - 44.97</b>	<b>0.004</b>
<b>Albumin (g/L)</b> <b>(n=94)</b>	<30	5	50.0	5	50.0	referent		
	30-38	52	74.3	18	25.7	0.39	0.14 - 1.05	0.063
	<b>&gt;38</b>	<b>13</b>	<b>92.9</b>	<b>1</b>	<b>7.1</b>	<b>0.10</b>	<b>0.01 - 0.83</b>	<b>0.033</b>
<b>Globulins (g/L)</b> <b>(n=93)</b>	<31	25	92.6	2	7.4	referent		
	31-34	9	90.0	1	10.0	1.32	0.12 - 14.53	0.822
	35-39	16	80.0	4	20.0	2.99	0.55 - 16.32	0.206
	40-45	13	76.5	4	23.5	3.43	0.63 - 18.71	0.155
	<b>&gt;45</b>	<b>7</b>	<b>36.8</b>	<b>12</b>	<b>63.2</b>	<b>13.24</b>	<b>2.95 - 59.47</b>	<b>0.001</b>
<b>Urea (mmol/L)</b> <b>(n=63)</b>	0-3.5	5	41.7	7	58.3	referent		
	<b>3.6-5.0</b>	<b>15</b>	<b>83.3</b>	<b>3</b>	<b>16.7</b>	<b>0.21</b>	<b>0.05 - 0.82</b>	<b>0.025</b>
	5.1-6.0	11	78.6	3	21.4	0.28	0.07 - 1.08	0.065
	>6.0	14	73.7	5	26.3	0.37	0.12 - 1.19	0.097

## **LIVER BIOPSY**

There are three fundamental aims of the investigation of cases of suspected liver disease: firstly, to differentiate subjects genuinely suffering from liver disease from those which are not; secondly, to determine the type of liver disease affecting the subject (and therefore selection of specific therapy); and thirdly, to differentiate those subjects which are likely to survive from those which are not. If liver disease is suspected on the basis of preliminary non-invasive tests then liver biopsy remains the 'gold-standard' technique by which to address these key questions.

Transabdominal ultrasonography should be used to guide liver biopsy and a site is usually chosen based on thickness of imaged hepatic tissue, absence of large vessels and, occasionally, focal presence of hepatic tissue with an abnormal ultrasonographic appearance. The widespread availability of diagnostic ultrasound makes the ongoing use of unguided biopsy techniques questionable.

The main potential problems associated with liver biopsy in the horse are collection of an unrepresentative biopsy and adverse effects of the procedure itself including haemorrhage, colic, peritonitis, pleuritis and pneumothorax. In reality all of these problems are rare, especially when the technique is performed under ultrasonographic guidance, and the requirement for pre-biopsy coagulation assessment is highly questionable. A quarter of human patients report some degree of abdominal pain following liver biopsy and therefore routine systemic analgesia should also be administered in horses.

### **Technique**

#### **A. With ultrasonographic guidance**

Guides are available for many transducers which fix the biopsy needle in the plane of the ultrasonographic image and may facilitate the procedure although some clinicians choose to operate the transducer and biopsy needle separately from each other.

1. The subject is sedated
2. Biopsy site and transducer are prepared for a sterile procedure.
3. 10 mL local anaesthetic is infiltrated subcutaneously and through the intercostal muscles to the parietal peritoneum using a 21 g 1½" needle.
4. A small stab incision is made through the skin using a no.15 (or 11) scalpel blade.
5. A small amount of sterile coupling gel or alcohol is applied to the skin/transducer.
6. A 14 gauge 16 to 20 cm biopsy needle is advanced perpendicularly to the skin into liver and the biopsy is collected.
7. The procedure is repeated if a suitable biopsy specimen is not obtained (2 or 3 attempts sometimes required).
8. Biopsy specimens are placed in 10% neutral buffered formalin for histopathologic examination and/or plain sterile containers for bacteriologic culture.
9. Samples from at least 2 separate sites are preferable.
10. Topical antiseptic spray is applied to the skin incision.
11. A single dose of 2 mg/kg phenylbutazone iv is administered.

