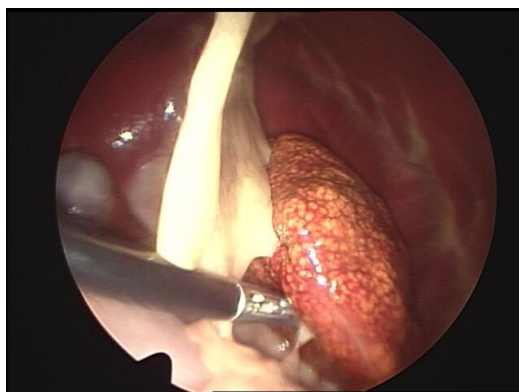


# **Liver and Pancreatic Disease 2017 Mini Series**

## **Session One: Understanding the Liver: How Can I Tell When It's Unhappy?**

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# Liver and Pancreatic Disease

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## Session 1: Approach to liver disease

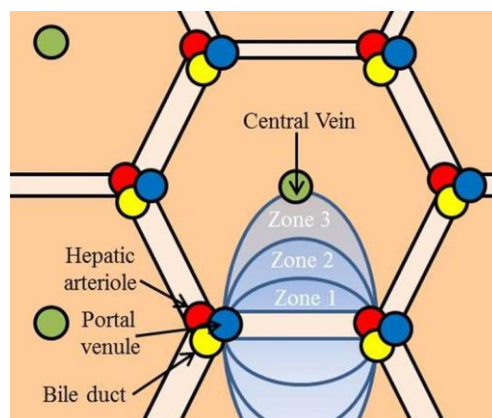
### Understanding the liver: how can I tell when it's unhappy?

Dogs and cats with liver disease present with a wide variety of clinical signs, which depend on the aetiology of the condition present and degree of any hepatic dysfunction that ensues. Clinical signs can be vague and include weight loss, lethargy and anorexia, or more specific with signs of hepatic dysfunction such as jaundice or hepatic encephalopathy. Elevated liver parameters may also be found during the course of routine evaluation, e.g. pre anaesthetic or geriatric blood panels, in animals with no obviously associated clinical signs. In this the first of the three sessions in this mini-series, we will review the approach to companion animals with liver disease, reviewing the utility of blood tests, imaging and biopsy techniques.

### Hepatic anatomy

The liver is an essential organ and has many functions. This includes breakdown of toxic metabolites, synthesis of hormones (e.g. insulin like growth factor-1), production of albumin, storage of vitamins and minerals, production of bile facilitating digestion and playing a key role in carbohydrate metabolism

The hepatic lobule is the basic anatomical unit within the liver. Blood enters the liver at the portal triads which are located at the each of the corners of its hexagonal shaped lobule. The portal triad consists of a hepatic arteriole, portal venule and a bile duct. The lobule is made up of cord of hepatocytes which run towards a central hepatic vein. The sinusoids between the hepatocytes contains vascular bundles (hepatic arterioles, hepatic venules and portal venules surrounded by epithelial cells) and between them and the hepatocytes is the space of Disse, in which lie hepatic lymphoid cells, Kupffer cells and hepatic stellate cells. Bile produced by hepatocytes moves from the central vein area towards the portal triads, with the ducts joining to form cholangioles (canals of Hering).



**Figure 1:** A schematic drawing of the hepatic lobules. The portal containing the hepatic arteriole, portal venule and a bile duct, are located at the points of the hexagonal lobule, with cords of hepatocytes running towards the central vein. The hepatic acinus describes the functional units of the liver, with zones 1 to 3 reflecting distance for the hepatic blood supply.

The hepatic acinus describes the functional, rather than anatomical, units of the liver, describing the movement of blood from each portal triad through the sinusoids towards the central veins. Dividing the acinus into zones reflects their relative distance from the vascular supply; zone 1 is nearest to the vascular supply at the portal triad and therefore has the greatest nutritional and oxygen supply, with zone 3 being the furthest away. Zone 1 is also first to encounter potentially harmful substances arriving from the portal circulation. Understanding the liver as functional units in this way, allows interpretation of the pathological patterns seen in many liver diseases.

### Approach to the patient with liver disease

As with any medical problem evaluation of the patient with suspected liver disease follows a problem solving approach, starting with a detailed history and physical examination, and where appropriate, followed by blood tests, imaging and often a biopsy. Signalment information can help guide possible causes for example both Doberman pinchers and Cocker spaniels have progressive breed associated forms of chronic hepatitis; as a result the finding of mild elevations in hepatic parameters may warrant further evaluation.

When a specific problem is identified (e.g. jaundice, ascites or hepatic encephalopathy) the list of differential diagnosis and diagnostic plan is clear, however for inappetence, weight loss and lethargy the list of possible differentials can be very long and it's appropriate to group disease into pathological processes (e.g. for weight loss, reduced intake, absorption or assimilation of nutrition) and perform some basic evaluation (e.g. complete blood count (CBC), serum biochemistry and imaging) before refining the problem list and diagnostic plan.

### Jaundice

Jaundice or icterus, is caused by excessive levels of bilirubin, which stain tissues. Bilirubin is formed during the process of haem metabolism during red cell breakdown in the spleen and is filtered from the circulation by the liver. If increased red cell breakdown can be excluded (e.g. due to immune mediated haemolytic anaemia) then hepatic (cholestasis) or post-hepatic (biliary tract obstruction) should be considered. Imaging normally allows evaluation of the biliary ducts and exclusion of obstructive disease. Jaundice is usually first seen in the sclera and is not normally detected until bilirubin levels reach 30-40µmol/l, although staining of tissues means rapid resolution of serum bilirubin levels can be associated with more gradual improvement in icterus. In dogs, particularly males, the renal tubules can metabolise freely filtered haemoglobin to bilirubin, leading to low level bilirubinuria being a normal finding in concentrated canine urine. Cat kidneys cannot metabolise haemoglobin and have a high threshold for bilirubin, so bilirubinuria is always an abnormal finding. The measurement of conjugated and unconjugated bilirubin is not clinically useful.

### Hepatic encephalopathy

Hepatic encephalopathy is a complex syndrome which describes neurological signs relating to hepatic insufficiency. Gastrointestinal flora produce many compounds which have been implicated in hepatic encephalopathy such as ammonia, short chain fatty acids, mercaptans, skatole and indoles. These substances cause neurotoxicity and alter brain neurotransmitter balance leading to altered neurological function. Clinical signs associated with hepatic encephalopathy are listed in table 1. Other factors such as gastrointestinal bleeding, hypokalaemia and alkalosis can precipitate and worsen clinical signs; this will be discussed in more detail in part 2 of this article.

Common Subclinical	Common Clinical	Rarer Clinical
Lethargy Listlessness Inappetence Ptyalism (particularly in cats) Vomiting Sensitive to dietary change Polydipsia / Polyuria	Personality changes Ataxia Weakness Aberrant drug responses Anorexia	Aimless pacing Circling Head-pressing Star gazing Amaurotic blindness Seizures

**Table 1:** Clinical signs associated with hepatic encephalopathy

### Ascites

Liver disease may lead to the formation of ascites through the development of portal hypertension, which may be contributed to by reduced albumin synthesis and resultant reduced oncotic pressure. Increased portal pressure leads to the development of ascites which varies in its nature depending on where the obstruction occurs. Portal hypertension may also lead to bowel wall oedema and associated diarrhoea. Pre-hepatic obstruction (e.g. portal thrombus or peri-portal fibrosis) tends to lead to a low protein fluid as the splanchnic vessels are relatively impermeable, whereas post-hepatic obstruction to blood flow (e.g. central vein fibrosis or hepatic vein obstruction) tends to lead to protein rich effusions as the sinusoids and lymphatics are much more permeable to albumin.

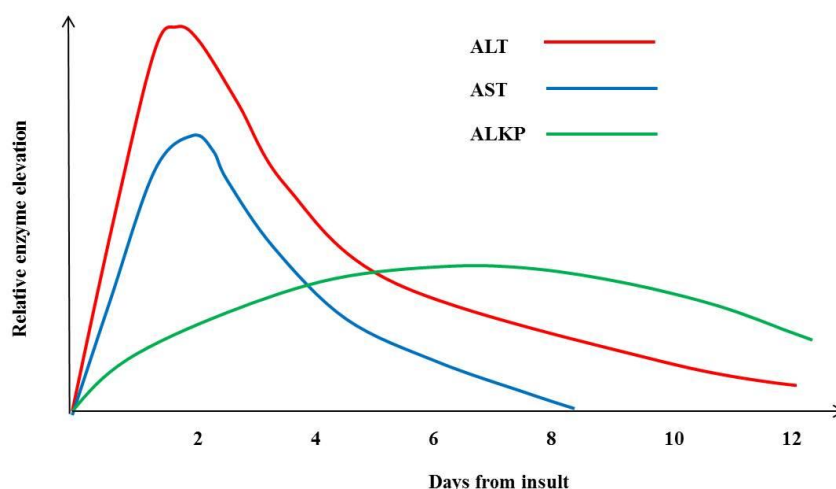
### Laboratory evaluation of liver disease

Blood tests are an essential starting point of the evaluation of liver disease. Liver enzymes give information about the degree and location of inflammatory changes within the liver, whereas functional tests such as bile acids and measurement of liver products such as albumin, cholesterol and glucose, allow assessment of hepatic function.

A CBC is useful as part of the general evaluation of patients with liver disease, but rarely reveals specific information. Evaluation of red cell numbers is essential to exclude pre-hepatic causes of icterus. In patients with congenital portosystemic shunts (PSS), abnormal iron metabolism often leads to a mild microcytic, hypochromic anaemia. Codocytes (target cells) occur in patients with liver disease (but also patients with regenerative anaemia, renal disease and lipid disorders). They occur due to excessive amounts of membrane to haemoglobin content and changes in membrane lipid content. Acanthocytes (spur or burr cells) can result from alterations in lipid metabolism and are seen in liver disease, but are also associated with the haemangiosarcoma and, splenic and renal disorders. The presence of acanthocytes and codocytes is not a specific finding for the presence of liver disease.