### Without ultrasonographic guidance

The right 13<sup>th</sup> intercostal space midway between two lines drawn between the point of the shoulder and the *tuber coxa* and the point of the elbow and the *tuber coxa* is preferred.

1. to 4. as above.
5. A 14 gauge 16cm biopsy needle is inserted slowly perpendicularly to skin until rhythmic movement of diaphragm is felt (typically between 5 and 10 cm from skin surface).
6. The needle is further advanced by about 2-3 cm and the biopsy is collected.
7. The procedure is repeated if a suitable biopsy specimen is not obtained (more than 5 or 6 attempts are not recommended).
8. to 10. as above.

### Interpretation

Marked periportal and bridging fibrosis have frequently been regarded as poor prognostic indicators although long term survival of cases with severe periportal and bridging fibrosis has been reported suggesting other factors are also important. In one study a scoring system was developed in order to attempt to attribute a prognostically useful broad comparative index of histopathologic severity (table 1). This results in a prognostic biopsy score between 0 (best prognosis) and 14 (worst prognosis). Biopsy scores from 73 cases showed a strong and statistically significant association with survival and survival times (table 2). The only two non-surviving cases with biopsy scores of 0 or 1 were diagnosed with biliary carcinoma and severe hepatic hydatidosis. In neither case was a biopsy score considered to be helpful or necessary as a firm diagnosis and prognosis was established prior to scoring. If these 2 cases were excluded on this basis, then the prognostic value of biopsy score would be increased further with 45/45 (100%) of horses with biopsy scores of 0 or 1 surviving for at least 6 months.

	<i>absent</i>	<i>mild</i>	<i>moderate</i>	<i>severe</i>
<b><i>Fibrosis</i></b>	<b>0</b>	<b>0</b>	<b>2</b>	<b>4</b>
<b><i>Irreversible cytopathology</i></b>	<b>0</b>	<b>1</b>	<b>2</b>	<b>2</b>
<b><i>Inflammatory infiltrate</i></b>	<b>0</b>	<b>0</b>	<b>1</b>	<b>2</b>
<b><i>Haemosiderin accumulation</i></b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>2</b>
<b><i>Biliary hyperplasia</i></b>	<b>0</b>	<b>0</b>	<b>2</b>	<b>4</b>

Table 1. Prognostic biopsy scoring system. Each individual parameter is scored and the total calculated. Minimum score = 0, maximum score = 14 (Durham *et al* 2003).

<b>Variable</b>	<b>Category</b>	<b>survivors</b>		<b>non-survivors</b>		<b>Odds ratio</b>	<b>95% confidence interval</b>	<b>P</b>
		<b>n</b>	<b>% total</b>	<b>n</b>	<b>% total</b>			
<b>TOTAL BIOPSY SCORE</b>	<b>0*</b>	<b>29</b>	<b>96.7</b>	<b>1<sup>Ω</sup></b>	<b>3.3</b>	<b>1.81</b>	<b>0.11 - 30.97</b>	<b>0.681</b>
	<b>1</b>	<b>16</b>	<b>94.1</b>	<b>1<sup>#</sup></b>	<b>5.9</b>			
	<b>2-6</b>	<b>8</b>	<b>66.7</b>	<b>4</b>	<b>33.3</b>			
	<b>7-14</b>	<b>2</b>	<b>14.3</b>	<b>12</b>	<b>85.7</b>			
						<b>14.50</b>	<b>1.42 - 148.57</b>	<b>0.024</b>
						<b>174.00</b>	<b>14.38 - 2104.85</b>	<b>&lt;0.001</b>

Table 2. Contingency table and results of logistic regression analysis demonstrating associations between total biopsy score and survival at 6 months post-biopsy (\* = reference category) (<sup>Ω</sup>case 14 – biliary carcinoma; <sup>#</sup>case 47 – severe hydatidosis) (n=73).