### Serum hepatic enzyme activities

Most routine biochemistry panels include measurements of alanine aminotransferase (ALT) and alkaline phosphatase (ALKP) and together with aspartate transaminase (AST) and gamma-glutamyl transferase (GGT) and glutamate dehydrogenase (GLD), are the commonly measured hepatic enzymes. They are relatively sensitive indicators of liver disease although not specific markers; for example extra-hepatic inflammatory disease can lead to elevations especially if associated with intestinal or pancreatic inflammation leading to reactive hepatitis. In dogs elevations over 3 to 4 times the reference interval usually reflects hepatic pathology. Given the much shorter half-life of the feline hepatic enzymes, any elevation is often significant. In dogs after resolution of a finite hepatic insult, liver enzymes will fall by approximately 50% every 4-6 days, which is important to consider when planning and interpreting follow up blood tests.



**Figure 2:** After a defined hepatic insult, the typical elevation in liver enzymes would be for the peak in ALT to be higher and earlier than AST, and for AST to normalise more rapidly. ALKP elevations are slower to peak and fall and are usually of a lower magnitude.

When interpreting liver enzyme elevations it is helpful to consider the relative elevations in terms of multiples of the reference interval, which can aid comparison between different laboratory machines. Elevations in ALT, AST and GLD suggest hepatocellular damage, with ALKP and GGT reflecting cholestasis. Comparison of elevations of more marked elevations in hepatocellular or choleostatic markers will help focus investigations, on parenchymal or hepatobiliary pathology. It is important to remember that elevations in liver enzymes do not allow interpretation of hepatic function and that functional hepatocytes need to be present to produce hepatic enzymes; in cirrhosis for example ALT and ALKP levels will decline over time as a result of reduced numbers of hepatocytes and it is important not to over interpret this as a response to treatment or improving prognosis.

Alanine aminotransferase (ALT) is found within the cytosol of hepatocytes with minimal amounts within skeletal muscle. Its serum half-life is long in the dog (45-60 hours) but much shorter in cats (3½ hours). Aspartate aminotransferase (AST) is also found within the cytosol of hepatocytes, however more severe hepatocyte damage is needed for its release compared to ALT. Its half-life is around 12 hours in the dog and 90 minutes in the cat. Glutamate dehydrogenase (GLD) is located with hepatocyte mitochondria and elevations usually reflect those of ALT. Its half-life is 18 hours in the dog.

Alkaline phosphatase (ALKP) has several isoenzymes. Hepatic L-ALKP is produced by hepatocytes and the biliary epithelium, in response cholestasis and biliary duct obstruction triggered by increased concentrations of bile acids. In dogs its elevation usually precedes an elevation in bilirubin and has a half-life of around 66 hours in the dog and 6 hours in the cat. Care needs to be taken with Scottish terriers as a breed related idiopathic hyperphosphatasemia has been reported which leads to raised ALKP in normal animals. B-ALKP is produced in bone and is associated with increased osteoblast activity, either in growing animals or in adults as a result of increased bone turnover as seen with osteomyelitis or osteosarcoma. Glucocorticoids (either endogenous or exogenous) can induce a steroid induced C-ALKP isoenzyme. Induction can often lead to very marked elevations in C-ALKP and care should be taken to distinguish C-ALKP induction from a steroid hepatopathy, which is associated with vacuolar change in hepatocytes.

Gamma-glutamyl transferase (GGT) is also produced by hepatocytes and the biliary epithelium as a result of elevated bile acids triggering increased production. Corticosteroids can also induce GGT but there is no bone isoenzyme. Elevations in GGT are generally more liver specific than ALKP. Although GGT elevations tend to follow the same pattern as ALKP, the magnitude is usually less marked

#### *Tests of hepatic function*

Bile acids are commonly used to measure liver function. Bile acids are produced in the liver from cholesterol and secreted into the gut via the biliary system. Conjugated bile acids are very efficiently absorbed by the ileum (95%) and are transported back to the liver in the portal circulation. Here 60-80% will be removed by first pass by the hepatocytes located in the periportal region. Bile acids act to provide osmotic force to promote biliary flow and act as a detergent in the small intestine to allow lipid uptake.

If a shunting vessel (either a congenital or acquired PSS) allows a proportion of the portal circulation to bypass the liver or if there are insufficient functional hepatocytes to extract bile acids from the circulation (due to severe liver disease) then bile acid concentrations will increase. Elevations in bile acids are often more pronounced in PSS than with chronic hepatopathies as the liver is very efficient at removing them from the circulation.

Fasting or post prandial samples >15-20µmol/l in the cat or 25-30µmol/l in the dog are supportive of hepatic dysfunction. To provide some scale of magnitude, animals with portosystemic shunting usually, though not always, have values >100µmol/l. Care needs to be taken in Maltese terriers who can have quite marked elevations in bile acids with no underlying hepatic pathology. Bile acids will be elevated in patients with hepatic or post hepatic jaundice, as bile acids share the same excretory pathways within the liver and physically through the biliary tree.

Ammonia is produced by bacteria in the gut and is metabolised to urea by hepatocytes via the urea cycle. Elevations in ammonia support the presence of both congenital and acquired PSS, as well as supporting the clinical suspicion of hepatic encephalopathy. Increased ammonia concentrations allow the development of ammonium biurate crystals, which are commonly seen with congenital PSS. The use of fasting ammonia levels has been shown to be more sensitive and specific for the detecting the presence of a PSS than fasting bile acids. Oral and rectal ammonia tolerance testing can be performed, but is not routine and can precipitate signs of hepatic encephalopathy. As ammonia is very labile measurement needs rapid following collection (<30 minutes) meaning in-house measurement is essential.

Albumin is produced by hepatocytes and they have a marked functional reserve for its manufacture. As a result hypoalbuminaemia is usually only seen in severely reduced hepatic function such as patients with congenital vascular anomalies and end stage cirrhosis. Other main differential diagnoses for hypoalbuminaemia are protein losing nephropathies and enteropathies.

The liver produces the majority of the coagulation and anticoagulant factors, as well as removing many of the products of fibrinolysis. A reduction in coagulation factors is common in both dogs and cats with liver disease, but elevations in coagulation times are rare. Reduced levels of coagulation factors can result from decreased coagulation factor production associated with diffuse hepatic disease or from reduced vitamin K absorption through extra-hepatic bile duct obstruction which will be associated with reduced levels of the vitamin K dependant coagulation factors II, VII, IX and X. In cats measurements of PIVKAs (proteins induced by vitamin K absence or antagonism) appear to be more sensitive than measurement of the activated partial thromboplastin time (APTT) and prothrombin time (PT) in the detection of vitamin K deficiency.

Hypoglycaemia is not commonly seen in animal with liver disease, with the exception of severe, acute fulminant hepatic failure and in animals with congenital PSS, especially in young and/or small breed dogs which may have reduced glycogen stores. Signs of hypoglycaemia can appear very similar to those of hepatic encephalopathy, thus should be checked early in investigations.

Blood urea nitrogen (BUN) is produced by hepatocytes metabolising ammonia produced in the gut via the urea cycle. Low BUN can be associated with reduced hepatic function (most commonly patients with congenital PSS) but is non-specific and can be reduced in patients with polydipsia / polyuria or those on diuretics or intravenous fluids.

Hypocholesterolaemia can be seen in animals with liver disease due to reduced hepatic production, both as a result of reduced hepatic mass and inhibition of cholesterol synthesis by bile acids. It is reported 60-70% of dogs and cats with congenital PSS have low cholesterol. Other differential diagnoses for hypocholesterolaemia include severe gastrointestinal disease (e.g. lymphangiectasia), hypoadrenocorticism and starvation. Hypercholesterolaemia can be seen with cholestasis.

## **Imaging the liver**

### *Radiography*

Survey radiographs are a good starting point for the evaluation of abdominal disease, however there assessment in hepatobiliary disease is limited to giving information about the external architecture of the liver. Radiographs give a good assessment of liver size, as well as an assessment of the shape and appearance of the liver margins, but are limited to the evaluation of the internal architecture and of the hepatobiliary tree. Areas of mineralisation within it in the wall of the biliary tree or choleliths will be detected, as will the consequences of liver disease such as peritonitis associated with rupture of the biliary tract or ascites secondary to portal hypertension.

### *Ultrasonography*

Due to the limitations of radiography and the cost associated with advanced imaging techniques, ultrasound is the most frequently used technique to evaluate the liver. It allows assessment of liver size (although this is more subjective than on radiographs), as well as assessment of the parenchymal architecture, portal and hepatic vasculature and the biliary tract. There is also the opportunity to use ultrasound to collect guided fine needle aspirate (FNA) samples.

The normal liver has a homogenous appearance, which should appear less echogenic than the spleen, with a slightly coarser appearance. The gallbladder is normally easy to locate, with a thin smooth wall. Sludge within the canine gallbladder is relatively common and not usually associated with hepatobiliary disease. Inspissation of bile and mucinous gland can lead to mucocoele formation, which has a very characteristic 'stellate' or 'kiwi fruit' appearance on ultrasound. It is usual to see the bile duct in cats and it can have a tortuous course, whereas in dogs is rarely seen. Obstruction to biliary flow should be suspected if the bile duct is >3mm in diameter in dogs and 5mm in cats. Evaluation of the portal vein allows evaluation of both congenital and acquired PSS's, portal vein thrombosis and measurement of portal pressure to confirm portal hypertension. Normal portal blood flow should be towards the liver, with a normal velocity of 15-20cm/sec in dogs and 10-18cm/sec in cats (D'Anjou and others, 2004). In animals with portal hypertension flow velocity is reduced (<10cm/sec) and may be reversed.

The hepatic echotexture may change with diffuse liver disease; however it can have a normal appearance, even with quite marked and advance hepatopathies. The diagnostic accuracy of ultrasound in diffuse liver disease has been reported between 36-39% for dogs and 55-58% for cats. Diffuse hypoechoic hepatic parenchyma has been associated with acute hepatitis, hepatic lymphoma and, amyloidosis and cholangitis in cats. Diffusely hyperechoic hepatic parenchyma has been reported with hepatic lipidosis, diabetes mellitus, steroid hepatopathy and hepatic lymphoma. A mixed pattern is commonly seen in chronic hepatitis as hypoechoic and hyperechoic regenerative nodules form.

Solitary mass lesions and metastatic neoplasia have a variable appearance on ultrasound, so it is difficult to determine their aetiology without a biopsy. Contrast-enhanced ultrasound allows assessment of tissue vascularisation and has high level of accuracy differentiating benign from malignant lesions.

### *Advanced imaging techniques*

Advanced imaging allows detailed anatomic evaluation of hepatic masses, which is useful, both for surgical planning allowing identification of margin and the major hepatic vessels, and in excluding metastatic disease. Magnetic resonance imaging (MRI) is also accurate in assessing if hepatic masses are benign or malignant. Computed tomography (CT) also allows excellent assessment of PSS as well as the hepatic, mesenteric and pancreatic vessels. As a result of rapid, practical and more accurate assessment of the portal vascular anatomy, CT angiography is recommended over ultrasound in the evaluation of PSS, especially in dogs >25kg where ultrasound evaluation can be challenging.

### **Liver sampling**

As bloodwork and imaging techniques will not define individual diseases, liver biopsies are required for definitive diagnosis in most cases. In general terms the bigger the biopsy is the more information can be obtained, especially where tissue architecture is important; thus in most cases tissue biopsies are more useful and accurate in the diagnosis of chronic hepatopathies than fine needle aspirates. Histopathology may also help with defining prognostic information such as the degree of fibrosis present or copper staining patterns in chronic hepatitis, which may also guide therapy. It is important to consider carefully the reasoning for liver biopsy as in some cases the clinical signs do not warrant complete evaluation to the point of liver biopsy and in these instances speculative therapy or a monitoring approach may be more appropriate.

Indications for taking liver biopsies include:

- Persistently elevated liver enzymes with no determined cause
- Evidence of hepatic dysfunction
- Jaundice of hepatic origin
- Ultrasound findings revealing diffuse changes in echogenicity
- Ultrasound findings of a discrete hepatic lesion or lesions
- Hepatomegaly of unknown aetiology
- Evaluation of the presence of a breed-specific hepatopathy

#### *Fine needle aspirates*

Fine needle aspirates are relatively easy, quick and safe to perform. They are usually taken with a 22G needle of an appropriate length, with a 'free needle' technique, rather than the application suction with a syringe, which can result in haemodilution. Although FNA's can be taken relatively safely in compliant patients, sedation is preferred. Ultrasound guidance is used to avoid the gallbladder and major hepatic vessels. Unless there has been cause for concern regarding bleeding (e.g. bruising or haemorrhage at a venepuncture site), it is not necessary to check coagulation times.

Hepatic FNA's are usually taken if there is a discrete mass lesion with in the liver and can be helpful to evaluate diffuse changes in echogenicity. Fine needle aspirates are very useful in disease processes where cells exfoliate easily, such as hepatic lymphoma, or where cellular criteria alone can guide treatment, such as hepatic lipidosis. However numerous studies evaluating cytology and histopathology from surgical biopsies have shown poor correlation, especially in chronic hepatopathies.

#### *Approach to hepatic biopsies*

Although FNA's are a good starting point biopsies for histopathology should be considered if cytology does not provide sufficient information. Tissue biopsies can be obtained either by using a needle (e.g. Tru-cut®) to collect a core biopsy under ultrasound guidance or at surgery. There are advantages and disadvantages to both techniques (see Table 2).

	Needle core biopsy	Laparoscopy	Laparotomy
<b>Invasiveness</b>	+	++	+++
<b>Cost</b>	££	£££	£££
<b>Operator experience</b>	++	++	+

**Table 2:** Relative differences between methods for obtaining liver biopsies

Prior to collecting surgical liver biopsies, a coagulation panel should be obtained, including aPTT, PT and platelet count. If coagulation times are prolonged, the administration of vitamin K and fresh frozen plasma should be considered and biopsies reconsidered once coagulation normalises. As haemostasis is more controlled with surgical techniques the risk of bleeding post procedure is considered less than with needle biopsies. Ascites and reduced liver volume are also relative contraindications of needle biopsies.

#### *Percutaneous ultrasound-guided needle biopsies*

There are several types of biopsies needle used in veterinary medicine, with semi-automatic needles being the most commonly used. These allow a notched stylet to be advanced under ultrasound guidance, into the area of interest avoiding major blood vessels. Once in position and hepatic tissue fills the notch within the needle, an outer sheath is triggered to advance over the stylet, which traps the sample within the notch. The sample obtained is small and several biopsies are often taken. In cats and small dogs 16G needles are recommended and 14G needles for larger dogs. Fully automated biopsies guns are also available, which fire both the stylet followed by the sheath into liver tissue. The speed of this process makes obtaining biopsies from a hard fibrotic liver easier. These automated devices should not be used in cats due to the possibility of potentially fatal vagal-induced shock associated with the shock of impact on the liver tissue.



### *Surgical biopsies*

Surgical liver biopsies can either be obtained via laparotomy or laparoscopy. Both allow multiple biopsies to be taken from normal and abnormally appearing areas of the liver. Laparotomy allows visualisation of all of the abdominal contents and is recommended when a surgical intervention could be expected, for example if there is biliary tract pathology, to perform portovenography or remove a solitary mass lesion. Several techniques are described to collect surgical liver biopsies, but no specialist equipment is needed.

Laparoscopy allows visual evaluation of the liver and multiple tissue biopsies to be taken, as well as the collection of bile under guidance. The samples obtained via laparoscopy are smaller than those obtained at laparotomy, but this does not appear to affect diagnostic accuracy as long as the biopsies contained 3-12 portal triads. Laparoscopy requires specialised equipment and training, but is a relatively less invasive technique.

### **Hepatic therapeutics**

Treatment of liver disease falls into three categories cytoprotective drugs and antioxidants, anti-fibrotic agents and steroids. The treatment of ascites, hepatic encephalopathy and coagulopathies will be discussed in session 2.

### **Cytoprotective medications and antioxidants**

Antioxidants are prescribed frequently in veterinary practice as part of the supportive treatment for a wide variety of hepatobiliary conditions. Oxidative damage plays an important role in the pathogenesis of liver disease in both people and animals. The liver is the main site of metabolism of gut based toxins and drugs, which means that the liver is routinely exposed to a large number of toxic intermediates, that can cause oxidative damage. Studies have shown that hepatic levels of one of the key antioxidants glutathione is reduced in inflammatory hepatitis in dogs and cats, as well as in feline hepatic lipidosis. Reduced glutathione concentrations likely predispose to oxidative hepatic injury, given the ability of glutathione to reduce reactive oxygen species. Thus it's widely suggested that antioxidants and therapies that help replenish glutathione stores are considered in the treatment of hepatobiliary disease. Many such supportive treatments are available and are often heavily advertised in the veterinary press. The interest in antioxidants as therapeutic agents is justified, given the theoretical benefits and the lack of definitive or effective treatment options for many hepatic conditions such as chronic hepatitis and cirrhosis. However, the use of antioxidants is sometimes seen in a controversial light, as with large areas of veterinary medicine, there are no large scale randomised placebo controlled studies evaluating their use. Such studies are hard to construct and would like need large case numbers, given that a uniform population of patients with liver disease would be hard to find. Small numbers of specific toxin based animal models and clinical veterinary studies are available, which are highlighted below.

### **S-Adenosylmethionine (S-AdoMet)**

S-Adenosylmethionine (S-AdoMet) is an endogenous molecule that is important in a number of metabolic pathways in the liver and is essential in the production of glutathione, which is an important free radical scavenger and antioxidant. S-AdoMet is produced from the amino acid methionine and ATP in a 2-step process catalysed by the enzyme methionine adenosyltransferase or MAT. MAT levels are known to be decreased in many types of liver disease in man, which result in reduced cellular levels of S-AdoMet.

S-AdoMet administration in hepatobiliary disease increases hepatocyte glutathione levels, thereby alleviating signs of oxidative stress and reversing signs of mitochondrial damage. S-AdoMet administration restores normal membrane fluidity which helps to restore normal mitochondrial glutathione transport. S-AdoMet also has anti-inflammatory actions through cytokine modulation, by modulating the activation of TNF- $\alpha$  and increasing production of IL-10, which is an anti-inflammatory cytokine. S-AdoMet has also been shown to protect normal hepatocytes against apoptosis and has growth regulatory effects on hepatocytes.

The effects of S-AdoMet have been evaluated in animal models, human clinical trials and a small number of veterinary studies. S-AdoMet has been shown to improve survival in animal models of

alcohol and paracetamol liver injury, and decreases hepatic fibrosis in mice given carbon tetrachloride. Meta-analysis of several placebo control human studies of patients with acute hepatitis, showed improvement in liver parameters and histopathological findings with SAME administration. Long-term administration of SAME (1200g/day for 2 years) to human patients with the early stages of alcohol induced liver cirrhosis increased survival and reduced the need for liver transplantation.

Oral administration of SAME has been shown to increase plasma SAME levels and increase hepatic glutathione concentrations, in both cats and dogs. Cats were also shown to have increased resistance to red cell osmotic fragility tests suggesting possible membrane stabilising effects. SAME administration has also been shown to reduce the effects of paracetamol induced red blood cell damage and liver injury in both cats and dogs. SAME administration to dogs receiving prednisolone therapy did not prevent the development of vacuolar hepatopathy or elevations in liver enzymes, although hepatic glutathione levels were increased suggesting SAME administration may mitigate the pro-oxidant effects of prednisolone. At present there are no published studies evaluating the effects of SAME on hepatobiliary disease in companion animals.

The recommended dose of SAME is 20mg/kg/SID for dogs and cats. Bioavailability is poor (around 3%) and preparations should be enteric coated to prevent inactivation by gastric acid; as a result tablets should not be split or crushed. Food will reduce absorption, thus SAME is best given on an empty stomach. SAME is very well tolerated however immediate post pill nausea, food refusal and anxiety have been reported in a small number of dogs and vomiting post administration has been seen in cats. SAME may increase the clearance of drugs that undergo hepatic glucuronidation (such as paracetamol, diazepam and morphine) and concurrent use with tramadol or monoamine oxidase inhibitors (such as selegiline) may lead to additive serotonergic effects.

As studies have not yet been published, the therapeutic potential of SAME in companion animals with liver disease is largely unknown. We know that liver disease reduces glutathione levels in most dogs and cats, however some animals had elevated levels. One study, by Center and colleagues revealed glutathione levels were low in 42% of dogs and 80% of cats with inflammatory hepatitis and 33% of dogs and 75% of cats with hepatic lipidosis. Given the important role of oxidative stress in the development of liver disease, restoring normal glutathione levels would be expected to be of benefit to small animals. In this respect SAME administration is likely to be of benefit to a wide range of hepatobiliary conditions including inflammatory, toxic, metabolic hepatopathies and cholestatic disease.

### **Silymarin**

Silymarin is derived from the fruit of *silybum marianum* or the milk thistle plant and has been used in Europe for more than 2000 years as a remedy for liver disease in man. Silymarin, the extract of milk thistle, is a mixture of flavonolignans which include silibinin (the major active component), isosilibinin, silidianin and silichristin. Silymarin has antioxidant effects by scavenging reactive oxygen species, protecting against glutathione depletion and reducing lipid peroxidation. Anti-inflammatory effects include inhibition of the leukotriene pathways and reduced NF- $\kappa$ B expression. Silymarin has also been shown to inhibit hepatitis C viral replication. Silymarin has anti-fibrotic actions through the inhibition of TGF- $\beta$  secretion and stellate cell activation. Silymarin has been shown to promote choleresis by increasing transporters in the apical membrane of hepatocytes, increase protein synthesis through stimulation of ribosomal RNA polymerase in hepatocytes and accelerate hepatocellular regeneration by increasing gene translation and transcription.

There is a wide range of in vitro and in vivo evidence that silymarin can protect the liver from a wide range of toxins (e.g. paracetamol, ethanol, *Amanita phalloides* (death cap mushrooms)) and ischemic, viral and radiation induced injury. Studies in the veterinary literature show that silymarin is protective against tetracycline induced hepatotoxicity in cats and carbon tetrachloride injury and *Amanita* mushroom toxicity in dogs. In the study by Vogel and colleagues, silibinin was found to be completely protective against the effects of *Amanita phalloides* at the LD50 dose, when given 50-150mg silymarin intravenously 5-24 hours after

ingestion. At present there is no information about the effectiveness of silymarin in the treatment of inflammatory liver disease in either cats or dogs.

Bioavailability of silymarin is low due to poor gastrointestinal absorption. Plasma half life is short and it is preferentially accumulated in the liver. Silymarin is excreted in bile and glucuronide and sulfoglucuronide conjugates, as a result biliary concentrations are roughly 100 times higher than those of serum. The exact dose requirements of companion animals is unknown with a wide range of doses reported, however most authors suggest silymarin doses in the region of 20-50mg/kg/day. Recently a form of silybin complexed with phosphatidylcholine, silyphos, has become available, which has a bioavailability 3-5 times higher than silymarin. A dose of 3-6mg/kg/day is suggested in dogs with a peak serum level seen 3 hours after administration. In cats bioavailability is around 7% with a dose of 10mg/kg, with a study showing this dose leads to increased neutrophil function and glutathione content.

No side effects have been reported with silymarin use in animals and extensive experience in the human field suggests very low toxicity, although gastrointestinal signs, pruritus and headaches have been reported. Toxicity studies using silybin-phosphatidylcholine complex in dogs appears to indicate a wide safety margin. Silymarin inhibits the activity of P-glycoprotein, glucuronide transferases and some cytochrome P450 enzymes. Although largely a theoretical consideration metabolism of drugs that use these pathways may be altered and this should be considered in patients receiving concurrent medications (e.g. P450 substrates such as beta blockers, calcium channel blockers and metronidazole and drugs that undergo hepatic glucuronidation such as paracetamol, diazepam and morphine).

A recent study, by Skorupski and colleagues in 2011, evaluated the effect of silybin (in a phosphatidylcholine complex) and SAME, on dogs receiving chemotherapy with the alkylating agent lomustine (CCNU), which can cause hepatotoxicity. The authors found that dogs receiving SAME and Silybin, were less likely to have elevations in liver enzymes and more likely to complete their course of chemotherapy. Although case numbers were low, and this was not a placebo controlled study, the results suggest that the combination of SAME and Silybin reduced lomustine induced hepatotoxicity.

Silymarin should be considered in dogs and cats with chronic inflammatory hepatitis due to its anti-inflammatory, choleoretic, anti-apoptotic and anti-fibrotic actions. Silymarin is also indicated in the treatment of Amanita mushroom toxicity, although high doses are needed to inhibit uptake of the toxin and the intravenous formulation is difficult to obtain. It may also be considered in dogs receiving chemotherapy with lomustine.

## **Vitamin E**

Eight isomers (vitamers) of vitamin E exist, with the most potent being  $\alpha$ -tocopherol. Its primary physiological role is as an anti-oxidant, forming a lipid soluble component of cell walls inhibiting lipid peroxidation. Vitamin E also modulates cell signalling pathways by altering the activity of lipooxygenases, cyclooxygenases and protein kinase C. It also inhibits platelet aggregation and adhesion, and suppresses inflammation by protecting against kupffer and stellate cell activation. The D stereoisomer of Vitamin E is synthesised by plants and is mainly found in vegetable oils, seeds and grains. It is not known if vitamin E levels are reduced in dogs and cats with liver disease and there is only one small pilot study reported by Twedt and colleagues in 2003, evaluating its therapeutic effect in dogs. This study showed increases in hepatic and serum vitamin E levels, as well as an improved redox status in 20 dogs with chronic hepatitis fed a vitamin E supplemented food over a 3 month period, however no changes in clinical or histological status were noted.

The suggested dose of vitamin for dogs and cats is 10-15 IU/kg/day or 100-400IU/dog/day. Traditionally over supplementation with vitamin E was felt harmless and no adverse effects of vitamin E supplementation are reported in the veterinary literature. However a recent meta-analysis revealed an increase in overall mortality in man with supplementation greater than 150IU/day, leading to recommendations that vitamin E administration be limited to <400IU/person/day. Absorption of other fat soluble vitamins may be inhibited when administered at high doses. For this reason it has been suggested that vitamin E

supplementation be avoided in patients with vitamin K deficiency (Flatland 2003). Vitamin E supplementation may also affect the absorption of ciclosporin and, if used concurrently, monitoring of ciclosporin levels is suggested.

Vitamin E supplementation should be considered for the management of hepatobiliary disorders that involve oxidative membrane injury (such as cholestatic hepatopathy and hepatic lipidosis in cats), specific hepatic toxins and inflammatory hepatopathies, although definitive studies proving their therapeutic effect are currently lacking from the literature.

### **Ursodeoxycholic acid**

Ursodeoxycholic acid (UCDA) is the predominant bile acid found in Chinese Black Bear bile, which has been used for centuries for its hepatobiliary healing properties and is now available in synthetic preparations. Bile acids are toxic to hepatocytes, causing membrane damage and in general terms the more hydrophobic the bile acid, the more potentially hepatotoxic it is. UCDA is less hydrophobic than the most common human bile acids, but of equivalent hydrophobicity to cholic acid, the most common bile acid in dogs and cats.

Ursodeoxycholic acid has many proposed actions which include. Promoting bile flow (choleresis) which is achieved through inducing membrane transporters necessary for promoting bile flow and increasing the osmotic gradient into the bile channels resulting in increased passage of water into the bile, which increases the volume of bile. UCDA has anti-apoptotic effects being protective to neurones, cardiac myocytes and intestinal epithelial cells. UCDA has anti-oxidant effects through increasing hepatocyte glutathione and possibly S-adenosylmethionine concentrations. UCDA has immunomodulatory effects, directly inhibits T and B lymphocyte activities. UCDA also binds to the glucocorticoid receptor in the cytoplasm. This UCDA-receptor complex can inhibit the production of pro-inflammatory cytokines

There are many suggested clinical uses for UCDA and there has been proven benefit in people with some specific hepatopathies, none of which have a veterinary equivalent. UCDA does induce choleresis in healthy dogs and causes measurable increases in circulating concentrations in healthy cats. There is one case report detailing its use (successfully) in a dog with chronic hepatitis.

In small animals UCDA might be useful in acute and chronic hepatopathies, especially if cholestasis is present. It is relatively contraindicated if there is extra hepatic biliary duct obstruction due to the potential for biliary rupture. Absorption improved if given with a meal. UCDA is generally well tolerated although rarely vomiting and diarrhoea have been reported. The suggested dose is 10-15mg/kg SID.

### **Anti-fibrotic agents**

Perisinusoidal cells can be activated to produce collagen either spontaneously (e.g. congenital forms of cirrhosis) or in response to any sustained inflammatory disease. Once collagen is cross linked in a stable helix, it is relatively insoluble. All anti-fibrotic therapies are most effective if used early on in the disease course before extensive formation of stable collagen has occurred. Any therapy that alleviated the underlying disease will have an indirect anti-fibrotic effect.

### **Colchicine**

Colchicine has an anti-fibrotic effect through interfering with microtubule assembly, this is the first intracellular step in collagen synthesis. It also blocks the secretion of procollagen and induces collagenase activity, which are the enzymes which breakdown collagen.

There is very limited data regarding its use in people but appears not to be uniformly effective. Currently there is no data regarding its use in dogs and cats. Gastrointestinal disturbance (mainly diarrhoea) and neutropenia have been reported following its use in dogs. In addition severe blood dyscrasias and myelofibrosis can result from prolonged use and careful monitoring is suggested when using this medication. The suggested dose is 0.01-0.03mg/kg

**Glucocorticoid therapy**

Steroids have a number of effects which are all mediated through the glucocorticoid receptor in cytosol. The glucocorticoid-receptor complex translocates to nucleus where it controls gene transcription. Glucocorticoids have anti-inflammatory, immunomodulatory, anti-fibrotic and cholera effect. Many side effects are possible and include PU/PD, increased protein catabolism which can worsen / aggravate encephalopathy, gastrointestinal ulceration (especially given that patients with portal hypertension are predisposed to this and thromboembolism).

In man, steroids are used for autoimmune hepatitis. This disease is becoming quite rare as infectious aetiologies (particularly viral infections) are increasingly recognised. One retrospective study of canine chronic hepatitis showed a distinct benefit of prednisolone therapy. This was a retrospective study, and it is likely that there was heterogeneous group of hepatopathies in the studied population.

Prednisolone should only be used in dogs with confirmed chronic hepatitis in which infectious and metabolic aetiologies (e.g. copper toxicosis) have been excluded via biopsy. Prednisone is metabolised to prednisolone in the liver, so it would seem logical to use prednisolone

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